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Evaluating the Effectiveness of Different Rhizobia Strains and Their Effect on Crop Yields in Acid Soils of Western Kenya

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ABSTRACT

Food insecurity in Sub - Saharan Africa (SSA) is on the rise due to soil fertility depletion and in Kenya, Nitrogen (N) is one of the widely deficient nutrients. Biological nitrogen fixation (BNF) can replenish N into the soil system. A study was carried out in acid soils at Koyonzo and Ligala sites of western Kenya to determine the effectiveness of different inoculants after agricultural lime application in enhancing BNF and yields of groundnuts (*Arachis hypogea* L.) and maize (*Zea mays* L.) intercrop. Red Valencia groundnut variety was intercropped with Hybrid 513D maize variety. A6w, W1w and V2w indigenous rhizobia strains were tested alongside a commercial rhizobia strain called biofix. Nitrogen treatment was included as a positive control. The results showed that inoculation significantly increased nodule number and weight per plant. There were significant differences among indigenous rhizobia in fixing N. Rhizobia inoculation accounted for 58.91% and 78.95% increase in the amount of N fixed above the control at Koyonzo and Ligala respectively. The strain that fixed the highest amount of N was A6w followed by V2w and W1w at both sites under the dolomitic soil amendment with the values of 14.67, 9.56, 3.53 and 11.37, 8.20 and 1.50 kg N ha^{-1}, respectively at Koyonzo and Ligala sites. Rhizobia inoculation accounted for 80.96% and 47.09% maize yield increase at Koyonzo and Ligala respectively. The best inoculant A6w, gave maize yields of 3.76 and 2.78 t ha^{-1} at Koyonzo and Ligala, respectively. In conclusion soil amendment with dolomitic lime and inoculating groundnuts with rhizobia strain A6w resulted in increased groundnut and maize yields. This practice can, therefore, be adopted by farmers in western Kenya to improve the productivity of the groundnut maize intercropping systems.

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Introduction

Rhizobia contribute to sustainable agriculture through symbiotic biological nitrogen (N) fixation (BNF) in legumes. Food security in the Sub Saharan Africa (SSA) largely depends on soil fertility replenishment (Yakubu et al., 2010). BNF is an attractive technology because it has the advantages of low cost of production and minimal environmental pollution (Van and Slinkard, 1994). Legumes and in particular, groundnut (*Arachis hypogaea* L.) seeds are rich in oil at 38-50%, protein at 25%, and traces of calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K) and vitamins making them important component of human diet. However, increased grain legume production depends on the successful legume inoculation with efficient strain of rhizobia. Groundnuts can naturally fix up to 206 kg ha^{-1} of N annually and are among the most important N fixing field crops (Giller, 2001). The N fixation process in groundnuts is facilitated by bacteria classified as *Bradyrhizobia japonicum.* This rhizobia functions efficiently under optimum pH ranges of 5.5- 7.0 (Rifat et al, 2008), therefore, soil acidity is a major constraint to N fixation process. Lime is used to reduce soil acidity and it is incorporated into the soil at least one month prior to planting. To guarantee food security, intercropping has been highly practiced in the recent past. Managing Beneficial Interactions in Legume Intercrops (MBILI) is an intercropping system that enhances crop yields due to reduced shading (Thuitha, 2007). The objective of the study therefore was to evaluate different rhizobia strains on their effectiveness in terms of the amount of N fixed from the atmosphere in two different soil types in Kenya.

Materials and Methods

A field experiment was carried out in two sites of western Kenya whose soils were acidic. Koyonzo soils characterized as acrisols with a soil pH of 5.20 and Olsen P of 9.20 mg kg^{-1} and Ligala soils characterized as ferralsols with a soil pH of 4.60 and Olsen P of 2.20 mg kg^{-1}. The experiment followed a Randomized Complete Block Design in a split plot arrangement where the lime treatment was the main treatment while the rhizobia inoculants were the sub plot treatment. These rhizobia treatment were identified as A6w, V2w W1w and one commercial rhizobia inoculant known as biofix. A6w and V2w were characterized as acid (pH<3.5) and aluminum (150uM) tolerant but W1w and biofix were not. The inoculant was introduced to the groundnut seeds on the planting date under a shade by using gum arabic as the sticker. The two types of lime were: calcitic ($CaCO_3$) and dolomitic [$CaMg(CO_3)_2$] lime both from Athi River Company in Nairobi, Kenya. These limes were applied three weeks prior to planting at the rate of 2 tha^{-1} (Hussein and Noorka, 2008) There was blanket application of P and K at the rate of 26 and 50 $kgha^{-1}$ respectively. The N fertilizer was supplied as calcium ammonium nitrate (CAN) in a split application at planting and at six weeks after planting at the rate of 34 kg N ha^{-1}.

Maize (*Zea mays* L.) and groundnuts (*Arachis hypogaea* L.) were intercropped in this experiment under the MBILI system. Hybrid maize variety, H513D, was bought from Kenya seed company while one groundnut variety, Red Valencia, was obtained from KARI Nthiwa at Kisii. Four rhizobia strains were tested. Three strains were native and were isolated at the Biotechnology center at the University of Eldoret and one commercial strain known as biofix was obtained from MEA limited in Eldoret, Kenya. Groundnuts were inoculated with different rhizobia strains at planting. Nodule number and weight were determined 42 days after planting. Aerial plant tissues sampled from field grown groundnuts were air dried in the greenhouse and ground for BNF analysis using the ^{15}N natural abundance method (Unkovich et al., 2008). Samples of maize grain, groundnut kernels, maize straws and groundnut shoots were taken from plots treated with different lime to determine yields of these crops.

All data was subjected to Analysis of Variance (ANOVA) and treatment means were separated using contrast analyses.

Results and Discussion

Effect of Inoculation on Nodule Number and Weight

Inoculation significantly increased nodule number and weight. Usually, inoculation increases the population of rhizobia in the soil. With a large population of rhizobia in the soil, there are higher chances of root infection and colonization hence more nodule formation. Rhizobia A6w significantly gave high nodule number and weight. This is attributed to its high intrinsic ability to compete for nodule occupancy. It also had a higher acid and aluminum tolerance compared to the other rhizobia and hence made it to compete favorably. The indigenous rhizobia inoculants, A6w and V2w, performed better than the commercial inoculant (Biofix) in terms of nodule number while W1w did not differ significantly from biofix in nodulation effectiveness. Apparently the two outstanding indigenous rhizobia are better adapted to the environment of western Kenya, making them to have higher competitive ability to occupy nodules. Recent studies indicate close relationship between rhizobia and legume yields (Yakubu et al., 2010; Yahui et al., 2011).

There were fewer nodules formed in the N treatment which was not significantly different from the control. These result shows that there was low rhizobia population in plots treated with N, minimizing infection of groundnut roots by rhizobia and subsequently limiting nodulation. Furthermore, high levels of mineral N in the soil hinder nodule formation (Hussain et al., 2008). Mineral N inhibits the rhizobia infection process and nitrogen fixation due to impairment of the recognition mechanism by nitrates (Fatima, 2007).

There were more nodules formed as a result of rhizobia inoculation in the second season (2011 SR) for both sites compared to the first season (2011 LR). This was probably as a result of increase in the population of inoculant rhizobia in the soil arising from the residual effect of inoculation from the first season and further inoculation in the second season. These results agree with work reported by Yakubu et al. (2010).

Table 1 Nodule number and weight of groundnuts as affected by different rhizobia strains at Koyonzo and Ligala sites in two cropping seasons

Strain treatment	Koyonzo				Ligala			
	2011 LR		2011 SR		2011 LR		2011 SR	
	Nodule $plant^{-1}$	Dry Wt (mg)	Nodule $plant^{-1}$	Dry Wt (mg)	Nodule $plant^{-1}$	Dry Wt (mg)	Nodule $plant^{-1}$	Dry Wt (mg)
V2w	13	10.1	16	11.1	10	7.9	13	9.7
A6w	16	10.8	18	11.2	12	9.6	16	9.5
W1w	9	5.1	10	5.8	7	3.5	8	5.1
Biofix	10	5.3	11	5.9	6	3.8	7	5.4
N	4	2.3	4	2.9	3	1.7	3	2.6
Control	2	1.4	3	1.7	2	1.3	2	1.6
SED Inoculant	0.26***	0.24***						
SED Season	0.15***	0.14***						

KEY: ***- Highly significant

Effect of Inoculation on Nitrogen Fixation

Generally, rhizobia inoculation led to higher amounts of N fixed. Approximately 11.56 kg N ha^{-1} was realized from indigenous rhizobia inoculant A6w when dolomitic lime was applied to ameliorate soil acidity. This was equivalent to 38.89% of Ndfa and gave indication that a farmer can cut down on the cost needed to buy inorganic fertilizers to supply N into the soil almost by half. These results agree with those of Okito et al. (2004), although in their case more N (55% Ndfa) was fixed. The difference is attributed to the fact that in Okito et al. (2004), a different soil type was used. They worked on a Nitisol which is characterized by higher fertility levels compared to the Ferralsols and Acrisols used in the present study.

Results from this study showed that the rhizobia strain A6w was the most superior of all the inoculants tested here for N fixation efficiency. The A6w strain fixed 27.6 % higher amount of N at Koyonzo than at Ligala and again this can be attributed to the relatively higher fertility status of the acrisols of Koyonzo as compared ferralsols of Ligala. There were very low amounts of N fixed in plots where N fertilizer was applied and further the amount of N fixed in this treatment was not significantly different from that fixed in the control. According to Hussain et al. (2008), the presence of combined forms of N in the soil hinders the BNF process. Such evidence was also reported by Vanlauwe et al. (2000), Van der Krift et al. (2001) and Wagner (2012).

Table 2 N yield and %ndfa as affected by different rhizobia strains in Koyonzo and Ligala sites of Western Kenya

Strain treatment	Koyonzo			Ligala		
	N yield ($kg\ ha^{-1}$)	Ndfa (%)	Amount of N ($kg\ ha^{-1}$)	N yield ($kg\ ha^{-1}$)	Ndfa (%)	Amount of N ($kg\ ha^{-1}$)
V2w	26.79	30.62	7.66	24.37	24.62	6.05
A6w	32.11	38.89	11.56	30.26	30.92	9.06
W1w	20.28	13.53	2.8	17.71	9.08	1.16
Biofix	20.52	13.06	2.58	17.38	7.15	1.22
N	21.19	6.38	1.08	18.92	3.19	0.63
Control	10.68	3.16	0.33	8.08	2.35	0.2
SED Site	0.271***	0.395***	0.158***			
SED Inoculant	0.469***	0.685***	0.273***			

KEY: ***- Highly significant

Table 3 Performance of different rhizobia inoculants on yield of groundnuts and maize in $Kg\ ha^{-1}$ at Koyonzo and Ligala during the two rainy seasons of 2011

Strain treatment	2011 LRs				2011 SRs			
	Groundnut Yields ($kg\ ha^{-1}$)		Maize Yields ($kg\ ha^{-1}$)		Groundnut Yields ($kg\ ha^{-1}$)		Maize Yields ($kg\ ha^{-1}$)	
	Koyonzo	Ligala	Koyonzo	Ligala	Koyonzo	Ligala	Koyonzo	Ligala
V2w	705.2	585.9	1264.0	1173.0	883.8	852.7	1966.3	1861.0
A6w	778.9	661.8	1363.0	1271.2	958.0	923.9	2064.9	1957.1
W1w	516.7	379.8	782.7	694.2	696.9	666.8	1483.3	1377.8
Biofix	522.9	404.2	747.8	657.9	702.3	600.9	1445.9	1341.8
N	715.0	596.6	1196.3	1109.0	894.0	859.1	1897.9	1792.1
Control	411.9	301.2	543.4	513.6	412.5	320.1	540.8	537.7
Mean	608.4	491.3	982.9	903.2	757.9	703.9	1566.5	1477.9

SED S: 5.9*** 2.7***; SED Sn: 5.6*** 2.7***; SED I: 9.7*** 4.7***; SED S*I: ns 6.6*; SED Sn* I: ns 6.6***; CV%: 6.3 5.6
Where, V2w, A6w, N, Control, Biofix and W1w are sub plot (rhizobia inoculant) treatments, ***-Significance at $P<0.001$, SED-Standard error of the difference, *-Significance at 95%, CV%-Coefficient of variation, LRs-Long rains and SRs- Short rains, S-Site, Sn-Seasons, L-Lime, I- Inoculant.

Effect of Amount of Nitrogen Fixed by Different Strains on Groundnut Yields

Significantly higher yields were realised in plots where rhizobia inoculation was done (Table 3). Inoculation increased rhizobia population which in turn occupied root nodules in high numbers and enhanced N fixation. Nitrogen is vital in chlorophyll formation. Improved N supply through BNF most likely contributed to more chlorophyll synthesis and an increased rate of photosynthesis in groundnuts generating food reserves that were translocated to the kernels and added up to their dry matter content (Alam et al., 2005). There were higher groundnut yields due to higher N fixation at Koyonzo than at Ligala that translated to more DM of the kernels. At both sites groundnut yields were higher during 2011 SR than in the 2011 LR. This could be due to the residual effect of rhizobia inoculation which resulted in higher N fixation in the second season. This result concur with Rifat et al. (2008) who conducted a research on BNF of summer legumes and their residual effects on subsequent rainfed wheat yields. Numerous publications have indicated the necessity of legume inoculation with effective and efficient rhizobia strains especially when the soil is void of the specific rhizobia agents (Jensen and Hauggaard, 2003; Verma et al., 2005).

The positive yield response of groundnuts to lime application can be attributed to P availability after liming. Phosphorus is important in ATP (adenosine triphosphate) formation and is very crucial for the nitrogenase enzyme that facilitated the N fixation process (Coyne, 1999). Results also showed that dolomitic lime gave the highest groundnut yields compared to calcitic lime. At Koyonzo it gave 17.11% yield increase above the control while at Ligala it gave 13.01 % above the control. Yield response of groundnuts to calcitic lime was as a result of Ca being available within the 10 cm of top soil where most pods were concentrated. According to Sorenson and Butts (2008), Ca supply in the podding zone is critical for the production of quality kernels.

Effect of Amount of Nitrogen Fixed on Maize Yields

Both lime and rhizobia inoculation increased maize yields on both sites (Table 3). Maize has extensive fibrous roots which tapped the nitrates into its root hairs for uptake. The N taken up was important for grain filling and chlorophyll formation which eventually translated to high DM (dry matter) content in maize grain. Enhanced N fixation by groundnut due to improved soil conditions via treatment application yielded nitrates that were also available for uptake by intercropped maize. However, maize yields in this experiment were lower than those reported by Thuita (2007) who worked in Bungoma site in Western Kenya. This is because, in the present study, there was no external source of N for maize use but it entirely depended on N fixed by the groundnuts which was low compared to the Fertilizer Use Recommendation Project (FURP) recommendations of 78 kg N ha^{-1} for optimum maize yields. This finding on cereals benefiting from N fixed by legumes is related to work done by Trannin et al. (2000). Available evidence indicates that N could be transferred from legumes to the associated cereal plants in an intercropping system via pathways of roots and nodular tissue decay (Ta and Faris, 1988; Trannin et al., 2000), via exuded compounds from legume plant roots (Van and Slinkard, 1994) or via transfer from mycorrhizal fungus (Hamel and Smith, 1991). Usually cereals have a stronger ability to absorb soil nutrients (Rynne et al., 1994) than legumes. Lime recorded yield increase of 131. 32% and 98.76% were recorded at Koyonzo and Ligala respectively. Rhizobia inoculation accounted for 80.96% and 47.09% yield increase at Koyonzo and Ligala respectively. These results of increased maize yields agree with those of Arnold and Wayne (2006).

Conclusion

In conclusion, the practice of inoculating groundnut seeds before planting significantly leads to high crop yields. Moreover, inoculating the groundnut seeds using the rhizobia strain A6w leads to great levels of nitrogen fixed in the soil as well as increased crop yields. Application of lime as well enhances the rhizobia activity. Therefore, these practices can be adopted for improved BNF and increases crop yield in the endeavor of alleviating poverty.

Recommendation for further research

- Molecular analysis on strain A6w should be carried out for further characterization.
- More work should be carried out to ascertain the lime rates that would be appropriate for superior strains in this research to fix high amounts of atmospheric nitrogen.

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