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Nitrogen fixation potential and residual effects of selected grain legumes in a Kenyan soil

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Abstract

Based on their performance attributes, butter bean (variety Ex-kasuku) and grass pea (Selection 1325) have been identified as potential alternative legumes for the maize-based cropping system in the cold semi-arid region of Laikipia County in Kenya. However, their nitrogen fixation potential and nitrogen residual effects have not been established. A green house experiment was therefore conducted to determine the nitrogen fixation potential and residual effects of the introduced legumes (butter bean and grasspea) relative to the local checks (common bean cv. Katumani 330 and chickpea cv. Desi). The legume seeds were planted in perforated polythene bags, measuring 14 cm diameter and 20 cm high, containing 3.6 kg air dried soil collected from Matanya in Laikipia County, Kenya. Barley, a non-nitrogen fixing reference crop, was also planted in polythene bags and used to determine the amount of nitrogen fixed according the N-difference method. Butter bean and grass pea significantly out-performed chickpea in total nodule number, active nodule number, total nodule dry matter, total plant dry matter, dry matter N yield, amount of N-fixed, percent N derived from the atmosphere and residual N effect, but were comparable to common bean in all these attributes. There was a significant, positive linear relationship between quantity of N-fixed and quantity of total plant biomass accumulated. Butter bean, grass pea and common bean significantly increased soil mineral nitrogen while chickpea had no influence on soil nitrogen. Butter bean and grasspea can therefore provide N to cropping systems in the cold semiarid region through biological N fixation.

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Introduction

The benefit of including legume crops in cropping systems is mostly associated with their ability to biologically fix atmospheric N (Cheminig'wa *et al.,* 2006; Walley *et al.,* 2007). Nitrogen fixation is reported to be affected by such factors as soil available N and effectiveness of the rhizobia-host association (Van Kessel and Hartley, 2000), soil pH, soil moisture, soil available P and plant management (Fosu *et al.,* 2004; Muza and Mapfumo, 1998; Peoples *et al.,* 1995; Thies *et al.,* 1995) and soil microbial populations (Dogbe *et al.,* 2000). It is reported to vary with legume species (Chemining'wa *et al.,* 2004; Mwangi and Wanjekeche, 1997;) and to be closely correlated with legume dry matter production (Kumar and Goh, 2000; Lelei *et al.,* 2009).

Generally soil N can be increased by biological nitrogen fixation (Mburu and Gitari, 2006). The amount of nitrogen added to a cropping system depends on the legume nitrogen yield and the proportion of N due to biological nitrogen fixation (Giller, 2001).

Butter bean (variety Ex-kasuku) and grass pea (Selection 1325) were identified as potential legumes for the cold semi-arid region of Laikipia district on the basis of biomass and grain yields, nitrogen yield and water use efficiency and were comparable to the local checks (common bean (variety Katumani 3330) and chickpea (variety Desi)) in most performance attributes (Mwangi, 2011). Intercrops of these legumes and maize were also demonstrated to have land use and monetary advantages over respective sole cropping (Mwangi, 2011). The nitrogen fixation potential and nitrogen residual effects of these legumes have however not been established.

Previous studies have shown that Kenyan soils have indigenous rhizobia strains that can fix atmospheric nitrogen in association with commonly grown legumes such as common bean, cowpea, green gram and pigeon pea (Chemining'wa *et al.,* 2011; Karanja *et al.,* 2002).

Presence of indigenous rhizobia that can effectively nodulate butter bean and grasspea in the cold semi-arid area of Laikipia has not been established. Legume species and varieties vary in their capacity to fix nitrogen and make it available to subsequent rotation crops (Van Kessel and Hartley, 2000; Giller, 2001; Graham *et al.*, 2004). In previous studies, for example, chickpea was reported to fix 64-138 kg N ha^{-1} with residual balance of up to 38 Kg N ha^{-1}(Fatima *et al.,* 2008)

Assessment of nitrogen fixation potential and nitrogen residual effects is critical in the selection of legumes for integration into the low nitrogen maize-legume based production systems in the cold semi-arid area of Laikipia county, Kenya. Therefore, a green house experiment was conducted to determine the nitrogen fixation potential and residual effects of the introduced legumes in a soil from a cold semiarid area of Laikipia.

Materials and methods

Experimental design, treatments and crop husbandry

The experiment was conducted in a greenhouse at the National Agricultural Research Laboratories (Kabete). The experiment was arranged in a randomized complete block design and replicated three times. Treatments were four legumes including: grass pea Selection 1325, butter bean variety Ex-Kasuku, common bean variety Katumani 3330 and chickpea variety Desi.

Perforated polythene bags measuring 14 cm diameter and 20 cm height were filled with 3.6 kg of air dried soil collected from the study site (Matanya) at 0-15 cm depth. Four seeds of each legume and barley (as a reference crop that cannot fix nitrogen (Soon and Arshad, 2004)) were planted in each bag. A total of 6 bags per legume were planted. The bags were watered regularly to maintain field capacity. Butter bean and grasspea had no history of cultivation in the site (Matanya in Lakipia county of Kenya) where the soil for the study was collected.

Data collection

Number of nodules per plant and nodule dry matter

At flowering, one bag of each legume (containing four plants) was flooded with water and the soil carefully poured out. All nodules from each plant were counted. Number of fixing nodules was determined by splitting the nodules and counting those with pink colouration. Percent of active nodules was calculated as the ratio of active nodules to total number of nodules. All nodules were dried to constant weight and nodule dry matter per plant determined.

Plant total dry matter and nitrogen yield

At physiological maturity, the other bag of each legume and barley was flooded and soil poured out. The materials (root and shoot) were carefully washed and dried at 700C to constant weight and the weight recorded. A composite sample of each material was ground to pass through a 2 mm sieve. Percent nitrogen in each sample was then determined using the Kjeldahl procedure (Okalebo et al., 1993). Nitrogen yield for each material was calculated as the product of percent nitrogen and dry weight.

Amount of fixed nitrogen and percent nitrogen derived from the atmosphere

The amounts of nitrogen fixed by the legumes were estimated using the nitrogen difference method (Brockwell et al., 1982; Soon and Arshad, 2004):

BNF = Nleg - Nref + (Nsoil – Nsoilref) where BNF (biological nitrogen fixation) = amount of nitrogen fixed, Nleg = legume nitrogen content, Nref = reference crop nitrogen content, Nsoil = soil mineral nitrogen before planting and Nsoilref = soil mineral nitrogen after harvesting the reference crop. Barley was used as the reference crop. Percent nitrogen derived from the atmosphere (% Ndfa) was determined as the ratio of BNF to plant nitrogen yield.

Soil nitrogen

Soil samples were analysed for soil available N (NO-3-N + NH+4-N) using the method described by Okalebo *et al.* (1993) before planting and at physiological maturity.

Data analysis

Collected data were subjected to analysis of variance using SAS statistical package (SAS Institute, 1993). Where the F values were significant, means were compared using the least significant difference (LSD) test, at p = 0.05. Regression analysis was used to compare the relationship between amounts of fixed nitrogen and legume total dry matter yield.

Results

As shown in Table 1, the number of nodules were not significantly different (p=0.05) between introduced legumes (butter bean and grasspea, with 25.6 and 25.5 nodules per plant respectively) and locally grown legumes (common bean and chick pea, with 23 and 27.3 nodules per plant respectively). Grass pea, butter bean and common bean had higher number and proportion of active nodules, nodule dry matter, total plant biomass yield and N yield than chickpea (Table 1). They also generally fixed significantly more atmospheric N, had higher percentage of N derived from the atmosphere (Table 2) and contributed more soil residual nitrogen after harvest than chickpea (Table 3). Linear regression relationship between amounts of fixed nitrogen and legume dry matter yield was positive and significant (Figure 1).

Discussion

Grass-pea and butter bean were adequately nodulated in soil from the cold semiarid site where they had not been previously grown, indicating the abundance of indigenous rhizobial strains compatible with these legumes in the region. Previous studies have demonstrated the widespread presence in Kenya soils of rhizobial strains that are compatible with a cross range of legume crop species (Chemining'wa *et al.*, 2006; Chemining'wa *et al.*, 2011; Karanja *et al.*, 2000).

Grass pea, butter bean and common bean fixed more nitrogen and had higher soil residual nitrogen after harvest than chickpea. These observations suggest that the former have greater nitrogen fixation capacity than the latter.

Genetic variation among legumes in nitrogen fixation potential has been reported in various studies (Giller, 2001; Lelei *et al*., 2009; Walley *et al*., 2007; Yusuf *et al*., 2008). Graham *et al*. (2004) reported that nitrogen fixation in legumes is a quantitively inherited trait. The results in the current greenhouse study suggest that grass pea, butter bean and common bean have the potential to contribute positively to the overall soil N economy over time.

Table 1. Nodule count, nodule dry matter (g), biomass yield (g/plant), nitrogen yield (g/plant) of butter bean, grass pea, common bean and chickpea planted in soil collected from Matanya (Laikipia county, Kenya).

Legume	Nodule number per plant	Active nodules per plant	Percent active nodules	Nodule dry matter (g)	Biomass yield (g/plant)	Nitrogen Yield (g/plant)
Grass pea	25.5a	22.6a	88.7a	0.5a	108.2a	3.9a
Butter bean	25.6a	22.4a	86.4a	0.6a	113.8a	3.7a
Common bean	23.0a	19.6a	86.4a	0.5a	96.3a	3.3ab
Chickpea	27.3a	1.7b	6.5b	0.2b	66.0b	2.1b
Mean	25.4	16.6	67.0	0.4	96.1	3.2
LSD (p=0.05)	Ns	9.3	11.4	0.2	23.9	1.2
CV (%)	24.9	29.0	8.8	20.2	12.9	18.7

The poor N fixation potential in chickpea could be attributed to the presence of inefficient but compatible native rhizobia in the site. This is supported by the fact that the total number of nodules per plant was not significantly different among the legumes while chickpea had significantly fewer active nodules than the other legumes. The observations imply the need to inoculate chickpea with commercial rhizobial strains and screen chickpea genotypes for capacity to effective nodulatewith native rhizobia. Currently there are no commercial inoculants strains for chickpea in Kenya hence there is need to screen for effective chickpea nodulating rhizobia strains. Higher N fixation capacities of grass pea, butter bean and common bean also suggest the presence of adequate and effective indigenous rhizobia infecting these legumes. Chemining'wa *et al*. (2011) reported that native rhizobia that nodulate common bean and cowpea were widespread in central Kenyan soils. According to Dogbe *et al*. (2000), for effective nodulation rhizobia population density should not be less than 50 cells per gram of soil.

The increased soil nitrogen after planting grass pea, butter bean and common bean could have been due to enhanced nitrogen supply by the legumes through nitrogen fixation and decaying nodules and roots. Several reports have suggested increased soil nitrogen by some pulse crops (Beckie and Brandt, 1997; Gan *et al*., 2003; Soper and Grenier, 1987; Van Kessel and Hartley, 2000).

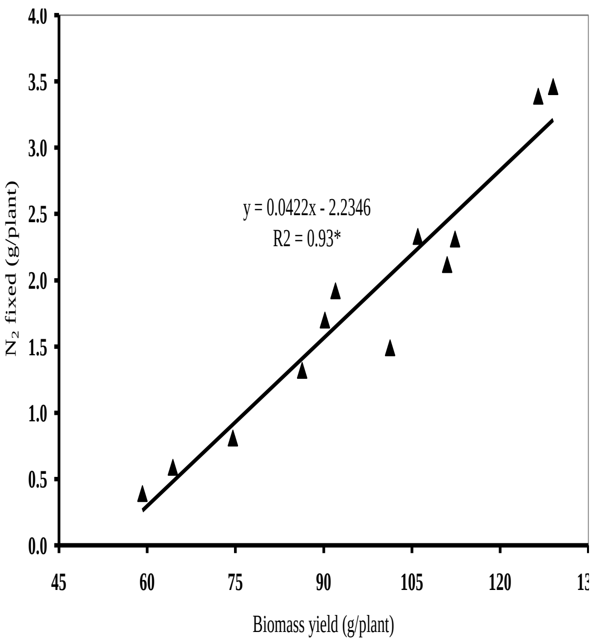
Table 2. Biologically fixed nitrogen (g/plant) and percent nitrogen derived from the atmosphere by butter bean, grass pea, common bean and chickpea planted in soil collected from Matanya (Laikipia county, Kenya).

Legume	Fixed N (g/plant)	Percent N derived from atmosphere
Grass pea	2.6a	64.1a
Butter bean	2.4a	61.3a
Common bean	1.8ab	55.7a
Chickpea	0.6b	29.8b
Mean	1.8	52.7
LSD (p=0.05)	1.2	14.8
CV (%)	32.8	14.4

Pulse crops are reported to have high grain protein content and frequently the net export of nitrogen to grain often exceeds the total amount of nitrogen fixed in the biomass (Beck *et al*., 1991). According to Van Kessel and Hartley (2000), a positive increment in soil nitrogen from fixation is only achieved when nitrogen fixation is relatively high and/or the NHI is relatively low. Low NHI on the other hand implies low grain yield or quality. The percentage of nitrogen derived from the atmosphere (%Ndfa) by grass pea, butter bean and common bean was higher than the corresponding NHIs (Mwangi, 2011) suggesting that they are likely to contribute to the soil nitrogen pool.

Table 3. Soil mineral nitrogen content before and after planting butter bean, grass pea, common bean and chickpea in soil collected from Matanya (Laikipia county, Kenya) and percent change in soil nitrogen.

Sampling period	Soil mineral N (mg/kg)	% change in soil N
Before planting	0.97c	
After grass pea	1.28a	31.6a
After butter bean	1.29a	33.4a
After common bean	1.23ab	27.1a
After chickpea	1.04bc	7.1b
Mean	1.16	24.8
LSD (p=0.05)	0.21	10.0
CV (%)	9.63	20.8



* significant at p=0.05

Fig. 1. Linear regression curve describing the relationship between amounts of biologically fixed N and above ground biomass yield of butter bean, grass pea, common bean and chickpea planted in a green house at the national agricultural research laboratories (Kabete) with soils collected from Matanya.

Legumes with high amounts of nitrogen fixed also had high biomass yield. The correlation coefficient (R^2) between amounts of nitrogen fixed and legume biomass yield was 0.93. This agrees closely with findings of Kumar and Goh (2000) who reported strong correlations between the amounts of fixed nitrogen for both legume dry matter yield (R2=0.96) and nitrogen accumulation. Giller (2001) had also reported that larger amounts of nitrogen fixed in broad bean (Vicia faba) resulted from better growth and high biomass accumulation. The positive correlation between dry matter and amounts of N fixed indicates that dry matter accumulation of N fixing legumes may be a good indicator of the amount of atmospheric N fixed and may be used as a selection criterion.

Conclusion

Butter bean (variety Ex-kasuku) and grass pea (Selection 1325) have the potential to contribute positively to soil N in cropping systems of the cold semi-arid region through biological N fixation.

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