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Effects of Liming on Nodulation, Nitrogen Fixation and Seed Protein Content in Soybean

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Soil acidity is influenced by high levels of aluminium, iron, manganese and low levels of Cation Exchange Capacity (CEC), plant nutrients availability such as phosphorus, affect the growth of symbiotic-fixing bacteria in soybean which is detrimental to nodule formation and functioning. In an attempt to evaluate liming effects on soil pH, nitrogen fixation and seed protein content, an experiment was set up using a randomized complete block design (RCBD) layout in a split-plot with four replications at the Crop Museum, the Sokoine University of Agriculture in Morogoro, Tanzania. Soybean genotypes (Bossier, Laela and Uyole soya-1) were used as the main plot, while lime levels (1560, 936 and 624 kg/ha) were used as the subplot. The analysis of variance (ANOVA) showed a significant influence of lime levels on soil pH, quantities of nitrogen fixed and seed protein content. There was a significant difference ($P = 0.001$) observed among soybean genotypes with the application of 1560 kg/ha of lime (pH 6.5), recording the highest (7.6) nodule counts and nodule dry weight (19.26). Among the different varieties used in the study, Bossier was observed to have a fixed 24.46 kgN/ha, while Laela produced the highest (10.60%) seed protein content. Application of 1560 kg/lime observed a significant increase in kgN/ha (35.71) as well as a minimal increase (15.66%) in seed protein content at pH 6.5. Interaction effects ($P = 0.05$), however, observed Laela has fixed the highest nodule (7.6) counts, nitrogen fixation (44.90 kgN/ha), with Bossier recording the highest (20.22%) seed protein. The study revealed that the physiochemical properties of acid soil, as well as soybean yield components such as nodules, N₂ fixation and seed protein content, can be maximally improved when limed with CaCO₃.

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INTRODUCTION

Soybean is a minor crop in Tanzania produced mostly by smallholder farmers. The crop is also grown by large-scale enterprises. In Tanzania, the crop is used as food for humans and is being processed and increasingly used in livestock feeds as a protein replacement for dried fish which has been the usual source of protein in the past (Wilson, 2018). Cultivation of the crop is rapidly gaining popularity in Africa, following high demand from the expanding livestock feed industry (which consumes about 70 - 80% of soybean produced per year) and its need to restore soil nitrogen in symbiosis with *rhizobium* makes it a very good colonizer of low-N soils (Mahasi *et al.*, 2011). As the richest plant source of protein, the crop is well placed to meet the growing demand for vegetable oil and animal feed in developing countries with a protein content (40%) and oil (20%) (Tukamuhabwa *et al.*, 2012). It provides the cheapest source of plant protein known to man in terms of accessibility, especially in developing countries (Idrisa *et al.*, 2010). It can be processed into animal feed and its seed contains oil which can be used for cooking, making margarine and other industrial uses such as bio-fuel (Mathu *et al.*, 2010).

Amongst the many legumes known and grown by farmers, soybean affects different aspects of the ecosystem including soil microbes. In association

with bacteria, the crop can fix nitrogen and aid in the amelioration of acidic soil conditions. Fageria *et al.* (2014) found that the amount of N₂ fixed by forage legumes on low-fertility acid soil is dependent on legume growth and persistence. At pH<4.5, *Rhizobium*-legume symbiosis, N₂ fixation, number of nodules, nitrogenase activity, nodule ultra-structure, and fresh and dry weights are greatly affected (Baijukya *et al.*, 2013). The increasing demand for soybean production in recent years has made the crop to be regarded as Africa's Cinderella crop (Kolapo, 2011). The crop is among the 16 major crops (barley, cassava, groundnut, maize, millet, potato, oil palm, rapeseed, rice, rye, sorghum, soybean, sugar beet, sugarcane, sunflower, and wheat) cultivated worldwide (Foley *et al.*, 2011).

The low soil fertility status is influenced by strong soil acidity (pH 5.1), low organic carbon (OC), low cation exchange capacity (CEC) and low exchangeable bases on smallholder farms in the study area as reported by (Merumba *et al.*, 2020), the result has been the low yield and yield components of most annual crops including soybean. However, liming acid soil remains an essential component in providing optimum conditions for biological activities to increase nitrogen fixation, nodule formation and functioning and increase the dry mass of the shoot in soybean (Goulding, 2016).

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Crop Museum, Sokoine University of Agriculture (SUA) farm in Morogoro, Tanzania. The study area is located between latitude 6°05' and longitude 37°39' East at an elevation of 568 m above sea level. The field site is located at the foot slopes of Uluguru Mountain in Morogoro Municipal, Tanzania. The rainfall distribution is bimodal with the first season (short rain) lasting from November to January while the second season (long rain) lasting from February to May. The annual rainfall ranges between 800 to 950 mm. The soils are sandy clay loam with a pH ranging between 5.0 – 5.2. The physiographic feature of the area is an undulating convex land, and the slope is about 4% (Kisetu *et al.*, 2013).

Experimental Design

Three soybean varieties (*Bossier*, *Laela* and *Uyole soya-1*) used in the experiment were obtained from Uyole Agricultural Research Institute in Tanzania. Lime (CaCO₃) was purchased from an agricultural inputs supplier in Morogoro, Tanzania. The experiment was laid out in a split-plot - randomized complete block design (RCBD) with four replications. The soybean varieties (Uyole soya-1, Bossier and Laela) were applied as the main plot and lime rates to change the soil pH to 5.5, 6.0 and 6.5 as the subplot. The crop was spaced at 0.5 x 0.2 m for inter-row and intra-row, respectively. The subplot size was 2 x 2 m while the main plot size was 9.5 x 2 m; between subplots was 0.5 m, and spacing between main plots and replications was 1 m resulting in 2 m². Each plot had four rows with ten plants per row. The length of the field was 32.5 m, and the width was 13 m. The effective area used for the experiment was 422.5 m².

Soil Analysis

Soil samples were collected at a depth of 0-30 cm as recommended by Pleyrier (1995) for the

physiochemical analyses according to Landon (1991). Soil Bulk Density was determined by the core method as described by Lal and Shukla (2004). Soil pH was determined electrometrically in 1:2.5 soil-water suspensions as described by Mclean (1982). Total nitrogen was determined by the micro-Kjedahl digestion method as described by Bremner and Mulvaney (1982). Soil available phosphorus was determined based on the Mehlich 3 extraction procedure (Mehlich, 1984). Exchangeable potassium was determined by the use of the ammonium acetate method (McLean and Watson, 1985). The CEC of the soil was determined by a compulsive exchange procedure (Gillman and Sumpter, 1986). Exchangeable aluminium was determined by the KCl method as described by Hiradate *et al.* (1998). Exchangeable calcium was determined by the buffer method as described by Adams and Evans (1962). Zinc was determined by the zincon method as described by Miller (1979). Exchangeable sodium was determined based on the method described by Blakemore *et al.* (1987). Iron, Magnesium and Manganese were determined by the Ethylene Diamine Tetra Acetic acid (EDTA) method as described by Schnug *et al.* (1996).

Data Collection

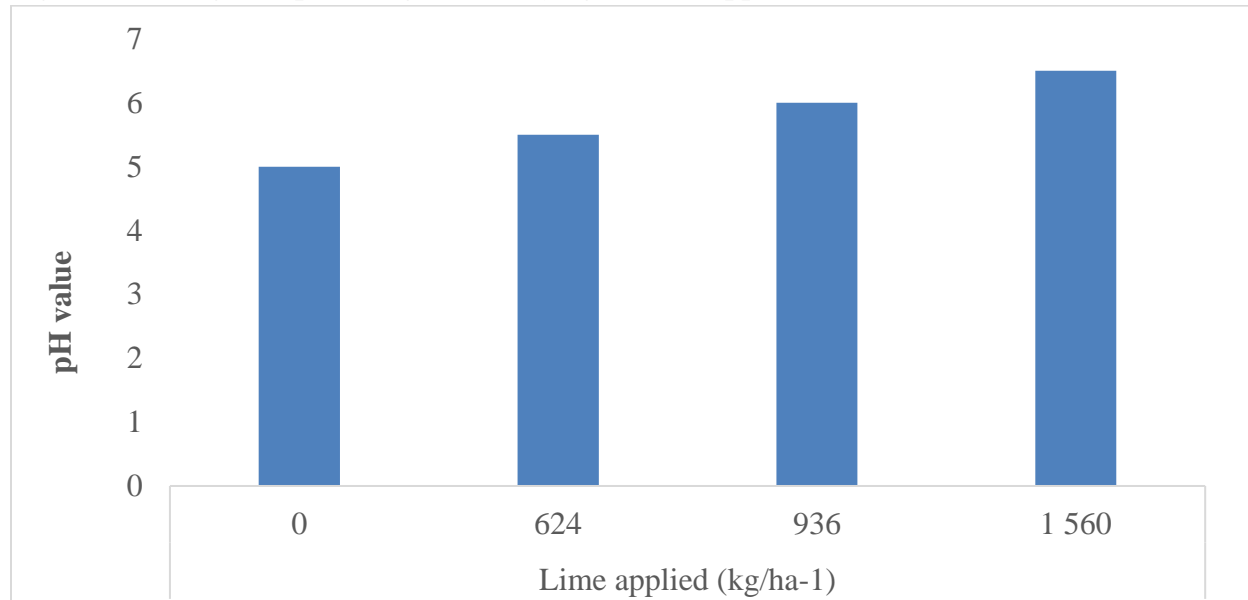
Determination of pH Response to Liming

Different rates of calcium carbonate (per two kg of soil equivalent to 1, 2, 3, 4 and 5 tons/ha assuming about 1.5 million kg of soil/ha) were established based on the bulk density of the soil under investigation. The soil in separate pots was moistened with 500 ml of distilled water to bring it to about field capacity and allowed the reaction to take place at room temperature, thus permitting weekly pH-lime determination for six weeks as described by Watson and Brown (1998). The different lime rates (2, 3 and 5 tons/ha) that raised the soil pH in the medium to 5.5, 6.0 and 6.5, respectively, were determined using the soil lime curve as described by Watson and Brown (1998). The bulk density of the experimental soil was found

to be 1.04 g/cm³. The weight of the soil/ha was determined as follows: Vol. of 1ha = 100 x 10 x 100 x 1.5 = 1 500 000 L x 1.04g/cm³ = 1 560 000 kg soil/ha. The weight of the soil was used to determine

the lime rates under the 2 x 2 m experimental unit. Using the same principle, the following amount of lime in kg/ha was applied to raise the soil pH from 5.0 to the desired soil pH (5.5, 6.0 and 6.5).

Figure 1: Average soil pH change with varying levels of applied Lime



Determination of Nodule Number

Three plants were sampled randomly from the 1.0 m² area plot at 50 % flowering. The three plants were carefully uprooted using a spade so as to obtain intact roots and nodules. Uprooting was done by exposing the whole-root system to avoid losses of the nodules. The soil was carefully removed by washing the roots with tap water and avoiding the detachment of the intact nodules. The number of nodules per plant was then determined by counting the number of nodules from all three uprooted plants per plot and then the average per plant Vincent (1970).

Determination of Nitrogen Fixation Characteristics

Plants were dried at 70°C for 72 hours and ground into fine powder. A 0.2 g sample of dried plant material was weighed and placed into a test tube. Total nitrogen in each sample was determined by

the use of the Kjeldahl digestion method as described by Bremner and Mulvaney (1982).

Estimation of N₂ fixed

The amount of N₂-fixed was determined by subtracting the total nitrogen of the control plants from that of the N₂-fixed plants, and the difference was assumed as the amount of N derived by Biological Nitrogen Fixation (BNF; N₂-fixed). Thus, N₂-fixed = Total N fixed – Total N in control as described by Viera-Vegas *et al.* (1995).

Total N in plant

$$= \frac{\text{Dry matter weight} \left(\frac{\text{kg}}{\text{ha}} \right) \times \% \text{ N in plant}}{100}$$

Determination of Seed Protein Content (%)

Seed protein content was determined using the Kjeldahl method. Total nitrogen content was then converted to seed protein content value (%) by a multiplication factor of 5.46, as described by Bremner and Mulvaney (1982).

Data collected were subjected to analysis of variance (ANOVA) using GENSTAT 15th edition and declared significant at $P < 0.05$ using the statistical model as described by Gomez and Gomez (1984). The mean separation test was done using Duncan's Multiple Range Test and conclusions were made at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSIONS

Soil Physiochemical Characteristics

Table 1: Physical and Chemical properties of soil at the study site

	Soil characteristics	Unit	Value	Remarks
Physical characteristics	Sand	%	67	Sandy Clay Loam* High**
	Silt	%	06	
	Clay	%	14	
	Bulk Density	g/cm ³	1.04	
Chemical properties	Soil pH		5.0	Acidic*
	Cation exchange capacity	cmol/kg soil	8.70	Low*
	Organic carbon	%	0.44	Very low*
	Calcium	cmol/kg soil	2.24	Low*
	Magnesium	cmol/kg soil	0.54	Low*
	Total Nitrogen	%	0.06	Very low*
	Phosphorus	Ppm	6.44	Low*
	Sodium	cmol/kg soil	0.13	Low*
	Aluminium	meq/kg soil	4.54	High**
Micronutrients	Zinc	Ppm	2.1	Low*
	Iron	Ppm	96.78	High*
	Manganese	Ppm	40.62	Very high**

Rating: *Landon (1991); **Lindsay and Norvell (1978)

The cation exchange capacity (CEC) in the current study was low (8.70 cmol/kg soil) according to a rating by Landon (1991). The total organic carbon was also low (0.44 %) according to the rating by Landon (1991). The low organic matter in the study area could be attributed to continuous cultivation, non-return of crop residues, and non-use/limited applications of soil amendments like farmyard manures. Total nitrogen was very low (< 0.06) according to the rating by Landon (1991). Effects of low soil N retards plant growth and reduce crop yield. The soil in the study showed low (6.44 ppm) availability of phosphorus according to the rating by Landon (1991). Soil analysis based on classification by Landon (1991) rated calcium low (2.24 cmol/kg

Soil analyses in the current study revealed that the soil textural class was classified as sandy clay loam (Ryan *et al.*, 1999) with a bulk density of 1.04g/cm³ and a soil pH of 5.0. The soil pH was considered strongly acidic according to a rating by Landon (1991), thus resulting in the application of lime (CaCO₃). Salvagiotti *et al.* (2008) reported that soybean thrives in the pH range of 6.0 to 6.8.

soil), magnesium low (0.54 cmol/kg soil) and potassium low (0.27 cmol/kg soil).

Iron (96.78 ppm) and manganese (40.62 ppm) contents in the study were high according to the rating by Lindsay and Norvell (1978), while available zinc (2.1 ppm) was low (Landon,1991). However, the application of calcium carbonate (CaCO₃) to acid soil supplies Ca²⁺ and neutralizes the toxicity effects of H⁺, Al³⁺ and Mn²⁺ and make nutrients readily available for plant root uptake. Ibrahim *et al.* (2011) reported that limed acid soils increased plant nutrient output, ameliorated acidic soil conditions, and improved plant growth, yield and yield components.

Aluminium in the current study was high (45.4 meq/kg soil) according to the rating by Landon (1991). A high level of Al occurs in soil when the pH values are equal to or less than 5.4. An excessive amount of Al^{3+} inhibits plant root development and limits crop growth. Limed acid soil, however neutralized Al^{3+} effects and reduced its concentration resulting in a conducive soil environment favourable for supporting plant growth and development. Kisinyo *et al.* (2012) reported that lime decreased exchangeable soil acidity and increased the pH hence displacing H^+ and Al^{3+} ions from the soil adsorption sites by adding Ca^{2+} ion contained in the lime ($CaCO_3$).

Soil pH Response to Liming

There was no significant difference observed among the genotypes used in the study (Table 2). With the application of different lime levels, a significant

increase ($P = 0.001$) was observed in the initial soil pH (5.0). At flowering, it was observed that 1560 kg/lime had raised the pH to 6.65, followed by 936kg/lime (pH 6.23) and 624kg/lime (5.49). At crop maturity, the study observed a minimal increase in the soil pH, contrary to what was recorded during flowering (Table 2). The study observed that the increase in soil pH could be attributed to the residual effects of the applied lime. Interaction effects observed significant difference ($P = 0.001$) among soybean genotypes and lime levels with all the genotypes well pH performed (Table 3). Similarly, Workneh *et al.* (2013) reported significant changes in soil pH when several quantities of lime were applied. Kisinyo *et al.* (2014) attributed the changes in soil pH, improvement in the availability of basic cations, reduction in the concentration of toxic levels of Al and increase in P levels as some of the many benefits associated with limed acid soils.

Table 2: Effects of liming on soil pH, soybean, N₂ fixation and seed protein content

Treatment		Initial pH	Soil pH at flowering	Soil pH at maturity	Nitrogen fixed (kg/ha)	Seed protein content (%)
Soybean varieties Factor (a)	Bossier	5.0	5.86a*	5.93a	24.46b	9.18a
	Laela	5.0	5.87a	5.95a	20.09ab	10.60a
	Uyole Soya-1	5.0	5.88a	5.96a	11.54a	8.33a
	Grand mean	5.0	5.87	5.94	18.7	9.4
	CV a (%)	NA	23.42	19.48	19.8	16.75
	P value	NA	0.89	0.76	0.04	0.66
Lime (kg ha) Factor (b)	0 (pH 5.0)	5.0	5.09a	5.18a	3.72a	5.49a
	624 (pH 5.5)	5.0	5.49b	5.53a	13.19ab	5.54a
	936 (pH 6.0)	5.0	6.23c	6.33a	22.16bc	10.79ab
	1,560 (pH 6.5)	5.0	6.65d	6.75b	35.71c	15.66 b
	Grand mean	NA	5.87	5.94	18.7	9.4
	CV b (%)	NA	3.61	3.79	12.6	9.92
P value	5.0	0.001	0.001	0.001	0.003	

*Means in the same column followed by the same letter do not differ significantly using the Duncan Multiple Range Test at $P \leq 0.05$

Table 3: Interaction effects of liming on soil pH, soybean, N₂ fixation and seed protein content

Treatments (AXB)	Initial pH	Soil pH at flowering	Soil pH at maturity	Nitrogen fixed (kg N/ ha)	Seed protein content (%)
Bossier x 5.0	5.0	5.1 a*	5.18 a	0.54a	3.48a
Laela x 5.0	5.0	5.53 b	5.53 b	3.96a	0.39
Bossier x 5.5	5.0	6.20 c	5.25 c	14.37ab	5.87ab
Uyole Soya-1 x 5.0	5.0	6.67 d	6.75 d	6.67a	5.14ab
Laela x 5.5	5.0	5.10 a	5.20 a	15.26abc	7.18abc
Uyole Soya-1x5.5	5.0	5.48 b	5.53 b	9.94a	5.95ab
Bossier x 6.0	5.0	6.25 c	6.35 c	33.74abc	10.80abcd
Laela x 6.0	5.0	6.60 d	6.73 d	16.26abc	10.20abcd
Bossier x 6.5	5.0	5.08 a	5.15 a	49.20c	20.22d
Laela x 6.5	5.0	5.48 b	5.53 a	44.90bc	15.97bcd
Uyole Soya-1 x 6.0	5.0	6.25 c	6.38 c	16.50abc	8.93abcd
Uyole Soya-1 x 6.5	5.0	6.68 d	6.78 d	13.04ab	18.31cd
Grand mean	NA	5.87	5.94	18.7	9.4
CV b (%)	NA	3.35	8.29	25.9	9.92
P value	NA	0.001	0.001	0.05	0.05

*Means in the same column followed by the same letter do not differ significantly using the Duncan Multiple Range Test at $P \leq 0.05$. NA: Data was not subjected to statistical analysis because 5.0 was the initial pH of the experimental site before liming.

Evaluation of Liming Effects on Nodulation, Nitrogen Fixation and Protein Content in Soybean

There was no significant difference observed in the number of nodules among soybean varieties. Control plots, however, showed an insignificant amount of nodule formation and counts.

Application of lime observed a significant difference ($P = 0.001$), thus resulting in an increase in nodule counts (Table 2). Hence, the application of 1560kg/lime observed nodule counts of 7.6, thus making it the highest of all the lime applied in the study. Interaction effects of lime and Laela also showed similar nodule counts, as shown in Table 2.

Table 2: Effects of soybean varieties and lime application on yield components

Treatment	No. of nodules	Nodule Color rating	Nodule dry weight (g)
Soybean varieties Factor (a)	Bossier	5.4a	2.11a
	Laela	5.7a	2.05a
	Uyole soya-1	5.5a	2.09a
	Grand mean	5.5	2.1
	CV a (%)	17.6	2.0
	P value	0.69	0.41
Lime (kg/ha) Factor (b)	0 (pH 5.0)	0.0a	0a
	624 (pH 5.5)	7.2b	2.71b
	936 (pH 6.0)	7.3b	2.76bc
	1 560 (pH 6.5)	7.6b	2.86c
	Grand mean	5.5	2.1
	CV b (%)	11.6	0.12
	P value	0.001	0.001

*Means in the same column followed by the same letter do not differ significantly using Duncan Multiple Range Test at $P \leq 0.05$.

The study showed significant effects ($P \leq 0.001$) of lime on nitrogen fixation and seed protein content among soybean genotypes. Bossier was observed to have fixed the highest nitrogen (24.46 kg/ha), followed by Laela (20.09 kg/ha) in the study. Also, Laela produced the highest seed protein content (Table 2). When different levels of lime were applied, 1560 kg/lime observed the highest nitrogen (35.71 kg/ha) fixed, followed by 936 kg/lime (pH 6.0) and 624 kg/lime (Table 2). Interaction of lime and soybean genotypes observed Bossier at pH 6.5, fixing the highest (49.20 kg/ha) nitrogen and highest (20.22 %) seed protein content among the three soybean genotypes used in the study (Table 2). The study observed significant changes in the initial soil pH (5.0) over time when different levels of lime were applied. The change in pH had significant effects on soybean nodulation, nitrogen fixation and seed protein content. The results are in conformity with those of Ebeniro *et al.* (2010) who reported a significant effect ($P \leq 0.001$) on soil pH levels and soybean yield components when lime at different levels (1 356 kg/ha lime, 1 437.80 kg/ha, 1 790.06 kg/ha) were applied. The minimal performance of nodule effectiveness, soybean growth, nutrient uptake, and biological nitrogen fixation could be attributed to the improvement of nutrients' availability following the application of lime, as previously reported by Lin *et al.* (2012). The study showed that the application of lime to acid soil raised the pH and made the soil favourable for soybean growth, yield and yield components.

Interaction effect, however, observed the highest (20.22 %) seed protein content in Bossier. The findings are contrary to Stephanie *et al.* (2015), who reported significant ($P = 0.05$) Protein content in soybean varieties when inoculated with Biodoz Rhizofilm. Besides, the low protein content observed in the study could be contributed to the low availability of phosphorus in the study area. Kisinyo *et al.* (2014) reported the importance of soil phosphorus in N metabolism and its associations with protein metabolism in leguminous crops. The

study observed that P limits symbiotic N_2 fixation, which is associated with protein content assimilation in legumes.

CONCLUSION

The effectiveness of the lime application is very important for successful soybean cultivation in soils that are believed to be acidic and have low nutrient availability. The study area revealed low soil nutrient output before lime was applied. With the application of lime, significant effects were observed on the soil pH, thereby influencing nodulation, nitrogen fixation and seed protein content among soybean genotypes. Application of 624 kg/ha lime observed a considerable increase in nodule counts with 1560 kg/ha at pH 6.5, evidently producing the highest nodule (7.6) counts. Bossier was observed to have fixed the highest (44.90 kgN/ha) quantity of nitrogen and seed protein content (20.22%) in the study. Bossier is considered the most efficient nitrogen and seed protein content crop of all the varieties used in the study. However, a combination of lime, inoculant and phosphorus application to strongly acidic soils will greatly aid in the formation and functioning of nodules, thus improving and influencing nitrogen fixation and seed protein content in soybean.

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