Original Research Article

Influence of P fertilizer on nodulation, growth and nutrient content of cowpea (*Vigna unguiculata* L.) in acidic soils of South Western Kenya

ABSTRACT

Aims: To determine the effects of liming and phosphorous (P) fertilizer on nodulation, growth, yield and nutrient content of cowpea in strogly and moderately acidic soils.

Study design: Randomized complete block design in a 2 x 3 x 3 factorial arrangement.

Place and Duration of Study: Field experiments: Kericho East (0° 24' S, 37° 1' E) and Bomet central (0° 47' S, 35° 21' E) between July 2012 and March 2014.

Methodology: Three cowpea varieties (KVU 27-1, M66 and Ngor) were each treated with: lime (0 t CaO ha^{-1} and 4 t CaO ha^{-1}) and P fertilizer (0 kg P ha^{-1}, 25 kg P ha^{-1} and 50 kg P ha^{-1}). Data collected were: nodule number and weight, leaf area index, shoot dry weight, shoot and grain N and P uptake, grain yield, tissue N and protein content.

Results: Liming had no significant ($P \leq .05$) effects on cowpea nodulation at experimental sites characterised by strongly acidic (pH 4.85) and moderately acidic (pH 5.58) soils, but increased shoot dry matter by 35% and grain N and P uptake in the strogly acidic soils of Kericho East by 1.8 kg ha^{-1} and 2 kg ha^{-1} respectively. In absence of liming or P fertilizer, grain yield was not recorded in two varieties at Kericho East. Application of 50 kg P ha^{-1} significantly enhanced nodulation at both experimental sites, it increased nodule dry weight at Bomet Central by 27% in the short rains than in the long rains season. Lower P rate (25 kg ha^{-1}) increased shoot dry matter by 46% at Bomet central, but 50 kg P ha^{-1} increased growth parameters of cowpea by over 100% at Kericho East in all seasons.

Conclusion: Liming is not beneficial to cowpea nodulation in soils with similar ecological conditions reported in this study. Application of 50 kg P ha^{-1} is required for cowpea production in strogly acidic soils.

Keywords: Cowpea, nodulation, liming, P fertilizer.

1. INTRODUCTION

Cowpea is one of the most important legume crop that is grown for grain while the leaves are used as vegetable (1). It has a high nutritional value; its seed contains 23% protein and 57% carbohydrate, its leaves contain 27 - 34% protein and also 20.1 mg, 290 mg and 410 mg of iron, calcium and vitamin C per 100 g respectively (2, 3). It is a potential export crop as its pods are currently being promoted for use as a vegetable in Eastern Europe (4). Integration of cowpea into existing cropping systems can enhance soil fertility as its rhizobia can fix up to 201 kg N ha^{-1} per season (5), and the crop can leave a net fixed N deposit of 60-70 kg ha-1 in soils (6). The area under cowpea production in the world is about 12.5 million hectares, and Kenya accounts for about 2.2 % of the world production area (3, 7).

Farmers in South Western Kenya (SWK) produce cowpea as a vegetable for use especially during the dry season. The production of cowpea in SWK is however constrained by low soil pH and phosphorous (P) deficiency (8). One of the causes of P deficiency in the region could be continuous cropping without replenishment of soil with external sources of fertilizer (9). Soil acidity in SWK could be another cause of P deficiency, due to a possibility of this element being fixed by aluminium ($Al^{3+)}$ or Iron ($Fe^{3+)}$ in such soils (10). Soil acidity is also known to adversely affect the survival and persistence of rhizobia, hence curtail their symbiotic efficiency (11). Phosphorous deficiency can be corrected by soil liming and application of organic or inorganic P fertilizer. Lime contains Ca^{2+} and/or Mg^{2+} that displaces Al^{3+} and Fe^{3+} hence P becomes available for plant use (12). Similarly, molybdenum which enhances the activity of the nitrogenase enzyme in nodules becomes available when acid soils are limed (10). Previous research work show that combined application of 45 kg P ha^{-1} and *Bradyrhizobium* inoculant increases cowpea grain yield by 54% in mildly acidic soils of Eastern Kenya (13). Maize plants grown at soil pH of 5.3 and supplied with 2 t ha^{-1} of lime and low rates of P fertilizer (30 kg P ha^{-1}) had the highest dry matter yield compared to those supplied with 100 kg P ha^{-1} at similar lime level in Western Kenya (14). However, the optimum lime and P level for production of various cowpea genotypes under acidic soils of Western Kenya has not been documented. The objective of this study was to determine the effects of liming and three levels of P fertilizer on nodulation, growth, grain yield and nutrient content of three cowpea varieties in acid soils of SWK.

2. MATERIAL AND METHODS

2.1 Experimental sites and soil analyses

A field experiment was conducted at two sites (Farmers training center at Bomet central- 0^{o} 47' S, 35^{o} 21' E and Kerego Kericho East- 0^{o} 24' S, 37^{o} 1' E) located in SWK. Bomet farmer training center is located at an altitude of 1920 m above the sea level, receives an average annual rainfall of 1300 mm and its agro-ecological zone is lower highland 2 (9). Kerego is located at an altitude of 2182 m above the sea level, with an average annual rainfall of 2090 mm and mean annual temperature of 17.2^{o}C, its agro-ecological zone is lower highland 1 (9). Before planting, soils were sampled at a depth of 20 cm from each site, and analysed for organic carbon, soil pH, total N, available P and exchangeable cations (K^{+}, Ca2^{+}, Mn^{2+}, Mg^{2+} and Al^{3+}). Available P was analysed using Mehlich-1 method (15). Organic carbon was analysed using Walkley-Black method, total N was analysed using Kjeldahl method, and finally exchangeable cations were extracted using ammonium acetate as documented by (16). Population of rhizobia was determined at the experimental sites using the most probable number (MPN) plant infection technique in germination pouches under glasshouse conditions, at the Department of Plant Science and Crop protection (University of Nairobi), following protocols described previously (17).

2.2 Treatments and experimental design

The cowpea genotypes used in this study were M66, KVU 27-1 and Ngor. Variety M66 is adapted to medium to high altitude; KVU 27-1 is adapted to medium altitudes (http://www.infonet-biovision.org/PlantHealth/Crops/Cowpea). Ngor is a landrace commonly grown by farmers in the study sites, and distributed through informal seed supply system. Each of the three cowpea genotypes received: P fertilizer in form of triple superphosphate (TSP) at rates of 0 kg P ha^{-1}, 25 kg P ha^{-1} and 50 kg P ha^{-1}; liming with calcium oxide (CaO), at rates of 0t ha^{-1} and 4t ha^{-1}. Lime was applied two weeks before planting. The experimental design used was randomized complete block design in 2x3x3 factorial arrangement, and treatments were replicated three times. The size of each experimental plot was 2.5 m x 2.5 m. All the plots received starter N fertilizer (in form of calcium ammonium nitrate) at the rate of 20 kg ha^{-1} during planting. Seed rate was 25 kg ha^{-1}, and seeds were sown at spacing of 50 cm between rows and 20 cm within rows.

2.3 Crop husbandry and data collection

Crops were weeded using hand hoe as from the 4th week after emergence until its canopy could smother weeds. Lambda-cyhalothrin and mancozeb were sprayed for crop protection against pests and diseases at rates of 0.5 ml and 2.5 g L^{-1} of water respectively. The data collected included: nodule numbers, nodule and shoot dry weight, leaf area index (LAI), shoot and grain N and P concentration, shoot and grain N and P uptake, shoot and grain protein content, and grain yield (kg ha^{-1}). Additional nutrient elements (K, Ca, Mg, Zn and Mn) on cowpea shoots were also analysed for correlation analyses. Data collection was done at R2 phenological stage (50% flowering stage), except grain yield, grain N and P concentration and uptake, and grain protein content, which were done at RH (harvest maturity). Data on nodulation and growth parameters at Kericho East were collected at early vegetative stage (V5) in long rains season of 2012 due to supernormal rains which would damage the crop.

Sampling for nodules and shoots were done as follows: Six shoots were harvested at random from the inner rows of each plot at each sampling period and put in paper bags. Immediately after harvesting the shoots, six cowpea root cores (6.5 cm in diameter and 15 cm deep) (18) were taken per plot, and then transported to the laboratory alongside shoots. Soil was then carefully removed with flowing water, and then roots were separated from the nodules. Active nodules with white-pink colour were counted, and then put in paper envelopes and oven dried alongside shoots at temperature of 60^{o}C to constant weight. Nodule and shoot dry weights were taken afterwards.

Six cowpea shoots were sampled for leaf area determination using the cork borer method (19), where leaf discs were punched using a cork borer, and the relationship between area and dry weight of the discs was used to estimate the leaf area. The leaf area was divided by the ground area occupied by the six plants in the field, to obtain the LAI. The oven dried plant shoots harvested at the 50% flowering stage (20) were used for plant tissue analyses. Shoot and grain analyses for N, P, K, Ca, Mg, Zn and Mn were done following procedures described previously (16). Protein content in plant tissues was determined using the following equation: Total Kjeldahl N* 5.45 (21). Cowpea N and P uptake was calculated by multiplying the total N and P concentration by the shoot dry weight (22). During crop harvest, 12 plants were selected at random from the three inner rows of each plot, and their pods were harvested, shelled and grains were oven dried at 60^{o}C to constant weight. Grain weights were taken using electronic balance, and then used to calculate grain yield per plot based on plant population, and then converted to grain yield in kg ha^{-1}.

2.4 Statistical analyses

Data collected were subjected to analysis of variance (ANOVA) using Genstat software 16th Edition (VSN International, U.K). Whenever treatment effects were significant, means were compared using Fischer's protected least significance difference test at *P*≤.05. Correlation analyses between shoot nutrient content and agronomic parameters were done using Pearson's correlation coefficient (*r*) in the same statistical software.

3. RESULTS

3.1 Soil chemical conditions in experimental sites

Soil pH in the study sites was strongly and moderately acidic (23) at Kericho East and Bomet central respectively (Table 1). Available P in both sites was below the 20 mg kg^{-1} required for optimum crop production (24), while mg levels of 0.47 cmol kg^{-1} in Kericho East (Table 1), was considered low for crop production (23). Rhizobial cells in soils of Kericho East were not detected by the MPN plant infection technique (Table 1).

Table 1: Soil chemical characteristics and rhizobial population at the farmer training center in Bomet central and Nile heritage farm in Kericho East

	Bomet central (FTC[a])	Kericho East (Nile heritage farm)
pH (H_2O)	5.58[b]	4.85[c]
Organic carbon (%)	2.57	3.98
Total N (%)	0.31	0.42
Available P (mg kg^{-1})	8.85	8.18
Exchangeable K (cmol kg^{-1})	0.8	0.6
Exchangeable Ca (cmol kg^{-1})	2.8	2.1
Exchangeable Mg (cmol kg^{-1})	0.93	0.47

Exchangeable Na (cmol kg^{-1})	0.35	0.35
Exchangeable Al (cmol kg^{-1})	0.3	0.75
Population of rhizobia	6 cells g^{-1} of soil	Undetected

[a] Farmer training centre, [b] moderately acidic soils, [c] strongly acidic soils

3.2 Effects of phosphorous fertilizer and liming on nodulation and growth of cowpea

Agricultural lime, P fertilizer and cowpea variety interactions for nodule numbers, nodule and shoot dry weights were significant ($p \leq 0.05$) at Bomet central but only during the long rains season of 2012 (Table 2). In absence of liming, 50 kg P ha^{-1} gave the highest nodule numbers in improved cowpea varieties (KVU 27-1 and M66), but lower P rate of 25 kg P ha^{-1} gave the highest nodule and shoot dry weights in the landrace (Ngor) and M66 respectively respectively at Bomet central (Table 2). These observations suggest that liming was not a requirement for nodulation and growth of cowpea in the mildly acidic soils.

Application of 50 kg P ha^{-1} consistently enhanced nodule numbers and dry weight in cowpea during all sampling periods at both Kericho East and Bomet central (Table 3 and 4). Compared to the control without P fertilizer, application of 50 kg P ha^{-1} increased nodule numbers by 2% in the long rains compared to the short rains, but increased nodule dry weight by 27% in the short rains compared to long rains at Bomet central (Table 2). The higher P rate (50 kg P ha^{-1}) increased cowpea nodule number and dry weight by 21% and 20% over the control in the long rains compared to the short rains at Kericho East (Table 4). In general, nodule numbers and dry weights were very low in the strogly acidic soil compared to mildly acidic soil.

Application of 25 kg P ha^{-1} significantly increased shoot dry matter of cowpea by 46% at Bomet central, but only during the long rains season (Table 5). In the strogly acidic soils at Kericho East, higher P rate (50 kg P ha^{-1}) significantly increased shoot dry matter and leaf area index of cowpea by over 100% compared to the control, irrespective of the rains season (Table 5). Lime application significantly increased shoot dry weight in Kericho East at rate of 0.2 g plant^{-1} per ton of lime applied, which was 35% higher compared to unlimed plants (Table 6).

Table 2: Influence of lime, phosphorous fertilizer and variety interactions on nodulation and shoot dry weight of cowpea during R2 stage of growth in a field experiment conducted at Bomet central during the long rains season of 2012

	Parameter		
Treatment	Nodule number plant^{-1}	Nodule dry weight mg plant^{-1}	Shoot dry weight (g plant^{-1})
0t ha^{-1} lime + 0 kg P ha^{-1} + KVU 27-1	11.56 bc	31.67 d	15.09 cd
0t ha^{-1} lime + 0 kg P ha^{-1}+ M66	5.00 c	24.00 d	14.82 cd
0t ha^{-1} lime + 0 kg P ha^{-1}+ Ngor	4.22 c	10.56 d	7.14 g
0t ha^{-1} lime + 25 kg P ha^{-1}+ KVU 27-1	14.44 abc	56.11 bcd	17.53 bc
0t ha^{-1} lime + 25 kg P ha^{-1} + M66	14.44 abc	41.33 cd	20.87 a

0t ha^{-1} lime + 25 kg P ha^{-1} + Ngor	21.78 ab	133.78 a	20.38 ab
0t ha^{-1} lime + 50 kg P ha^{-1} + KVU 27-1	27.44 a	108.22 abc	18.81 ab
0t ha^{-1} lime + 50 kg P ha^{-1} + M66	27.78 a	80.00 abcd	13.21 def
0t ha^{-1} lime + 50 kg P ha^{-1} + Ngor	5.78 c	37.11 d	14.03 de
4t ha^{-1} lime + 0 kg P ha^{-1} + KVU 27-1	7.11 bc	11.89 d	10.03 fg
4t ha^{-1} lime + 0 kg P ha^{-1} + M66	11.33 bc	22.57 d	15.16 cd
4t ha^{-1} lime + 0 kg P ha^{-1} + Ngor	7.67 bc	33.56 d	13.08 def
4t ha^{-1} lime + 25 kg P ha^{-1} + KVU 27-1	18.00 abc	67.44 abcd	13.11 def
4t ha^{-1} lime + 25 kg P ha^{-1} + M66	12.11 bc	33.89 d	20.37 ab
4t ha^{-1} lime + 25 kg P ha^{-1} + Ngor	8.44 bc	36.67 d	17.83 abc
4t ha^{-1} lime + 50 kg P ha^{-1} + KVU 27-1	9.67 bc	24.56 d	18.96 ab
4t ha^{-1} lime + 50 kg P ha^{-1} + M66	16.22 abc	38.67 cd	11.36 ef
4t ha^{-1} lime + 50 kg P ha^{-1} + Ngor	19.00 abc	119.00 ab	12.07 def
Mean	13.40	50.60	15.21
$LSD_{0.05}$	14.89	70.14	3.27
CV (%)	20.70	21.80	13.00

Means followed by same letter in a row are not statistically significant at $P \leq .05$ (Fischer's protected LSD test).

Table 3: Effects of phosphorous fertilizer on nodule numbers and weight during the R2 growth stage of cowpea plants in a field experiment conducted at Bomet central between 2012-14

	Active nodules $plant^{-1}$			Nod dry weight (mg $plant^{-1}$)		
Treatment	Long rains (2012)	Short rains (2012)	Short rains (2013)	Long rains (2012)	Short rains (2012)	Short rains (2013)
0 kg P ha^{-1}	7.81b	2.96b	3.22b	22.37b	5.37b	4.71b
25 kg P ha^{-1}	14.87a	4.24b	7.32a	61.54a	20.91ab	21.59a
50 kg P ha^{-1}	17.65a	6.97a	7.82a	67.93a	33.69a	23.00a
Mean	13.40	4.73	6.12	50.61	20.00	16.40
$LSD_{0.05}$	6.08	2.65	3.89	28.63	16.83	16.69

CV (%)	20.70	26.10	30.00	21.80	30.00	30.00

Means followed by same letter in a row are not statistically significant at *P*≤.05 (Fischer's protected LSD test).

Table 4: Effects of phosphorous fertilizer on nodule numbers and weight of cowpea at V5 and R2 growth stages in a field experiment conducted at Kericho East between 2012-14

	Active nodules plant^{-1}			Nod dry weight mg plant^{-1}		
Treatment	Long rains (2012- V5 stage)	Short rains (2012 -R2 stage)	Short rains (2013- R2 stage)	Long rains (2012-R2 stage)	Short rains (2012-R2 stage)	Short rains (2013-R2 stage)
0 kg P ha^{-1}	0.192b	2.76b	0.73b	0.15b	3.00b	1.92b
25 kg P ha^{-1}	1.041b	5.93a	1.45b	1.20b	11.69a	3.07b
50 kg P ha^{-1}	2.483a	8.26a	2.96a	4.80a	15.30a	6.61a
Mean	1.24	5.65	1.71	2.05	10.00	3.87
$LSD_{0.05}$	0.99	2.53	1.36	1.75	5.12	3.28
CV (%)	20.50	23.40	19.70	25.30	28.50	30.00

Means followed by same letter in a row are not statistically significant at *P*≤.05 (Fischer's protected LSD test).

Table 5: Effects of P fertilizer on shoot dry weight and leaf area index of cowpea plants during the active V5 and R2 growth stages in a field experiment conducted at Bomet central and Kericho East between 2012-14

	Bomet central	Kericho East				
	Shoot dry weight (g plant^{-1})				Leaf area index	
Treatment	Long rains (2012- R2 stage)	Long rains (2012- V5 stage)	Short rains (2012- R2 stage)	Short rains (2013- R2 stage)	Short rains (2012- R2 stage)	Short rains (2013- R2 stage)
0 kg P ha^{-1}	12.55c	0.20c	1.20c	0.76b	0.03c	1.52b
25 kg P ha^{-1}	18.35a	0.30b	2.91b	1.34a	0.07b	2.37ab

50 kg P ha^{-1}	14.74b	0.52a	4.12a	1.54a	0.13a	3.34a
Mean	15.21	0.34	2.74	1.21	0.08	2.41
$LSD_{0.05}$	1.34	0.09	0.73	0.42	0.04	1.06
CV (%)	13.00	30.00	13.60	15.70	1.70	23.2

Means followed by similar letters in a column are not statistically different at $P \leq .05$ (Fischer's protected LSD test).

Table 6: Influence of lime rate on a shoot dry matter and nutrient uptake of cowpea plants in a field experiment conducted in Kericho East in two rains season between 2012-14

	Short rains (2012)	Short rains (2013)	
Lime rate	Shoot dry matter plant^{-1}	Grain N uptake (kg ha^{-1})	Grain P uptake (kg ha^{-1})
0 t ha^{-1}	2.33b	2.60b	3.25b
4 t ha^{-1}	3.15a	4.38a	5.30a
Mean	2.74	3.49	4.27
$LSD_{0.05}$	0.6	1.26	1.45
CV	13.6	26.4	27.5

Means followed by different letters in a column are statistically different at $P \leq .05$ (Fischer's protected LSD test)

3.3 Effects of liming and P fertilizer application on nutrient content and grain yield of three cowpea varieties at Bomet central and Kericho East

P fertilizer rate and variety interactions were significant for shoot and grain nitrogen and protein content (Table 7). Cowpea variety KVU 27-1 required application of 50 kg P ha-1 to enhance its shoot N and crude protein content in the moderately acidic soils at Bomet central, but the same variety had the highest grain N and protein content in absence of P fertilizer under strongly acidic soil conditions at Kericho East (Table 7). It was further observed that cowpea variety Ngor required supply of 25 kg P ha^{-1} so as to give significantly higher grain N and crude protein content in the strongly acidic soil conditions at Kericho East (Table 7). In general, application of P fertilizer (25 kg ha^{-1} and 50 kg P ha^{-1}) significantly increased N and P uptake of cowpea plants at Kericho East (Fig. 1). Liming significantly increased grain N and P uptake by 1.8 kg ha^{-1} and 2 kg ha^{-1} respectively, at the strongly acidic soils (Table 6).

Cowpea variety M66 had highest grain yield at Bomet central without any treatment applied, but no grain was harvested from the same variety and also KVU 27-1 at Kericho East without soil amendments with lime or P fertilizer (Table 8). Cowpea variety KVU 27-1 had the highest grain yield at Kericho East when soil was limed and supplied with the highest P rate (Table 8). In general, grain yield at Bomet central was 1.19 tons higher than Kericho East at same season (Table 8).

Table 7: Influence of P fertilizer and variety interactions on shoot and grain nutrient content of cowpea plants in a field experiment conducted at Bomet central and Kericho East during the short rains season of 2013

	Bomet central		Kericho East	
Treatment	Shoot N (%)	Shoot CP[a] (%)	Grain N (%)	Grain CP (%)
0 kg P ha^{-1} + KVU 27-1	1.75_{abcd}	9.54_{abcd}	1.90_{a}	10.37_{a}
0 kg P ha^{-1} + M66	1.52_{cd}	8.27_{cd}	1.39_{d}	7.59_{d}
0 kg P ha^{-1} + Ngor	1.75_{abcd}	9.54_{abcd}	1.77_{ab}	9.65_{ab}
25 kg P ha^{-1} + KVU 27-1	1.58_{bcd}	8.58_{bcd}	1.72_{abc}	9.37_{abc}
25 kg P ha^{-1} + M66	1.87_{abc}	10.17_{abc}	1.67_{bc}	9.08_{bc}
25 kg P ha^{-1} + Ngor	1.63_{bcd}	$8.9b_{cd}$	1.90_{a}	10.34_{a}
50 kg P ha^{-1} + KVU 27-1	2.04_{a}	11.13_{a}	1.71_{abc}	9.37_{abc}
50 kg P ha^{-1} + M66	1.48_{d}	8.04_{d}	1.56_{cd}	8.48_{cd}
50 kg P ha^{-1} + Ngor	1.93_{ab}	10.49_{ab}	1.68_{bc}	9.15_{bc}
Mean	1.73	9.41	1.7	9.27
$LSD_{0.05}$	0.39	2.12	0.2	1.067
CV (%)	19.2	19.2	9.8	9.8

[a] Crude protein; means followed by similar letters in a column are not significantly different at $P \leq .05$ (Fischer's protected LSD test).

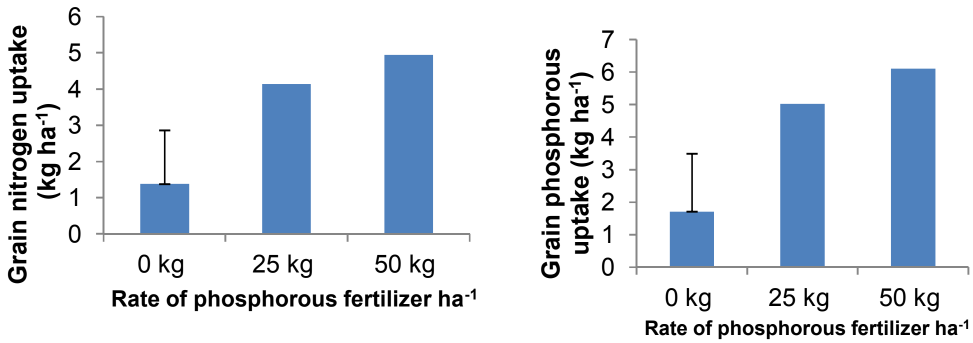

Fig 1: Effects of phosphorous fertilizer on grain nitrogen and phosphorous uptake of cowpea plants at harvest in a field experiment conducted in Kericho East during the short rains season of 2013. LSD bars show differences in means of P fertilizer rates at *P*≤.05 (Fischer's protected LSD test).

Table 8: Influence of P fertilizer and variety interactions on grain yield of cowpea plants in a field experiment conducted at Bomet central and Kericho East during two rain seasons between 2012-14

	Grain yield (tons ha^{-1})		
	Bomet central		Kericho East
	Long rains	Short rains	Short rains
Treatment	(2012)	(2013)	(2013)
0t ha^{-1} lime + 0 kg P ha^{-1} + KVU 27-1	1.48 bc	0.85	0.00 f
0t ha^{-1} lime + 0 kg P ha^{-1}+ M66	2.49 a	1.66	0.00 f
0t ha^{-1} lime + 0 kg P ha^{-1}+ Ngor	0.43 e	0.91	0.14 cdef
0t ha^{-1} lime + 25 kg P ha^{-1}+ KVU 27-1	0.91 cde	0.94	0.29 abc
0t ha^{-1} lime + 25 kg P ha^{-1} + M66	1.75 abc	1.60	0.06 ef
0t ha^{-1} lime + 25 kg P ha^{-1} + Ngor	1.79 ab	1.54	0.13 cdef
0t ha^{-1} lime + 50 kg P ha^{-1} + KVU 27-1	1.68 abc	1.03	0.18 cdef
0t ha^{-1} lime + 50 kg P ha^{-1} + M66	0.99 bcde	2.81	0.23 bcde
0t ha^{-1} lime + 50 kg P ha^{-1} + Ngor	1.06 bcde	1.32	0.40 ab
4t ha^{-1} lime + 0 kg P ha^{-1} + KVU 27-1	1.33 bcd	1.10	0.19 cdef
4t ha^{-1} lime + 0 kg P ha^{-1} + M66	1.77 abc	1.31	0.07 def
4t ha^{-1} lime + 0 kg P ha^{-1} + Ngor	0.51 de	0.99	0.07 def
4t ha^{-1} lime + 25 kg P ha^{-1} + KVU 27-1	1.52 bc	0.95	0.28 abc

4t ha^{-1} lime + 25 kg P ha^{-1} + M66	1.65 abc	1.46	0.27 abcd
4t ha^{-1} lime + 25 kg P ha^{-1} + Ngor	1.05 bcde	1.35	0.33 abc
4t ha^{-1} lime + 50 kg P ha^{-1} + KVU 27-1	1.03 bcde	2.16	0.47 a
4t ha^{-1} lime + 50 kg P ha^{-1} + M66	1.81 ab	2.17	0.32 abc
4t ha^{-1} lime + 50 kg P ha^{-1} + Ngor	1.04 bcde	0.88	0.21 bcde
Mean	1.35	1.39	0.20
$LSD_{0.05}$	0.87	NS	0.21
CV (%)	30.00	20.40	7.00

Means followed by similar subscript letters in a column are not significantly different at *P*≤.05 (Fischer's protected LSD test).

3.4 Correlation analyses

There were significant positive correlation between protein content and N uptake as well as shoot K, N, Ca, Mg and Mn content (Table 9). Leaf area index of cowpea was positively correlated with N and P uptake, shoot dry weight and content of K and Mg. There were significant positive correlation between N uptake and P uptake, shoot dry weight and K, N and Mg content (Table 9). Nodule dry weight of cowpea was significantly positively correlated with shoot concentration of Mn; P uptake of cowpea also had a significant positive correlation with shoot content of K, P and mg; shoot dry weight of cowpea had significant positive correlation with shoot Mg content (Table 9).

Table 9: Pearson correlation coefficient for shoot nutrient content and agronomic parameters of three cowpea varieties in a field experiment conducted at Bomet central in the short rains season of 2013

	K (%)	P (%)	N (%)	Ca (%)	Mg (%)	Zn (mg kg^{-1})	Mn (mg kg^{-1})
Protein (%)	0.27*	0.19	1.00***	0.41***	0.33*	0.23	0.30*
LAI[a]	0.25*	0.10	0.15	-0.05	0.27*	0.02	-0.17
N uptake (g plant^{-1})	0.29*	0.14	0.35**	0.01	0.43***	0.19	-0.09
Nodule dry weight plant^{-1}	-0.17	-0.12	0.04	0.01	-0.15	-0.11	0.35**
P uptake (g plant^{-1})	0.30*	0.47***	0.07	0.05	0.35**	0.16	-0.25
Shoot dry matter plant^{-1}	0.15	0.04	-0.06	-0.19	0.29*	0.11	-0.21

[a] Leaf area index; * *P*≤ .05, ** *P*≤ .01, *** *P*< .001

3. DISCUSSION

Liming had no significant effects on nodulation in the study sites characterised by soil pH of above 4.85 and 5.65. In soils with pH of 5.65 at Bomet Central, lime application had no significant effect on cowpea growth and grain yield, but increased shoot dry weight, N and P uptake in the strongly acid soils (pH 4.85) of Kericho East. Application of 50 kg ha^{-1} consistently increased cowpea nodulation at both sites. Nodulation response of cowpea due to the higher P rate was over 20% higher in the long rains compared to the short rains at Kericho East, but increased nodule dry weight by 27% in the short rains compared to long rains at Bomet central. Application of 50 kg ha^{-1} increased growth parameters of cowpea at Kericho East in all seasons. The physiological basis of these findings and other observations are discussed in detail.

Lack of nodulation response of cowpea due to lime application in acidic soils contradicts findings from Nigeria where liming enhanced nodulation and growth of cowpea in soils with pH of 5.4 (25). Similarly, lime application enhanced growth of common bean (*Phaseolus vulgaris*) and also growth and nutrient content of *Sesbania sesban* under conditions of soil acidity (12, 26). At pH of 4.85 recorded at Kericho East, it would be expected that Al^{3+} would become soluble and cause plant toxicity which is characterised by inhibition of uptake, translocation and utilisation of P by plants (10, 27), hence decline in physiological processes such as N_2 fixation. Liming would often reverse these conditions in soils (12) and consequently plant growth and nodule formation would increase. The non significant effects of lime on nodulation and minimal effects on growth of cowpea in the site with strongly acid soils may suggest that Al concentration of 0.75 cmol kg^{-1} may not cause toxicity in cowpea. Nodulation was responsive to application of 50 kg P ha^{-1} in general at both sites. Enhanced nodulation in cowpea in response to high rate of P fertilizer is in agreement with findings reported in Ghana (28). However, nodulation response to P fertilizer rate was genotype dependent; similar findings had been reported in Nigeria (29). Lower P rates (25 kg ha^{-1}) enhanced nodule weights in the local variety Ngor, but 50 kg P ha^{-1} enhanced nodule numbers in improved varieties (M66 and KVU 27-1) at Bomet central; this suggest that the cowpea landrace requires low P input for effective nodulation at this site with moderate soil acidity. It was further observed that application of 50 kg P ha^{-1} increased cowpea nodule dry weight by 27% in the short rains compared to long rains. Short rains seasons in this study was characterised by moisture stress because they extended into drought seasons. Phosphorous fertilizer is reported to enhance moisture tolerance in plants possibly due to its role in increasing leaf relative water content and net photosynthetic rate (30). This may explain the high nodule dry weight of cowpea during the short rains season.

Cowpea plants responded to 50 kg P ha^{-1} for growth at Kericho East in all the seasons, but showed increase in shoot dry matter with the lowest P rate (25 kg P ha^{-1}) at Bomet central in only one season. Although both sites had slight variation in available Mehlich 1- P (8.19 mg kg-1 and 8.85 mg kg^{-1} respectively), the possible explanation for cowpea response to low P rate at Bomet central could be lower solubility of Al at its pH of 5.58, hence minimal P fixation (10, 31). Consequently, shoot P uptake was not enhanced by liming or P fertilizer at Bomet central. However, lime application and P fertilizer enhanced grain N and P uptake of cowpea in the strongly acidic soils of Kericho East. Liming may have raised the soil pH thus increasing the available P (12), hence increase in its uptake. The possible role of P in N uptake is increased root growth which would facilitate N absorption (32). Nonetheless, the increased grain N uptake due to liming in Kericho East did not translate into increased grain N and protein content. Application 25 kg P ha^{-1} significantly increased grain protein content of local cowpea variety Ngor at Kericho East. Similar observations were reported in some varieties of finger millet in Kenya (33). P is important component of ATP and nucleic acids, which are essential for protein synthesis (34, 35). In contrast, cowpea variety KVU 27-1 had higher grain N and protein content in plots without P fertilizer at Kericho East. Previous research work in South Africa also reported varietal differences in protein content of cowpea in absence of fertilizer application (36). Nonetheless, KVU 27-1 responded to the highest P rate and liming for grain yield at Kericho East, but there is an inverse correlation between grain yield and protein content (37, 38). Therefore on smallholder farms with minimal application of P fertilizer, families can still obtain sufficient protein from cowpea variety KVU 27-1 under similar ecological conditions as Kericho East. At Bomet central, M66 gave the highest grain yield in absence of P fertilizer or liming. Available P in this site was low (8.85 mg kg-1), thus M66 may have high phospatase activity, which is associated with high P acquisition in soils low in P (39). However, this variety had very low protein content (8.3%), therefore there is need to investigate whether there are other varieties with high nutrient content under minimal input of P fertilizer.

In general, cowpea plants at Bomet central had higher nodulation, shoot dry weight and 1.19 more tons of grain ha^{-1} compared to Kericho East at similar growth stages. Kericho East had very low population of rhizobia undetected by MPN technique. However, cowpea plants were able to nodulate, but rhizobial cells in this site may have low symbiotic efficiency, which is characterised by low nodule and shoot dry weight (40). Correlation analyses showed significant positive correlation between Mg^{2+} and N and P uptake, growth parameters and protein content of cowpea. Soils of Kericho East were deficient in Mg^{2+}, and this may partly explain the poor growth and yield of cowpea in this site. Mg^{2+} is important for chlorophyll and nucleic acid synthesis, a co-factor in many enzymes controlling physiological processes in plants and enhances crop tolerance to abiotic stress (41, 42). Potassium had positive correlation with N and P Uptake, LAI and protein content of cowpea. It is involved in many plant physiological processes such as photosynthesis, regulation of enzymes synthesis, cell signaling and tolerance to biotic and abiotic stress (43). Manganese also had significant positive correlation with nodule dry weight, which is consistent with findings of previous authors (44). Fertilizers in Kenya contain mainly N and P, but there is need to include other nutrient elements such as K, Mg and Mn for optimum production of leguminous crops.

4. CONCLUSION

It was concluded that liming is not a requirement for nodulation under similar soil conditions reported in this study. In soils with strongly acidic soils and available P less than 10 mg kg^{-1} such as those found in Kericho East, liming or P fertilizer is a requirement for grain filling in improved cowpea varieties such as KVU27-1 and M66. Application of 50 kg ha^{-1} enhances nodulation of cowpea at strongly to moderately acidic soil conditions, and is a requirement for cowpea growth in strongly acidic soils. Effects of P fertilizer on protein content of cowpea depend on genotype and site. Variety KVU 27-1 required high rate of P fertilizer to enhance its protein content under ecological conditions prevailing at Bomet central, but its protein content was higher in absence of the fertilizer at Kericho East.

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