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Limestone application effects on common bean (*Phaseolus vulgaris* L.) yield and grain iron and zinc concentration on a Ferralsol soil in Uganda

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Soil acidification has major ramifications on crop production because low pH soils are less productive. The objective of this study was to determine the effect of limestone application on yield and grain iron (Fe) and zinc (Zn) concentration in newly released high iron and zinc, drought resilient varieties of common bean (NAROBEAN 1 and 3). Using a split-plot in time design, an experiment was set up using three common bean varieties (NABE 15, NAROBEAN 1 and NAROBEAN 3) as split plots and seven rates of limestone as main plots. The experiment was done for two rainy seasons on a Ferralsol soil in central Uganda. The results showed that soil pH, cation exchange capacity (CEC) and soil concentration of Ca and Na increased with greater amounts of limestone applied to the soil. On average, NAROBEAN 1 had 30 and 48% greater yield than NABE 15 and NAROBEAN 3 in both seasons, respectively. Grain Fe and Zn concentrations were not affected by limestone application. However, across seasons, NAROBEAN 3 seeds contained 12 and 15% more zinc and, 10 and 20% more iron than NAROBEAN 1 and NABE 15, respectively. Overall, limestone application did not impact yield or yield components of common bean.

Key words: Micronutrients, acidic soils, liming, biofortification, soil reaction.

INTRODUCTION

It is well documented that as soil pH declines, so does the supply of several essential plant nutrients, including calcium, magnesium and phosphorus (Goulding, 2016; Miller, 2016; USDA-NRCS, 2019). In many soils, this decline occurs alongside an undesirable increase in aluminum to levels toxic to plants (Harter, 2007; Miller, 2016). Free aluminum ions replace plant nutrient ions, such as potassium, calcium, and magnesium, on negatively charged soil colloids (Harter, 2007; Miller, 2016). While this process frees these minerals and makes them more available to plants in the short run, it also makes those nutrients more susceptible to loss from the soil due to leaching since they are unbound (Harter, 2007). In the tropics, most soil acidification is attributable to weathered soils associated with high rainfall (Kuylenstierna et al., 2001). These acidic soils are found

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where rainfall amounts exceed the level of evapotranspiration (Kamprath and Smyth, 2005). As excess water moves through the soil, Ca^{2+} and Mg^{2+} to which the balancing of negatively charged soil soluble anions (such as NO_3^- and Cl^-) is attributable, are leached in conjunction with soluble anions. Additionally, there is decrease in the percentage of basic cations on exchange sites. These sites, once occupied by Ca and Mg are then initially replaced with H^+ from soil organic matter decomposition and plant residues which then decompose and form Al-clays (Kamprath and Smyth, 2005). With highly weathered soils, phosphate fixation commonly occurs (Harter, 2007).

Highly weathered, poorly fertile soils are predominant in Masaka district of central Uganda, where years of continuous cropping, erosion and poor soil management have contributed to soil acidification. With average farm sizes of about 0.8 to 1.2 hectares per household in many farming communities in Masaka, continuous cropping through subsistence farming has become unavoidable (FAO, 2015). Low yields contribute to the common occurrence of food insecurity, hunger, and malnutrition. Masaka district is one of the major common bean (*Phaseolus vulgaris* L.) growing regions of Uganda (Kilimo Trust, 2012; Akpo et al., 2020; CASA, 2020). Common bean is embedded in the culture of the region and beans are a staple food and major source of protein. Although the majority of farms remain subsistence, beans also provide household income (Kilimo Trust, 2012; The Gatsby Report, 2014). However, common bean productivity is often constrained by highly acidic soils (MAAIF, 2019). While critical soil pH may vary with soil texture and crop cultivars (Goulding, 2016), common bean is reported to grow best in soils at a pH of 6 to 8 (Myers, 1999; Long et al., 2010). Studies done in Wisconsin by Fageria (2008), for instance reported achieving maximum grain yield when soil pH was 6.5 although chlorosis due to iron and zinc deficiencies could arise in some common bean varieties grown in soils at a pH above 7.2 (Hardman et al., 1990).

The negative impact of low soil pH on legume yield among other effects may be attributed to its interference with effective nodulation including reduction in the formation of nodules and their dry weight (Ferguson et al., 2013). Overall, yield and nodulation in common bean, faba bean (*Vicia faba* L.) and lupin (*Lupinis* spp.) was reported to be negatively affected by low soil pH (Frey and Blum, 1994; Denton et al., 2017). Frey and Blum (1994) explained that reduction in nodulation in low pH soils may be attributable to reduction in competitiveness of inoculant strains in acidic soils. Additionally, when soils are acidic, Ca^{2+} and Mg^{2+} loading are outcompeted by high concentrations of H^+ and Mn^{2+} (Horst, 1983). This affects nodulation by creating a steep concentration gradient between the rhizodermal cell and rhizosphere and thus favors anion uptake over Ca^{2+}, Mg^{2+} and K^+ (Horst, 1983). Vargas and Graham (1988) and Dejene et al. (2016) also explained that soil acidity associated with aluminum and manganese toxicity limited common bean nodulation because low soil pH affected abiotic and biotic factors for nodule formation except where acid-tolerant rhizobia strains existed. Hungria and Vargas (2000) added that soil acidity affected nodulation by limiting the survival and persistence of inoculant rhizobia. Slattery et al. (2001) also related root stunting to reduced nutrient and water uptake and overall decreased crop productivity to aluminum toxicities in acidic soils.

Liming is therefore recommended for common bean production where soil pH is below 5.5 (Long et al., 2010), as well as the use of wood ash to neutralize soil acidity (Demeyer et al., 2001; Park et al., 2005; Goulding, 2016; Dida and Etisa, 2019). Lime application is a long-established soil management practice to increase and maintain soil pH for optimal crop production with reports of positive impact on yield of most arable crops (Fageria et al., 2007; Connor et al., 2011; Goulding, 2016; Holland et al., 2019), including common bean (*P. vulgaris* L.) (Dida and Etisa, 2019). Lime is reported to improve soil structure and hydraulic conductivity by increasing Ca^{2+} concentration and ionic strength in the soil solution which in turn leads to flocculation of clays (Haynes and Naidu, 1998). Improved soil biodiversity was also reported following lime application due to increased biological (earthworm) activity leading to improved macro porosity and soil tilth over time (Bolan et al., 2003).

The global ramifications of mineral deficiencies on human health such as disease and health complications make the development of pulse crops with high seed mineral/nutrient concentration a necessity (Vandemark et al., 2018). Biofortification is one of the suggested strategies to reduce macro and micronutrient deficiencies. This may be achieved by application of agricultural management practices aimed at increasing mineral concentration in plant edible parts, development of new varieties with high concentrations of desired/target nutrients through conventional breeding, or the combination of both management practices and genetic approaches (White and Broadley, 2005).

The effects of iron and zinc deficiency are life long and contribute tremendously to the vicious cycle of poverty in many developing countries including Uganda. Iron Deficiency Anemia (IDA) is reported to affect mothers' mental health and mother-child interactions (Black et al., 2013). Maternal Iron Deficiency Anemia (IDA) during pregnancy also increases incidences of infant post and neo-natal deaths, affects child development and their general intelligence and cognitive functioning (Dibley et al., 2012; Black et al., 2013). Zinc deficiency also leads to preterm births and has long term effects on growth, and immunity of infants (King, 2011). In Uganda, iron deficiency affects one in two non-pregnant women and at least 50% of children below age five (HarvestPlus, 2016) and, about 20-69% and 21-29% of children and adults, respectively are zinc deficient in the country (Srinivasan, 2007). Good early nutrition is essential for children to attain their full development potential from which long-term human capital gains may be achieved alongside overall economic development of developing countries like Uganda (Black et al., 2013).

Supplementation programs are often used to overcome nutrient deficiencies and show promising results. Iron supplementation in children older than seven years resulted in improvement in their mental development (Sachdev et al., 2005). Other studies on iron also showed its benefits to motor development and some benefits to language in children below four years (Stoltzfus et al., 2001; Friel et al., 2003; Lind et al., 2003; Black et al., 2004). Additionally, zinc supplementation in pregnant mothers also reduced preterm births by up to 14% (Mori et al., 2015). Alongside supplementation programs, biofortified crops such as high iron and zinc common bean could be used to alleviate the burden of malnutrition. However, there are uncertainties about the impact of low soil pH on nutrient accumulation in edible plant parts especially on iron and zinc concentration in common bean grain of biofortified varieties. Therefore, combination of improved (biofortified) germplasm and improved agronomic management practices such as limestone and fertilizer application are important to ensure availability of these nutrients in the soil for plant root uptake. Grain iron for instance, is loaded in the seeds either via xylem vessels or phloem sieve tubes (Grillet et al., 2014). About 60-70% of iron loaded into seeds is as a result of root uptake from the soil and xylem transportation whereas 30-40% of total seed iron content is via the phloem stream from senescing leaves (Waters and Grusak, 2008; Grillet et al., 2014). This emphasizes the importance of soil conditions such as low pH and its effect on nutrient solubility and availability for plant uptake and, overall human nutrition and health.

An experiment was therefore set up using different rates of limestone for raising the pH of acid soil with the objective of determining the effect that these rates would have on grain yield and, grain iron and zinc concentrations in newly released high iron-high zinc, drought-resilient common beans.

MATERIALS AND METHODS

Site description

Experimental plots were established in Masaka district, at Kamenyamigo, Mukono Zonal Agricultural Research and Development Institute (MUZARDI) (0°18'12.1"S 31°39'56.0"E, 1242 m above sea level), Uganda. The site is located within the Buganda-Catena with predominantly shallow and skeletal soils which are believed to have developed from either summit or upper slope ironstone of quartzite and deep red/reddish brown clay loams occurring on pediments (ESG et al., 2001). Although not characterized by the US Soil Taxonomy, FAO characterizes the soils at the experimental site as Ferralsols (TAXOUSDA, 2014; Bulyaba et al., 2020). These high iron Ferralsols are vulnerable to erosion under poor management. The site receives annual averages of about 367 to 291 mm of rainfall in the MAM (March-April-May) and SON (September-October-November) growing seasons, respectively (Mugume et al., 2016). The experimental site had previously been under maize (*Zea mays* L.) production. Pretreatment (before limestone and NPK application) soil samples were collected from 0 to 30 cm from each plot and analyzed for pH, nitrate, available P and K (Mehlich-3) and organic matter at Crop Nutrition Laboratory Service Ltd (CropNuts) in Nairobi, Kenya (Table 1) before starting the experiment.

Experimental design

The experiment used a split plot in time design with three replications (blocks) done over two years (2017 and 2018). Treatments included seven rates of limestone and three common bean varieties. The limestone rates were the main plots whereas the bean varieties were the split plots. The first year of the experiment (2017) was late planted at the end of a season typically characterized by long rains (referred to as season A in this study) whereas the second year (2018) was planted early in a typically short rain season (referred to as season B in this study).

Limestone samples were sent to an independent chemical analysis lab (CropNuts, Nairobi, Kenya) for chemical and physical analyses (Table 2). The concentration of Ca and Mg in lime were determined using spectroscopy and particle size gradation using mesh screens (Goodwin, 1979) (Table 2). Fertilizer NPK (17:17:17) was applied alongside all the limestone treatments, except the control, at 124 kg ha^{-1} according to NARO recommendations for pulse production in Uganda and adjustments made for Ferralsols (Sunday and Ocen, 2015). The limestone and NPK (kg ha^{-1}) treatments were 0, 0 (control), (0, 124), (1236, 124), (2471, 124), (4942, 124), (9884, 124), (19768, 124). Limestone treatments were applied once to the whole plots and the three improved common bean varieties were randomly assigned to sub-plots.

In 2017 (Season A: September, to November) and 2018 (Season B: March, to May), the three common bean varieties used were NABE 15, NAROBEAN 1 and NAROBEAN 3. These were released by National Agricultural Research Organization (NARO) in 2010 (NABE 15) and 2016 (NAROBEAN 1 and 3). NAROBEAN 1 (large-sized seeds that are white/greyish with dark black stripes) and 3 (medium-sized seeds that are light yellow) were bred and released for drought tolerance and high iron and zinc concentrations whereas NABE 15 (medium-sized seed that are red with dark red stripes) was released for drought tolerance and yield.

Site management

Prior to planting, the experimental site was deep ploughed with a tractor followed by harrowing to produce fine tillage and cultivation by hand hoeing. Limestone was applied and ploughed into the soil about 5-10 cm deep by hand hoeing three weeks before planting to allow for reaction time (Ball, 2002). Fertilizer NPK (17:17:17) was applied at planting to the furrow rows by banding and covered with a thin layer of soil to prevent seed-fertilizer contact. Fertilizers were applied at 124 kg ha^{-1} at planting. A peat-based Mak-bio-N fixer inoculant (Makerere University, Kampala, Uganda) was used to inoculate seeds just prior to planting in both seasons. Plot size was 7.6 m long by 3 m wide, and each individual plot had four rows. Season A plots were planted on 21 November 2017 and season B plots were planted on 3 March 2018. Seeds were planted in furrowed rows 50 cm apart. The furrowed rows were 3.8 cm deep and made using the string and stake technique (Lunze et al., 2012). Seeds were planted at 10 cm from seed to seed, one seed per hole and covered with soil.

Weeding in season A (2017) and B (2018) was done two weeks

Table 1. Pre-treatment soil chemical properties obtained at 0-15 cm and 15-30 cm soil depths.

Parameter soil depth	pH	EC ($\mu S\ cm^{-1}$)	CEC (meq 100 g^{-1})	OC (%)	Ca (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})
0-15 cm	5.3	41.7	10.2	2.2	825	170	8	60	26
15-30 cm	5.3	38.4	9.9	2.1	817	167	6	49	27

Table 2. Physical and chemical characteristics of limestone used in the experiment.

Parameter	Unit	Limestone sample 1	Limestone sample 2
Calcium and magnesium content			
Calcium	%	36.7	37.2
Magnesium	%	0.32	0.34
Purity			
Calcium carbonate equivalent	%	87.7	88.4
Effective calcium carbonate equivalent	%	59.7	63.1
Speed of reaction/fineness			
Particle size (0.3-2 mm)	%	19.7	19.2
Particle size (< 0.3 mm)	%	48.5	52.2

after planting, before flowering, and additionally later in the both seasons as needed. Weeding was done by hand hoeing and pulling. Black bean aphids (*Aphis fabae* Scopoli) early in Season A were controlled using insecticide Dudu-Cyper® 5% EC (cypermethrin ((±) α-cyano-(3-phenoxyphenyl) methyl(±)-*cis-trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate). Application was done at 2.5 L ha^{-1} in 625 L H_2O ha^{-1}. A foliar wettable powder fungicide TATA MASTER® 72 (mancozeb 64% + metalaxyl 8%) was also applied that season at 2.5 kg ha^{-1}. Pesticides were sprayed using a knapsack sprayer. Other pests, including bean fly (*Ophiomyia phaseoli* Tryon.), which is an important pest in these areas was not observed in either season.

In 2017, the Season A experiment relied on natural rainfall and supplemental irrigation later in the season due to late planting that season. Hand-watering using watering cans was done on 19 and 23 December 2017 and 2 and 15 January 2018 (on the two middle rows) at 52,794 L ha^{-1}; the 2018 Season B experiment relied solely on natural rainfall.

Data collection

Soil samples were collected before the first season (before liming, before planting season A in 2017) and again before the second season (after 2017 liming and season A harvest, before 2018 season B planting) to determine the effect of limestone application on the soil (Table 3). Stand counts were taken at V4 (fourth trifoliate leaf stage) and again at R8 (full maturity) (Schwartz and Langham, 2010) stages of development. At V4 (fourth trifoliate leaf stage), stand counts were done using a randomly placed 5.3 m measuring rod between the two center rows along the length of each row whereas at R8, stand counts were done using a 1 m^2 quadrat.

Aboveground biomass was determined at harvest (R8-R9) by hand-clipping plants from 1 m^2 of each plot. Aboveground biomass samples were placed in a forced air oven at 60°C (NARO, Kawanda), dried to 0% moisture and weighed. Yield components (grain yield (kg ha^{-1}), pods (no. m^{-2}), seed (no. m^{-2}), seeds (no. pod^{-1}), seed (mg $seed^{-1}$)) from 1 m^2 at R8/R9 were determined from all plants. Pods were counted and hand threshed to remove seeds. Seeds were counted, oven dried at 60°C until dry, and weighed. Seeds were then packaged, labelled, and sent to CropNuts Laboratory for Fe and Zn analysis.

Data analysis

Data were analyzed by PROC GLIMMIX using SAS®9.4 (SAS institute Inc., Cary, NC). During analysis, blocks were

Table 3. Effect of different rates of limestone application on soil properties in the 0-15 cm and 15-30 cm soil horizons for soil samples collected October 15th 2017 and February 16th 2018.

Parameter	pH	EC (µS cm^{-1})	CEC (meq 100g^{-1})	OC (%)	Ca (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})
0-15 cm soil									
Control (untreated)	5.1	98.7	10.1	2.3	775	178	9	58	6
Limestone rate (kg ha^{-1})									
0	4.9^{d}	254.7^{NS}	11.6^{c}	2.1^{NS}	837^{c}	160^{NS}	24^{NS}	124^{NS}	7^{c}
1236	5.1^{d}	300.3^{NS}	13.8^{bc}	2.4^{NS}	928^{c}	171^{NS}	26^{NS}	139^{NS}	8^{c}
2471	5.2^{cd}	250.4^{NS}	13.4^{bc}	2.2^{NS}	1256^{c}	167^{NS}	23^{NS}	125^{NS}	10^{c}
4942	5.8^{bc}	275.3^{NS}	15.3^{bc}	2.2^{NS}	1805^{bc}	171^{NS}	30^{NS}	141^{NS}	13^{bc}
9884	6.1^{ab}	236.2^{NS}	18.0^{ab}	2.0^{NS}	2568^{ab}	179^{NS}	27^{NS}	117^{NS}	19^{ab}
19768	6.5^{a}	279.1^{NS}	21.2^{a}	2.2^{NS}	3309^{a}	171^{NS}	30^{NS}	132^{NS}	23^{a}
Significance									
Lime (L)	***	NS	***	NS	***	NS	NS	NS	***
Covariate	**	NS	0.06	NS	*	***	NS	**	NS
15-30 cm soil									
Control	5.2	74.6	10.2	2.2	794	168	6	29	5
Limestone rate (kg ha^{-1})									
0	5.0^{b}	111.6^{NS}	10.1^{b}	2.3^{NS}	769^{b}	155^{NS}	10^{NS}	55^{NS}	5^{NS}
1236	5.0^{b}	157.1^{NS}	12.1^{ab}	2.1^{NS}	882^{b}	164^{NS}	10^{NS}	53^{NS}	6^{NS}
2471	5.1^{b}	195.2^{NS}	11.5^{ab}	2.2^{NS}	935^{b}	147^{NS}	12^{NS}	65^{NS}	7^{NS}
4942	5.2^{ab}	157.5^{NS}	13.2^{ab}	2.2^{NS}	1086^{ab}	166^{NS}	9^{NS}	48^{NS}	8^{NS}
9884	5.3^{ab}	129.1^{NS}	13.2^{ab}	2.1^{NS}	1273^{ab}	160^{NS}	9^{NS}	37^{NS}	9^{NS}
19768	5.6^{a}	162.5^{NS}	15.3^{a}	2.2^{NS}	1839^{a}	166^{NS}	13^{NS}	59^{NS}	10^{NS}
Significance									
Limestone (L)	**	NS	**	NS	**	NS	NS	NS	NS
Covariate	***	NS	*	**	*	***	NS	NS	NS

Means followed by the same letter (s) within a column indicate no significant difference at $p \le 0.05$ by the least square means test. *Significant at $p \le 0.05$, **Significant at $p \le 0.01$, ***Significant at $p \le 0.001$ and NS = not significant.NPK was applied at a rate of 124 kg ha^{-1} in all plots at planting.

treated as random elements in the model whereas lime rate and common bean variety were treated as fixed effects. Since seasons differed greatly, they were analyzed separately. The PDIFF procedure was used to test for differences among means when F-tests were significant for main effects or their interactions. Differences between treatments were evaluated at a significance level of $p \le 0.05$, unless otherwise stated and covariate analysis done. The covariate in the model statement in SAS was the 2017 soil property such as, pH or EC. Soil samples used for SAS analysis for the covariate therefore included samples that were collected before limestone or NPK was applied in 2017 (limestone and NPK were then applied after soil sampling and season A planted) and, soil samples in 2018 that were collected after the first season A was harvested but before season B was planted. Linear regression was done using PROC REG for parameters that were influenced by limestone rate.

Table 4. Regression functions for limestone application rates predicting upper (0-15 cm) and lower (15-30 cm) soil pH, CEC, and Ca and Na concentrations, and grain yield in common bean.

Parameter	Function	r^2 or R^2
Upper soil pH	5 + 0.0002x	0.886
Upper soil CEC	12.609 + 0.0011x	0.957
Upper soil Ca concentration (mg kg^{-1})	959.57 + 0.3191x	0.949
Upper soil Na concentration (mg kg^{-1})	8 + 0.0021x	0.938
Lower soil pH	5 + 8E-05x	0.983
Lower soil CEC	11.174 + 0.0005x	0.836
Lower soil Ca concentration (mg kg^{-1})	797.71 + 0.1289x	0.995
NABE 15 (2017)	468 + 0.013x	0.341
NAROBEAN 3 (2017)	169 + 0.010x	0.644
NABE 15 (2018)	$431 + 0.318x – 0.000037x^2$	0.612
NAROBEAN 1 (2018)	877 + 0.110x	0.835

RESULTS AND DISCUSSION

Effect of limestone on soil properties at 0-15 cm and 15-30 cm

There were significant differences in the effects of treatments among the two soil horizons. Changes in pH were more prominent in 0-15 cm of soil than 15-30 cm depth. Regression analysis showed that soil pH increased with increases in limestone application rates (upper soil, r^2= 0.885; lower soil, r^2=0.983) (Table 4). In upper soil, the greatest increment in soil pH was observed when 19,768 kg ha^{-1} of limestone was applied and the lowest pH increments when limestone was applied at rates of 0 and 1,236 kg ha^{-1}. Similarly, in the deeper soil horizon, the highest limestone application rate of 19,768 kg ha^{-1} also had the greatest pH increment whereas, 2471, 1236 and 0 rates of limestone had much lower effects.

Agricultural limestone application did not affect electrical conductivity (EC) (Table 3). This may be because the soil did not have high levels of soluble salts. Although acceptable thresholds for these exist, they are also dependent on a number of factors such as crop, soil texture, among others (Gruttadaurio et al., 2013; Sonon et al., 2015). Provin and Pitt (2001) reported that saline soils often had an EC of 40,000 μS cm^{-1}. The EC of soils at our experimental site was between 98.7 μS cm^{-1} in topsoil and 74.6 μS cm^{-1} in subsoil therefore, compared to Provin and Pitt (2001), our experimental site had low EC. Low EC often exists alongside no/low salts in soils (Hanlon, 2015). Change in EC varied with soil depth and limestone application rate. Upper and lower soil EC ranges from our study were less than 1000 μS cm^{-1} and thus the soil at the experimental site would be considered non- saline after limestone was applied (USDA-NRCS, 2019). Thus, the conditions would not negatively impact crop growth and other important soil microbial processes such as nitrogen cycling, respiration and decomposition among others (USDA-NRCS, 2019). Low EC levels may also indicate low availability of plant nutrients. Optimal soil EC levels range between 1.1 to 5.7 μS cm^{-1}.

Limestone influenced CEC in 0-15 cm depth and 15-30 cm depth (Table 3). Regression analysis showed that upper and lower soil CEC increased with limestone addition (0-15 cm depth, r^2= 0.957; 15-30 cm depth, r^2=0.836) (Table 4). Similarly, Edmeades (1982) reported that effective cation exchange capacity (ECEC) increased with increasing soil pH. Lemire et al. (2006) reported that CEC increased as a direct function of the amount of lime added, if the final pH of the solution remained below 7. They further reported that a 1 cmol (+) kg^{-1} increase in CEC for every 2.1 t $ha^{–1}$ of limestone added to all soils regardless of texture, organic matter or other soil properties. Similarly, Aitken et al. (1990) reported a linear relationship between pH and CEC for pH ranges between 4 to 6.5 for all soils in their study.

Regression analysis for the relationship between CEC and pH in our study had similar values to those of Aitken et al. (1990). However, they added that the relationship between CEC and pH became curvilinear in upper ranges with CEC increasing distinctly with relatively small pH increments. Bartlett and McIntosh (1969) explained that the increase in pH due to increasing lime rates leads to neutralization of positively charged polynuclear Al-OH complexes which further unblocks negatively charged sites. This process contributes to ECEC increase and the increased pH may also induce deprotonation of pH-dependent sites leading to a proportional increment in CEC as well as charge density (Goedert et al., 1975).

Agricultural lime rate had no influence on soil organic carbon (OC) in either soil depth (Table 3). Haynes and Naidu (1998) mentioned that few studies, if any, have found a causal link between effects of lime application and soil organic matter. Additionally, increase in soil OC is largely due to residue accumulation especially where mineralization and decomposition are lower than organic matter addition. Such a causal link could not easily be attained especially given the short duration of the experiment. Mehlich-3 Ca was significantly affected by limestone application in both soil depths (Table 3). Overall, Ca concentration in upper and lower soils increased with incremental rates of limestone (0-15 cm depth, r^2= 0.949; 15-30 cm depth, r^2=0.995) (Table 4). The Ca concentration in plots where 0, 1236 and 2471 kg ha^{-1} of limestone were applied did not differ in either depth. Chimdi et al. (2012) attributed the increase in exchangeable Ca and CEC when lime rates increased, to the enhancement of Ca^{2+} ion concentration and their replacement of H^+ and Al^{3+} from the soil solution and exchange complex in the soil.

Mehlich-3 extractable Mg was not affected by application of different rates of limestone (Table 3). This is because the magnesium concentration of the limestone used was less than 0.5% which is quite low compared to the Ca in percentage in the limestone. Simard et al. (1994) and Riggs et al. (1995) reported that calcitic limestone had no significant effect on Mg. However, unlike our study, they reported that the values of exchangeable Mg decreased after the first growing season to values lower than those recommended for agricultural crop productivity, values that were lower than exchangeable Mg values in the soil before limestone addition. Edmeades (1982) also reported a decrease in soil Mg concentrations following lime application. The author attributed this Mg decrease to an increase in the exchangeable Ca: Mg ratio following addition of calcitic limestone. Contrary to our findings, other researchers found an increase in exchangeable Mg following agricultural/calcitic lime application (Grove et al., 1981; Grove and Summer, 1985; Mayfield et al., 2001).

No differences were observed in Mehlich-3 soil P in upper or lower soil with different rates of limestone (Table 3). Reeve and Sumner (1970) also reported that liming had no effect on P sorption in Oxisols. Haynes (1982) explained that liming highly weathered acid soils could result in either increased, decreased and even sometimes no change in available soil phosphorus. The author explained that increases in phosphorus availability occurred following liming if there was formation of various phosphate compounds. Haynes (1982) reported that if limed soils desiccated before reaction with phosphate, a decrease in phosphorus sorption and increase in its availability would occur due to crystallization of amorphous hydroxy-Al polymers. Several of these processes are extremely slow and therefore changes in available soil phosphorus may be difficult to observe in the absence of long-term study.

Application of different rates of limestone had no significant effect on Mehlich-3 K in both soil depths (Table 3). Simard et al. (1994) reported that lime had no significant effect on K extractability under different tillage intensities. This is contrary to other studies that reported either decreases or increases in K levels when limestone was applied. Phillips et al. (1988) explained that liming could increase K concentrations in the soil solution although this could eventually lead to K loss through leaching over time. Further, the effect (increase or decrease) or no effect of lime application on K in strongly acidic soils was dependent on the initial degree of soil base saturation (Schmehl et al., 1950). MacIntire et al. (1927) reported a decline in supply of available K when lime was applied on three soils as did Bartlett and McIntosh (1969). Such K declines were attributed to the opening up of K-selective exchange sites, previously blocked by Al when soil pH was low (Nemeth and Grimme, 1972) or, due to a reduction in percentage of K saturation triggered by an increase in CEC when lime was applied (Bartlett and McIntosh, 1969). In contrast to these previous studies, we observed an increase in CEC with increased limestone rates, although this did not affect K levels in our experiment.

Agricultural lime application rates had significant impact on Na concentration in upper soil ($p \leq 0.001$) and no impact on Na in subsoil (Table 3). Regression analysis showed that the greater the lime application rate, the greater the Na concentration in upper soil (r^2=0.938) (Table 4). It is possible that Na was a constituent of the limestone and therefore increased when lime was added.

Common bean productivity season A

The interaction of limestone and variety was significant for yield but not for stand density (V4 and R8), aboveground biomass, pods m^{-2}, seeds m^{-2}, seeds pod^{-1}, seed weight and seed iron and zinc concentration (Table 5). Stand density at V4 and V8 did not differ for limestone rate or amongst the varieties. This may be attributable to good crop management practices such as adoption of recommended inter-row spacing (50 cm), timely weeding to avoid competition for sunlight and nutrients, and management of potential insect and disease infestations (MAAIF, 2019). Seeding in rows facilitates cultivation and weeding by mechanical methods (Goulden, 1975). However, high density stands can lead to greater incidences of foliar diseases (Heard et al., 1990, Sandoval-Avila et al., 1994) and increased intra crop competition.

Aboveground biomass differed amongst the three varieties (Table 5). NABE 15 and NAROBEAN 1 had similar biomass, 57 and 65% greater at R8-R9 than NAROBEAN 3, respectively. The greater aboveground biomass obtained from NAROBEAN 1 may be because of the indeterminate growth habit of this variety (Table 5). Kelly et al. (1987) reported that determinate dry bean cultivars had lower stability across environments compared to indeterminate cultivars under rainfed conditions. Generally, there is a positive relationship between biomass and grain yield. This may also be reflected in their yield.

Variety and the interaction of limestone rate by variety

Table 5. Stand density at V8 and R8, above ground biomass, yield and yield components and seed iron and zinc, season A (longer rainy season).

Parameter	Stand density V4 (no. m^{-2})	Stand density R8 (no. m^{-2})	Biomass R8-R9 (kg ha^{-1})	Grain yield (kg ha^{-1})	Pods (no. m^{-2})	Seed (no. m^{-2})	Seed (no. pod^{-1})	Seed (mg seed^{-1})	Seed Fe (mg kg^{-1})	Seed Zn (mg kg^{-1})
Control (untreated)	32	19	411	334	32	94	3	361	69	31
Limestone Rate (kg ha^{-1})										
0	33^{NS}	20^{NS}	456^{NS}	398^{NS}	36^{NS}	113^{NS}	3^{NS}	358^{NS}	68^{NS}	31^{NS}
1236	34^{NS}	20^{NS}	511^{NS}	455^{NS}	40^{NS}	134^{NS}	3^{NS}	346^{NS}	60^{NS}	29^{NS}
2471	32^{NS}	19^{NS}	433^{NS}	408^{NS}	37^{NS}	131^{NS}	3^{NS}	337^{NS}	62^{NS}	29^{NS}
4942	34^{NS}	20^{NS}	483^{NS}	431^{NS}	42^{NS}	137^{NS}	3^{NS}	328^{NS}	63^{NS}	31^{NS}
9884	33^{NS}	20^{NS}	594^{NS}	465^{NS}	44^{NS}	142^{NS}	3^{NS}	332^{NS}	73^{NS}	30^{NS}
19768	32^{NS}	19^{NS}	561^{NS}	523^{NS}	45^{NS}	148^{NS}	3^{NS}	338^{NS}	64^{NS}	33^{NS}
Variety										
NABE 15	33^{NS}	20^{NS}	572^{a}	510^{a}	40^{b}	138^{b}	3^{a}	371^{a}	60^{NS}	28^{b}
NAROBEAN 1	32^{NS}	20^{NS}	703^{a}	634^{a}	57^{a}	205^{a}	4^{a}	309^{b}	69^{NS}	29^{b}
NAROBEAN 3	34^{NS}	19^{NS}	244^{b}	196^{b}	25^{c}	59^{c}	2^{b}	340^{ab}	66^{NS}	34^{a}
Significance										
Limestone (L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Variety (V)	NS	NS	***	***	***	***	***	**	0.08	***
L × V	NS	NS	NS	*	NS	NS	NS	NS	NS	NS

Means followed by the same letter (s) within a column indicate no significant difference at $p \leq 0.05$ by the least square means test. *Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$, ***Significant at $p \leq 0.001$ and NS = not significant. NPK was applied at a rate of 124 kg ha^{-1} in all plots before planting.

influenced grain yield (Table 5). Yields of NABE 15 and NAROBEAN 1 did not differ, but were 62 and 69% greater than for NAROBEAN 3, respectively. Kelly et al. (1987) reported that determinate dry bean cultivars had lower seed yield than their indeterminate counterparts. Stebbins (1974) explained that indeterminate plants have inherent flexibility which enables them to form either few or many flowers depending on how long the growing season may be. This in turn provides a buffer against changes that may occur in environmental conditions, especially in rainfed cropping systems (Stebbins, 1974). Acosta-Gallegos and Adams (1991) further explained that indeterminate bean cultivars with early vigorous establishment, greater dry matter as the seed-filling period commenced and the potential for assimilate transfer during seed development stages were better suited for growing under rainfed conditions compared to cultivars that are determinate. Beaver et al. (1985) and Nleya et al. (1999) also reported that indeterminate bean cultivars were higher yielding and more productive than determinate cultivars. For the interaction of limestone rate and variety with regard to grain yield, two comparisons were fit by functions using a simple linear model. Regression modeling using limestone rate to predict yield was successful at explaining yield variation for NABE 15 and NAROBEAN 3 but not NAROBEAN 1 (Table 4). Regression analysis showed that NABE 15 and NAROBEAN 3 grain yield increased with greater limestone addition rates (NABE 15, $r^2 = 0.341$; NAROBEAN 3, $r^2 = 0.644$). Varietal increase in yield with greater limestone rate may be attributable to crop favorable changes in soil properties due to liming such as an increase in soil pH, improvement in soil biodiversity, structure and hydraulic conductivity (Haynes and Naidu, 1998; Bolan et al., 2003).

Agricultural limestone rate did not impact pods m^{-2} or seeds pod^{-1} (Table 5). However, pods m^{-2}

and seeds m^{-2} differed among the three common bean varieties (p ≤0.001). NAROBEAN 1 had the greatest number of pods m^{-2} and seeds m^{-2}, followed by NABE 15; NAROBEAN 3 had the least. NAROBEAN 1 had 30 and 56% more pods m^{-2} as well as, 33 and 72% more seeds m^{-2} than NABE 15 and NAROBEAN 3, respectively. NAROBEAN 1 had 30 and 56% more pods m^{-2} than NABE 15 and NAROBEAN 3, respectively. These differences also may be attributed to the indeterminate growth habit of NAROBEAN 1. Limestone application did not impact seeds pod^{-1}. However, the number of seeds pod^{-1} differed by variety. NAROBEAN 1 and NABE 15 had the greatest seeds pod^{-1} and were similar whereas NAROBEAN 3 had the least. NABE 15 and NAROBEAN 1 had 33% and 50% more seeds per pod than NAROBEAN 3, respectively. Seed weight was not impacted by limestone application rate. However, seed weight was significantly different among bean varieties (p ≤ 0.01). NABE 15 had the highest individual seed weight whereas NAROBEAN 1 seeds had the least weight. NABE 15 seeds weighed 8 and 17% more than NAROBEAN 3 and NAROBEAN 1 seeds, respectively. Perin et al. (2002) reported that large seeds often increased plant shoot and root biomass as well as plant height and leaf area index during early plant development stages. The authors postulated that larger seeds had larger reserves that enabled for more vigorous initial development such as biomass production compared to smaller seeds. However, initial plant vigour was not measured in our study.

Seed zinc concentration differed among the three bean varieties. NAROBEAN 3 seeds had the greatest zinc concentration whereas NABE 15 and NAROBEAN 1 had the least. Zinc concentration in NAROBEAN 3 seeds was 15 and 18% greater than that in NAROBEAN 1 and NABE 15, respectively. Additionally, the zinc concentration in the latter two varieties did not differ. NAROBEAN 1 zinc concentration from our study corresponded to that expected/predicted by NARO-Uganda, 31.4-34.3 ppm (Agona, 2017) although our NAROBEAN 3 grain zinc concentration was slightly lower than the predicted 35-38 ppm by NARO (Agona, 2017). We do not know why the biofortified NAROBEAN 3 had low zinc concentration. We did not observe differences in grain zinc concentration due to limestone application rate. Additionally, seed concentration of Fe did not differ for limestone application rate or variety (Table 5).

Common bean productivity season B

Plant stand density at V4 and V8 differed by variety but was not affected by limestone application rate or the variety × limestone rate interaction (Table 6). NAROBEAN 1 had the greatest plant stand density whereas NAROBEAN 3 had the least at both V4 and R8 stages. At V4, the stand density of NAROBEAN 1 was 10 and 13% more than NABE 15 and NAROBEAN 3, respectively. At R8, the stand density of NAROBEAN 1 was 11 and 17% more than NABE 15 and NAROBEAN 3, respectively.

Agricultural limestone rate, variety, and their interaction had significant effects on aboveground biomass at R8-R9 (Table 6). Regression analysis showed that aboveground biomass increased with increments in limestone application rates ($y = 192.77x + 1876.5$; $r^2 = 0.744$). NAROBEAN 1 had 37% and 41% more aboveground biomass than NABE 15 and NAROBEAN 3, respectively. This may be attributable to the NAROBEAN 1 having an indeterminate growth habit as explained in Kelly et al. (1987).

Common bean varieties differed for yield and there was a significant interaction of variety and limestone rate (Table 6). Yield of NAROBEAN 1 was 37% more than NABE 15 and NAROBEAN 3. Pods and seeds m^{-2} differed by variety. NAROBEAN 1 had 41 and 30% more pods m^{-2} than NABE 15 and NAROBEAN 1, and 37 and 35% more seeds m^{-2}, respectively. These differences in yield may be attributable to NAROBEAN 1 having an indeterminate growth habit which enables the variety to have yield superiority (Beaver et al., 1985; Acosta-Gallegos and Adams, 1991; Nleya et al., 1999). To examine the interaction between limestone rate and variety for grain yield, simple linear regression was done and limestone rate was used to predict yield. A simple linear model was successful at explaining yield variation for NAROBEAN 1 and a better prediction with a quadratic model/equation was used for NABE 15 although neither of the models was successful for NAROBEAN 3 (Table 4). Regression analysis showed that grain yield increased with greater limestone addition rates (NAROBEAN 1, r^2= 0.835; NABE 15, R^2= 0.612). This may be due to increases in pH, consequent increment in soil nutrients such as calcium, improvement in other soil properties and even fertilizer use efficiency. Holland et al. (2019) associated an increase in spring bean (*Vicia faba* L.) yield to an increase in soil pH following lime application. Liming low pH soils was also reported to increase fertilizer use efficiency leading to increased yield in barley and wheat (Von Tucher et al., 2018).

Seeds pod^{-1} and weight $seed^{-1}$ were not affected by either lime treatment or variety (Table 6). Seed concentration of iron and zinc was significantly different among varieties (Table 6). NAROBEAN 3 seeds contained 10 and 20% more iron than NAROBEAN 1 and NABE 15, respectively. Additionally, NAROBEAN 3 seeds contained 9 and 13% more zinc than NAROBEAN 1 and NABE 15, respectively.

Conclusions

Our study demonstrated that soil pH increased with greater limestone application rates along with increases in soil calcium and CEC, thus limestone application can raise soil pH for improved bean productivity. Despite

Table 6. Stand density at V8 and R8, aboveground biomass, yield and yield components and seed iron and zinc, season B (shorter rainy season), 2018.

Parameter	Stand density V4 (no. m^{-2})	Stand density R8 (no. m^{-2})	Biomass R8-R9 (kg ha^{-1})	Grain yield (kg ha^{-1})	Pod (no. m^{-2})	Seed (no. m^{-2})	Seed (no. pod^{-1})	Seed (mg seed^{-1})	Seed Fe (mg kg^{-1})	Seed Zn (mg kg^{-1})
Control	27	17	1350	976	52	165	3	522	65	31
Limestone rate (kg ha^{-1})										
0	31^{NS}	17^{NS}	1811^{b}	560^{NS}	67^{NS}	212^{NS}	3^{NS}	262^{NS}	71^{NS}	30^{NS}
1236	31^{NS}	16^{NS}	2639^{ab}	912^{NS}	90^{NS}	303^{NS}	4^{NS}	324^{NS}	63^{NS}	30^{NS}
2471	24^{NS}	15^{NS}	2461^{ab}	830^{NS}	82^{NS}	307^{NS}	4^{NS}	271^{NS}	61^{NS}	28^{NS}
4942	27^{NS}	18^{NS}	2528^{ab}	830^{NS}	87^{NS}	283^{NS}	3^{NS}	295^{NS}	64^{NS}	31^{NS}
9884	29^{NS}	17^{NS}	2844^{ab}	1036^{NS}	100^{NS}	385^{NS}	4^{NS}	269^{NS}	64^{NS}	30^{NS}
19768	28^{NS}	17^{NS}	3024^{a}	996^{NS}	105^{NS}	350^{NS}	3^{NS}	275^{NS}	63^{NS}	29^{NS}
Variety										
NABE 15	28^{ab}	16^{ab}	2170^{b}	721^{b}	68^{b}	255^{b}	4^{NS}	281^{NS}	57^{b}	28^{b}
NAROBEAN 1	31^{a}	18^{a}	3450^{a}	1139^{a}	116^{a}	403^{a}	3^{NS}	281^{NS}	64^{ab}	29^{b}
NAROBEAN 3	27^{b}	15^{b}	2033^{b}	721^{b}	81^{b}	262^{b}	3^{NS}	286^{NS}	71^{a}	32^{a}
Significance										
Limestone (L)	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
Variety (V)	**	**	***	***	***	***	NS	NS	**	***
L × V	NS	NS	*	*	NS	NS	NS	NS	NS	NS

Means followed by the same letter (s) within a column indicate no significant difference at $p \leq 0.05$ by the least square means test. *Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$, ***Significant at $p \leq 0.001$ and NS = not significant. NPK was applied at a rate of 124 kg ha^{-1} in all plots at planting.

increasing soil pH and CEC, limestone addition did not improve bean yield in our two-year experiment. However, the newly released common bean varieties, NAROBEAN 1 and NAROBEAN 3 had greater yields and seed Fe and Zn concentrations than the older variety, NABE 15. These newer varieties should be used to improve livelihoods through better yields and human nutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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