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Limestone application on a Ferralsol soil and genotype by environment effects on yield and grain nutrient composition in common bean

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Limestone application on a Ferralsol soil and genotype by environment effects on yield and grain nutrient composition in common bean

by

Rosemary Bulyaba

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Crop Production and Physiology

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2019

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DEDICATION

I dedicate this dissertation to God, to my husband, Ivan and to my beautiful daughter, Adeline.

You have given me purpose, strength and reasons to work hard and keep going daily.

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ABSTRACT

Food and nutritional insecurity are prevalent in sub-Saharan Africa, where years of poor soil management have left most agricultural lands mineral deficient and low in organic matter. In Uganda, soil acidity is a major contributor to low agricultural productivity, hunger, malnutrition and the vicious cycle of poverty. To meet yield demands and nutritional needs in Uganda, publicly funded breeding programs released biofortified common bean varieties such as NAROBAN 1 and 3, that are high yielding, drought resilient and high in commonly deficient nutrients such as iron and zinc. The main objective of this study was to determine the effect of limestone application as a soil amendment strategy on grain yield and grain iron (Fe) and zinc (Zn) concentration in NAROBAN 1 and 3 grown on Ferralsol soils in central Uganda. Findings from the study showed that soil pH, CEC and soil concentration of extractable Ca and Na increased when more limestone was applied to the soil. On average the yield of NAROBAN 1 was 30% and 48% greater than NABE 15 and NAROBAN 3, respectively. Grain nutrients, Fe and Zn, were not affected by limestone application rates, however, their concentrations differed among bean varieties. We were also interested in how common bean varieties performed in different environments and how these interactions influence yield and nutritional value of the grain. We conducted another experiment in Colorado, Michigan, Iowa and Uganda using four common bean varieties ('Montcalm'/dark red kidney, 'Taurus'/great northern, 'Eclipse'/black turtle and, 'MY06326'/yellow Mayocoba). The objective of the study was to determine the effect of environment on total yield overall, yield components and grain nutritional composition. It was observed that yield and seed weight differed across locations and among varieties. Mineral concentration of bean seed P, K, and Mg and crude protein (CP) differed significantly among the varieties but were unaffected by environment. Although soil nutrients (such as Mehlich-3

extractable Fe, Zn, K, P), pH and organic matter to differed among the four experimental locations, we did not observe significant variety \times elemental composition effects across environments.

CHAPTER 1. GENERAL INTRODUCTION

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

Masaka district in central Uganda is one of leading common bean producing and trading districts in Uganda (Kilimo Trust, 2012). The district predominantly has weathered low pH soils due to long term poor soil management, erosion and continuous cropping. While most farms in the region are subsistence level, producing for food and selling the surplus. Common bean serves as a staple food, major source of protein and income in many households (Kilimo Trust, 2012; The Gatsby report, 2014). Most common bean grown in Uganda is consumed in-country with only about 20% exported (Kilimo Trust, 2012). Common bean is therefore an important target crop that could be used for alleviation of food and nutritional insecurity and income generation. Currently, national bean programs in Uganda and worldwide breed and release biofortified common bean varieties to meet yield demands and nutritional needs. These biofortified varieties include among others, varieties NAROBAN 1 and NAROBAN 3 released in Uganda in 2016 with high grain iron and zinc. NAROBAN 1 is locally known as “Masavu” and has white/greyish and dark black striped and large seeds. The variety grows well in low-medium altitude areas (1000-1600m). It has an indeterminate growth habit, matures within 60-68 days and yields about 1500-2500 kg/ha. The variety is resistant to bean rust and bean common mosaic virus and is tolerant to root rots, anthracnose, angular leaf spot and common bean blight and is drought tolerant especially because of early maturity. Variety NAROBAN 3, locally known as “Kyenvu”, is light yellow with medium seed size. The variety grows well in low-medium altitude areas (1000-1600m). It has a determinate growth habit, matures within 58-75 days, and yields about 2000-2500 kg/ha. The variety is resistant to bean common mosaic virus and

common bean blight, is tolerant to root rot, anthracnose, angular leaf spot and bean rust and is drought tolerant due to early maturity. On the other hand, NABE 15 (red with dark red striped seeds) was released in 2010 and though not biofortified, it is high yielding (1800-2000 kg/ha) and drought tolerant. Locally known as “Kanyebwa”, the variety is also disease tolerant to bean rust, root rots, common bean blight and angular leaf spot and resistant to Anthracnose. This medium seed size variety grows well in low-medium altitude areas (1000-1600 m), is a determinate bush bean, matures within 60-65 days and yields about 1800-2000 kg/ha.

Biofortification of common bean with micronutrients such as iron and zinc is vital for the alleviation of those commonly deficient micronutrients among the population. 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 IDA (Iron Deficiency Anemia) during pregnancy also increases incidences of infant post and neonatal 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, 2000). 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 (Srinivansan, 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 (Idjradinata and Pollitt, 1993; Moffatt *et al.*, 1994; Stolfus *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.*, 2012). Alongside supplementation programs, biofortified crops such as high iron and zinc common bean could be used to alleviate the burden of malnutrition. However, even with access to seeds of these nutrient dense crops, farmers in Uganda are challenged by soil acidity among other challenges. 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 lime and fertilizer 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.

Soil acidification is a common problem in crop production systems worldwide. In numerous countries, soil acidification is attributable to factors including acidic precipitation and acidifying gas/particle deposition such as ammonia and sulphur dioxide from the atmosphere, crop nutrient uptake and organic matter mineralization (Goulding, 2016). Soil acidification in agricultural systems is also caused when ammonium-based fertilizers, urea, and elemental S

fertilizers are applied as well as legume production (Guo *et al.*, 2010; Goulding, 2016). In tropical regions such as Uganda, most soil acidification is attributed to weathered soils due to high rainfall (Kuylenstierna *et al.*, 2001).

Highly weathered soils have low mineral nutrient holding capacity and as soil pH decreases below 5.5, dissolution of aluminum oxides begins hence increasing free aluminum ions in the soil solution and increasing aluminum toxicity to plants. High aluminum ion concentration in soil solution at low soil pH competes with cations such as calcium and potassium for negative sites and the cations are then often leached from the soil resulting in low fertility in many tropical soils (Harter, 2007). Phosphate fixation is also typical in tropical soils. This usually occurs due to reaction of aluminum and iron with phosphate on cation exchange sites to form insoluble compounds (Harter, 2007). These effects of low soil pH on soil nutrients and nutrient availability also affect yield and may influence crop nutritional composition. In common bean and lupin, for instance, low soil pH negatively affected nodulation and overall crop performance (Frey and Blum, 1994; Denton *et al.*, 2017). Low soil pH limits the survival and persistence of rhizobia in the soil and consequently nitrogen fixation by legumes (Slattery *et al.* 2001).

A number of strategies have been recommended and used over time to overcome soil acidity including use of lime and wood ash (Clapham and Zibilske, 1992; Demeyer *et al.* 2001; Park *et al.*, 2005; Risse and Gaskin, 2013). However, when wood ash is the selected strategy for increasing soil pH, it is not recommended to use offsite wood ashes because of their high potassium to calcium ratio which could easily lead to unbalanced plant nutrition (Harter, 2007). Liming is therefore one of the major and broadly used strategies for overcoming soil acidity (Goulding, 2015). Some of the most popularly used liming materials are crystalline compounds, calcium carbonate also known as calcitic limestone and calcium magnesium carbonate, also

known as dolomitic limestone (Kamprath and Smyth, 2005). Other liming materials include calcium oxide (quick lime), calcium hydroxide (hydrated lime) and basic slag (calcium silicate) (Kamprath and Smyth, 2005, Goulding, 2016). A number of factors influence the quality of the liming material and thus its effectiveness at raising soil pH and these include purity (measured as calcium carbonate equivalent) and fineness (particle size) (Kamprath and Smyth, 2005; Goulding, 2016; Hellmuth, 2016; McCauley *et al.*, 2017). The source of lime may also influence whether lime increases soil Mg^{2+} relative to Ca^{2+} (Cifu *et al.*, 2004). These changes in soil minerals such as Mg and Ca will be more observable in topsoil compared to subsoil (Holland *et al.*, 2019).

When applied to the soil, lime neutralizes the high concentration of H^+ , Al^{+3} and Mn^{+2} which are usually toxic to plants when in the soil solution. Lime removes the negative effect of H on Ca, K and Mg adsorption in acid soils below pH 5.5 and also neutralizes exchangeable Al and replaces it with Ca and/or Mg on exchange sites making the latter more available for plant uptake (Kamprath and Smyth, 2005). Liming acidic soils to pH 5.6 can also potentially reduce soil solution exchangeable aluminum to zero (Kamprath and Smyth, 2005). Experiments done in Rothamsted and Woburn also showed that lime significantly increased soil pH at those UK sites (Holland *et al.*, 2019). Additionally, incremental lime application rates also resulted in increases in pH. The authors reported the largest increments in pH to correspond to the highest lime treatments. Liming soils also increases soil productivity and biodiversity. Goulding (2016), explained that increased soil solution Ca^{2+} concentrations and ionic strength due to lime application led to flocculation of clays and overall improvements in soil structure and hydraulic conductivity, increased biological (earthworm) activity and overall soil tilth and macro porosity (Bolan *et al.*, 2003). Additionally, increased crop yield due to liming, leads to increased residue

returns from harvest which increases soil carbon content (Paradelo *et al.*, 2015) and ultimately liming serves as an effective climate change mitigation strategy. Fornara *et al.* (2011) explained that increased biological activity due to liming led to the processing of plant C inputs and their effective incorporation into resistant soil organo-mineral pools.

The combination of lime effects on soil chemical, biological and physical properties ultimately leads to increased yield in field crops. Several studies report incremental yield response associated with liming in several field crops such as barley, wheat, faba beans and canola among others (Liu *et al.*, 2004; Farhoodi and Coventry, 2008; Holland *et al.*, 2019). However, differences may be observed in how different crops or varieties of the same crop tolerate soil acidity (Slattery and Coventry, 1993), as well as their response to lime application and various lime application rates (Cifu *et al.*, 2004). In legumes grown on acidic soils for instance, liming helps to overcome low soil pH associated with aluminum and manganese toxicity which affect abiotic and biotic factors for nodule formation (Vargas and Graham, 1988). Increasing pH from liming thus increases the competitiveness of inoculant strains and hence increases their ability to fix nitrogen and overall increase in legume yield (Frey and Blum, 1994). Liming low pH soils is also reported to increase fertilizer use efficiency (Von Tucher *et al.*, 2018). The authors observed increased yield in barley and wheat plots that had lime and fertilizers applied compared to those that received fertilizers alone for soil amendment. Sole application of fertilizers may not be very helpful for raising pH on acidic soils. Without lime and only applying fertilizers, Goulding (2016) reported that soil pH continued to decline and this decrease in pH occurred faster on poorly buffered sandy loam soils.

Although, several research findings show that lime increased soil pH and subsequent crop yield, there is still insufficient information on how soil pH particularly affects crop quality such

as nutrient composition of grain (Holland *et al.*, 2019). Additionally, there are often uncertainties about how much lime to apply to observe changes in pH. For instance, contrary to common perception, in theory, the amount of lime required does not increase linearly as soil pH decreases (Goulding, 2016). The author explained that <5 t/ha of lime would increase soil pH from 6-7 over a period of 5 years. However, about 20 t/ha of lime were needed to increase soil pH from 5 to 7. Goulding (2016) further explained that lime requirement also varied with texture of the soil, its organic matter content and buffering capacity. This implies that to achieve the same required pH change, soils which have a low buffering capacity, such as sandy soils have a lower lime requirement than soils with higher buffering capacity, such as clay soils. Additionally, over liming may lead to molybdenum toxicity and plant deficiency in zinc, copper, boron and manganese as well as soil structure destabilization leading to breaking apart of soil aggregates and thus reduced permeability and poor soil drainage (Harter, 2007). Haynes and Naidu (1997), also reported that liming resulted in other unwanted soil physical properties, such as soil surface crust formation, soil pore clogging and slow and poor water penetration through the soil due to dispersed colloidal clays that arose from liming. They explained that as liming raised soil pH, soil surface net negative charge was increased alongside an increase in the negative to positive charges ratio. Simultaneously, Al^{3+} activity declined as Al precipitated as hydroxy-Al polymers resulting in repulsive forces between soil particles and dispersion of clay colloids.

Strategies to ameliorate global nutrition are invaluable given the existence of nutrient deficiencies of iron, zinc and vitamin A, among others, leading to stunting, decreased physical and cognitive development and death of infants and children worldwide (Black *et al.*, 2013), accompanied by vicious cycles of poverty. The global ramifications of mineral deficiencies such as disease and health complications make the development of pulse crops with high seed

mineral/nutritional concentration a necessity (Vandemark *et al.*, 2018). Biofortification is one the suggested strategies to reduce macro and micronutrient deficiencies. This may be achieved by application of agricultural management practices in order to increase mineral concentration in plant edible parts, development of new varieties with higher concentrations of desired or target nutrients through conventional breeding, or the combination of both management practices and genetic approaches (White and Broadley, 2005). However, even with genetic modification and conventional breeding of pulses for greater yield and nutritional composition, a lot of emphasis needs to be placed on agronomic management practices since these may very well affect the outcomes of the improved crop varieties.

Genotype by environment impact on common bean yield and grain nutrient composition

Common bean is one of America's oldest and earliest cultivated crops (Mensack *et al.*, 2010) and is now a major protein and calorie source in Eastern and Southern Africa (Moraghan *et al.*, 2002; Broughton *et al.* 2003; Alemu, 2017). Globally, common bean occupies about 30 million ha of cropland and about 7.6 million ha in Africa every year (Buruchara *et al.*, 2011; FAOSTAT, 2014). According to the US dry bean council (2019), the United States of America (USA) is the sixth leading common bean producer in the world with about 1.5 to 1.7 million acres planted annually. Some of the leading common bean producing states in the United States of America include Michigan and Colorado which produce about 17% and 5% of USA's common bean, respectively (US Dry Bean Council, 2019). Other states such as Iowa are not major common bean producers and the crop is mostly grown as an alternative crop (Delate, 2013). Adzuki beans, for instance, are grown in some parts of Iowa (USDBC, 2016). Africa on the other hand, produces about 17% of common beans in the world and 70% of that production is in East Africa. Uganda is the second top common bean producing country in East Africa just behind Tanzania (FAOSTAT,

2010). The crop is consumed extensively in Uganda (Laroche *et al.*, 2016). It is grown on about 1,060,000 ha per year in Uganda and yields at least 425,400 tons (FAOSTAT, 2016). Overall, about 9.8 and 3.4 kgs of common bean are consumed annually per capita in Uganda and USA respectively.

Unlike the Americas, common bean is not indigenous to Africa and was brought to the continent by Spanish and Portuguese traders (Jansa *et al.*, 2011). Despite the challenges of pests, diseases and drought among others which lower yield potential from about 5 tons/ha to 0.5 tons/ha (Verdoodt *et al.* 2004), the crop continues to be a major staple food and source of income in at least 100 million African households (Buruchara *et al.*, 2011; FAOSTAT, 2014). Common bean domestication was started by indigenous people during pre-Columbian times in regions of the Americas including, Mexico, Argentina and South America in the Andean region (Kaplan and McNeish, 1960; Kaplan and Kaplan, 1988; Gepts and Debouck, 1991). The common bean varieties spread worldwide today are classified into two major gene pools mostly on the basis on their origins in terms of center of domestication and diversity (Singh *et al.*, 1991). There are two primary common bean Centers of Domestication (COD). The Middle American COD from Central America and North America and, the Andean COD from South America from which races are divided based on their geographical origin and genetic ancestry (Mensack *et al.*, 2010). The Andean races include Chilean, Peruvian and Nueva Granada whereas the Middle American COD races include Durango, Jalisco and the Mesoamerican (Mensack *et al.*, 2010). Market classes including dark and light red kidney beans, white kidneys, cranberry beans, sugar beans and calima beans among others belong to the Andean COD and Middle American COD includes market classes such as great northern, pinto, small red, small white, pink, navy and black bean (Mensack *et al.*, 2010). Beans from Andean and Middle American COD also characteristically

differ genetically and in plant morphology, seed size and retention, maturity and protein expression (Gepts *et al.*, 1988; Koinange *et al.*, 1996; Blair *et al.* 2009b; Mensack *et al.*, 2010).

Studying the genetic variation of common bean genotypes is important because it may influence their tolerance or the lack thereof to unfavorable environments such as climate and soil nutrient deficiencies. For instance, studies have compared various common bean genotype tolerance to low soil P availability on a number of soils around the world (Singh *et al.*, 1989; Thung, 1990). Common bean genotypic differences in yield, soil and climate tolerance may be attributable to differences in several genotype mechanisms and their influence on nutrient utilization efficiency. These include differences in genotype phenology, differences in seed nutrient reserves especially among large and smaller seeded genotypes, differences in root exudates and root activity and architecture among others (Lynch and Beebe, 1995, Fageria *et al.*, 2010; Mourice and Tryphone, 2012). There is also genetic variability in nutrient accumulation and distribution in the seed in either seed coats or cotyledons. Seed coats of black seeded common bean genotypes contained between 17 to 40% of total seed iron content whereas the seed coats of white seeded genotypes contained only about 5 to 11% total seed iron content (Moraghan *et al.*, 2002). Seed tannin concentrations also varied with differences in seed color (Beebe *et al.*, 2002); white colored bean seeds contained lower condensed tannins than their colored counterparts (Welch *et al.*, 2000). Genotypic differences in seed mineral composition may also be attributable to differences in mineral allocation (Wu *et al.*, 2010). The authors explained that seed Zn concentration in rice for instance, was associated with the ability of the crop to translocate Zn from older/mature to newer tissues such as phloem Zn remobilization from leaves and stems to seeds. Some plants also have the ability to alter their rhizosphere by releasing H⁺ ion or organic acid root exudates to boost micronutrient availability and root uptake (Zhang *et al.*, 2010; Marschner, 2012).

However, numerous studies also reported that both environment and genotype may influence common bean yield and nutritional value (Beebe *et al.*, 2002; Frossard *et al.*, 2000). Acidic soils with low organic matter, low extractable Ca, Mg, K and soils with high extractable Al dominate most African crop land (Wortmann *et al.* 1998; Thung and Rao 1999) and together these reduce common bean yield by at least 60-75% (Jansa *et al.*, 2011). Aluminum toxicity alone leads to about 50-60% yield loss in Central, East and Southern African (Thung and Rao 1999; Broughton *et al.* 2003). In cropping systems with low solubility of iron in soil due to alkalinity crop yield may also decrease. Irmak *et al.* (2012) explained that crop yield decreased with soil iron deficiency especially because iron catalyzes many oxidation and reduction reactions, takes part in the production of chlorophyll and is an enzyme activator during electron transport. Moraghan *et al.* (2002) also reported that common bean seed grown on low pH acidic soils had more iron than those grown on calcareous soils. In their study with 16 common bean genotypes, Mukamuhirwa *et al.* (2015) found that genotype \times environment interactions influenced seed iron and zinc content by 32 and 22%, respectively. Both genotypes and environment also impacted common bean seed iron and zinc concentration (Beebe, 2000; Nchimbi-Msolla and Tryphone, 2010). Ortiz-Monasterio *et al.* (2007) explained that the interaction of environmental factors with plant gene expression influenced seed micronutrient accumulation and environmental differences across locations such as soil pH and weather conditions also influenced solubility of soil minerals such as iron and zinc for plant growth, uptake and accumulation (Mukamuhirwa *et al.*, 2015). Pirzadah *et al.* (2010) emphasized that iron solubility particularly rather than its abundance influenced its availability to plants. Therefore, crops grown in locations with either soluble or abundant soil minerals may differ in their accumulation of those nutrients in grains. Additionally, the presence of other soil nutrients such as nitrogen, phosphorus and calcium facilitated absorption and accumulation of

micronutrients like iron and zinc in common bean and wheat (Hao *et al.*, 2007; Cakmak *et al.*, 2010; Kutman *et al.*, 2010; Mukamuhirwa *et al.*, 2015). The presence of soil phosphorus is also reported to either promote root growth and uptake of Zn although application of phosphorous fertilizer could also lead to Zn precipitation, its deficiency in soil and interfere with its translocation from roots (Singh *et al.*, 1988, Zingore *et al.*, 2008). However, although some genotypes (Zn efficient genotypes) are capable of growing and producing good yield on soils that are nutrient deficient (Pearson and Rengel, 1997; Lonergan *et al.*, 2009).

Environmental factors and their interaction with genotypes may also influence common bean seed culinary quality traits such as cooking time (Kigel, 1999; Cichy *et al.*, 2019). Kigel (1999) reported that environmental differences, especially soil mineral concentration affected common bean seed characteristics. Paredes-Lopez *et al.* (1989) and Stoyanova *et al.* (1992) also observed that seeds grown in locations rich in Ca and Mg and with high average annual temperatures of about 59-75°F, were harder and took longer to cook compared to those grown in locations whose soils were low available concentrations of Mg and P and with lower temperatures of about 52-64°F. Stamboliev *et al.* (1995) explained that thinner coated seeds with shorter cooking time were associated with higher rainfall environments because rain altered the characteristics of seed coats and their water permeability.

Soil factors such as low soil pH, low organic matter content and mineral deficiencies not only negatively impact crop productivity and nutritional quality but also human nutrition and overall health from consumption those crops (Sanchez and Swaminathan, 2005). This may explain why regions of the world such as sub-Saharan Africa that have soil fertility problems on about 75% of their arable land (Toenniessen *et al.*, 2008) also have some of the highest malnutrition incidences in their population because soils with insufficient nutrient availability may also lead to

poor crop nutritional quality (Hurst *et al.*, 2013; Kumssa *et al.*, 2015). By understanding environment by genotype interactions, strategies may be developed, and efforts invested in areas such as agronomic biofortification to ensure that agricultural systems meet human population food demand and nutritional needs. Numerous studies done in the past justify these assumptions. Welch *et al.* (2013) postulated that agricultural systems played a major role in the existence of global malnutrition especially if agricultural systems produced nutrient deficient foods. For instance, the historical “Goiter belt” region in the USA that led to iodine deficiency disorders in the people and livestock was associated with low levels of iodine in the water and soils (Johnson *et al.*, 2003; Leung *et al.*, 2012). Nutrient deficient food leads to malnutrition in the population and increases morbidity and mortality rates which lowers workforce productivity and stagnates overall development (Bouis *et al.*, 2012; Graham *et al.*, 2007). Therefore, in these areas there is need for soil amendment and improved water management rather than improved crop varieties for improvement of food production systems (Sanchez and Swaminathan, 2005).

Research done by Cakmak *et al.* (2010) on soil Zn fertilization in Turkey for common bean, pea, soybean, canola, maize, wheat, sorghum and barley reported an increase in yield and grain Zn concentrations. Joy *et al.* (2015b) also showed increased Zn concentration in maize, rice and wheat by 23, 7 and 19% and 30, 25 and 63% following Zn soil and foliar application respectively in ten African countries. Shahzad *et al.* (2014) also observed an increase in cereal grain Fe concentrations following iron foliar sprays to wheat and rice. Direct effects of Se agronomic biofortification in Finland also resulted in increased maize and wheat grain Se concentrations and increased intake in the human and animal populations in the country (Alfthan *et al.*, 2015). Additionally, 30 to 45-fold increases in Zn concentration in soil solution were reported for every unit increase in soil pH at pH ranges between 5.5 to 7.0 (Marschner, 1993).

Given widespread common bean production and consumption worldwide, biofortification of common bean to improve concentrations of vital nutrients especially in grain is an important strategy to overcome nutritional deficiencies in human populations. Although several researchers have worked on the analysis of common bean inheritance of grain nutrient concentration within and across various genepools (Blair *et al.* 2009a; Cichy *et al.* 2009), there are still some uncertainties about how common bean genotypes would perform in diverse environments with regard to yield and seed mineral accumulation. This is because nutritional traits are mostly quantitative and moderately influenced by environment although genotypic variations may occur (Cichy *et al.*, 2009; Blair *et al.* 2009a). It is also worth mentioning that, bioavailability of nutrients in crop edible parts to humans may be influenced by a number of factors that are food or host related (Gibson, 2007) including food processing, gut health, age and gender among others that extend beyond the scope of our study. Studies of cereals like wheat and rice and their processing have been done to further explain how processes such as milling and soaking affect food nutrients (Lyons *et al.*, 2005; Ajiboye *et al.*, 2015; Prakash *et al.*, 2016; Hotz *et al.*, 2015). Studies on the how nutrient bioavailability is influenced by the gastrointestinal tract, enhancers like ascorbic acid, individual health, infections and parasites have been reported on by Sandström (2001), Hallberg (2000), Gibson (2007), Katona and Katona-Apte, (2008), Clemens (2014), to mention but a few.

By understanding environment by genotype relationships, nutrition sensitive strategies such as agronomic biofortification can be used alongside improved genotypes to increase yield and grain nutrient concentrations for human health. Although genetic biofortification is already in place, breeding programs are usually very expensive and require a considerable time to release improved varieties. Agronomic biofortification offers a short-term solution for soil nutrient availability and would greatly complement genetic biofortification. Therefore, agronomic and

genetic biofortification should be used hand in hand (Welch *et al.*, 2013). When poor soils impact yield and crop nutrient accumulation in edible parts such as grains, poor nutrition leads to poor health and overall, the population's wellbeing deteriorates and poverty increases especially when food production and consumption are local (Stewart *et al.* 2010; Oliver and Gregory, 2015).

Common bean is widely grown and consumed, is high in protein and other essential micronutrients such as iron and zinc and possesses the ability to retain those nutrients throughout harvesting and post-harvest handling unlike cereal grains (Wang *et al.*, 2003; Beebe *et al.*, 2000). Therefore, common bean is a great target crop for overcoming food and nutritional deficiencies.

1.1 Overall Objectives

Since limestone has long been used to raise pH dating back as far as 2000 years ago when the Romans used it to offset 'sourness' on agricultural farm land (Goulding *et al.*, 1989; Connor *et al.*, 2011), I set out to use different rates of limestone for raising the pH of acid soils with the objectives of determining the impact that these rates may have on common bean yield and grain iron and zinc in newly released high iron-high zinc drought resilient beans and also provide a baseline for lime recommendation rates for optimum bean productivity and yield in Uganda for farmers and researchers. Additionally, with a lot of emphasis placed on nutrient sensitive agriculture, there is need for combination of genetic and agronomic biofortification strategies which requires an understanding of environment by genotype relationships. I therefore set up experimental plots with four common bean varieties (Montcalm, Taurus, Eclipse and MY06326) in three diverse locations in the United States (Colorado, Michigan and Iowa) and Uganda to determine how environment may influence yield and common bean grain mineral concentration.

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CHAPTER 2. LIMESTONE APPLICATION EFFECTS ON COMMON BEAN (*PHASEOLUS VULGARIS* L.) YIELD AND GRAIN IRON AND ZINC ON A FERRALSOL SOIL

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Core Ideas

- Soil pH, Ca and CEC of Ferralsol increases as limestone application rates increase
- Limestone application may not impact common bean yield
- Common bean grain Fe and Zn concentration was not affected by limestone application rates
- Biofortified common bean varieties (NAROBAN 1 and NAROBAN 3) yielded more and had higher grain Fe and Zn concentration than regular variety (NABE 15)

Keywords

Common bean, Limestone, Yield, Iron, Zinc

Abbreviations

a.s.l., above sea level; CEC, cation exchange capacity; EC, electronic conductivity; ECEC, effective cation exchange capacity; OC, organic carbon; pH, potential of hydrogen.

Abstract

Soil acidification, particularly in developing countries like Uganda, has major ramifications on crop production as low pH soils are less productive. The objective of the study was to determine the effect of limestone application on grain yield and grain iron (Fe) and zinc

(Zn) concentration in newly released high iron and zinc, drought resilient beans (NAROBAN 1 and 3). Using a split plot in time design, we set up an experiment using three local common bean varieties (NABE 15, NAROBAN 1 and NAROBAN 3) and seven rates of limestone to raise soil pH for two rainy seasons on a Ferralsol in central Uganda. The results showed that soil pH, CEC and soil concentration of Ca and Na increased with greater amounts of limestone applied to the soil. On average, common bean variety, NAROBAN 1 had 30% and 48% greater yield and 35% and 47% more seeds m^{-2} than NABE 15 and NAROBAN 3 in both seasons, respectively. Common bean grain nutrients, Fe and Zn, were not affected by limestone application rate, however, their concentrations differed among bean varieties. In both 2017 and 2018, grain zinc concentration in NABE 15 and NAROBAN 1 did not differ. Across seasons, NAROBAN 3 seeds contained 12% and 15% more zinc and 10% and 20% more iron (2017) than NAROBAN 1 and NABE 15 seeds, respectively. Overall, the addition of limestone did not impact yield or yield components of these common bean varieties.

2.1 Introduction

It is well documented that as soil pH declines, so does the supply of several essential plant nutrients including calcium, magnesium and phosphorus. In many soils, this occurs alongside an undesirable increase in aluminum to levels toxic to plants (Harter, 2007). Free aluminum ions replace plant nutrient ions, such as potassium, calcium, and magnesium, on negatively charged soil surfaces (Harter, 2007). This makes those nutrients more susceptible to loss from the soil due to leaching (Harter, 2007). In the tropics, most acidic soils are attributable to weathered soils associated with high rainfall (Kuylenstierna *et al.*, 2001). These acid soils are found 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) explained that phosphate fixation occurred when aluminum and iron released during weathering became more accessible on cation exchange sites both in solution and on exposed surfaces and thus reacted readily with phosphate to form relatively insoluble compounds. Similar highly weathered soils are predominant in the 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 2 to 3 acres per household in many farming communities in Masaka, continuous cropping through subsistence farming remains inevitable. The soils are highly weathered and acidic with low fertility thus occurrence of food insecurity, hunger, and malnutrition are common. Masaka district is one of the major common bean (*Phaseolus vulgaris* L.) growing regions of Uganda. Although the majority of farms remain subsistence, common bean is embedded in the culture of the region. Beans are a staple food and major source of protein as well as income (UNDP, 2012; The Gatsby report, 2014). However, common bean productivity often is constrained by highly acidic soils. 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, 1990; Long *et al.*, 2010). Studies by Fageria (2008) 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).

Important to the negative impact of low soil pH on legume yield may be attributable to its interference with effective nodulation. Nodulation and overall yield in common bean, faba bean and lupin was reported to 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. Vargas and Graham (1988) also explained that soil acidity associated to 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.* (2000) 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). 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 (Goulding, 2016; Holland *et al.*, 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 also was 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 positive impact of lime is further backed by reports of immediate increase in soil pH every year at Rothamsted and Woburn sites in the UK where lime was applied (Holland *et al.*,

2019). The authors reported the largest increase in soil pH (7-8) following the greatest (15 t ha⁻¹) lime applications whereas low (5 t ha⁻¹) and medium (10 t ha⁻¹) lime applications had varying pH values of 5 and 7. They further reported an associated increase in spring bean (*Vicia faba* L.) yield to an increase in soil pH following lime application. Liming low pH soils is also reported to increase fertilizer use efficiency (Von Tucher *et al.*, 2018). The authors observed increased yield in barley and wheat plots that had lime and fertilizers applied compared to those that received fertilizers alone for soil amendment. Sole application of fertilizers may not be very helpful for raising pH on acidic soils. Without lime and only application of fertilizers, Goulding (2016) reported that soil pH continued to decline and this decrease in pH occurred faster on sandy loam soils. Liming also was reported to increase the availability of phosphorus in soil (Haynes, 1982) and therefore overall P uptake by plants. Hanlon (1993) explained that as soil pH decreased, plant availability of nutrients, such as phosphorus also decreased due to numerous precipitation reactions with iron and aluminum.

Although several research findings show that lime increased soil pH and subsequent crop yield, there is still insufficient information on how soil pH particularly affects crop quality, including grain nutritive value (Holland *et al.*, 2019). Additionally, there are often uncertainties about how much lime to apply to observe changes in pH and crop yield. For instance, contrary to common perception, in theory, the amount of lime required does not increase linearly as soil pH decreases on peaty soils (Goulding, 2016). Goulding (2016) explained that < 5 t ha⁻¹ of lime would increase soil pH from about 6 to 7 over a period of 5 years. However, about 20 t ha⁻¹ of lime were needed to increase soil pH from 5 to 7. Goulding (2016) further explained that lime requirement also varied with soil texture, organic matter, and soil buffering capacity. This implies that to achieve the same required pH change, soils with a low buffering capacity, such as

sandy soils have a lower lime requirement than soils with a higher buffering capacity, such as clay soils. It is also worth mentioning that the effectiveness of any liming material is influenced by its reactivity and particle size (Goulding, 2016; Hellmuth, 2016). Differences may also be observed in how different crops or varieties of the same crop tolerate soil acidity (Slattery and Coventry, 1993), as well as their response to limestone application and various lime application rates (Cifu *et al.*, 2004). The source of limestone may also influence whether it increases soil Mg^{2+} relative to Ca^{2+} (Cifu *et al.*, 2004) and such changes in those nutrients will be more robust in topsoil compared to subsoil (Holland *et al.*, 2019).

Strategies to ameliorate global nutrition are invaluable, given the existence of nutrient deficiencies such as iron, zinc and vitamin A, among others, and how these lead to stunting, decreased physical and cognitive development and, death of infants and children worldwide (Black *et al.*, 2013), as well as vicious cycles of poverty in many developing countries. The global ramifications of mineral deficiencies such as, disease and health complications make the development of pulse crops with high seed mineral/nutritional concentration a necessity (Vandemark *et al.*, 2018). Biofortification is one 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). However, even with genetic modification and conventional breeding of pulses for greater yield and better nutritional composition, a lot of emphasis needs to be placed on agronomic management practices since the latter have tremendous impact on the outcomes achieved with improved crop varieties.

Limestone has long been used to raise soil pH, dating back as far as 2000 years ago when the Romans used it to offset 'sourness' on agricultural farmland (Goulding *et al.*, 1989; Connor *et al.*, 2011). An experiment was therefore set up using different rates of lime 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.

2.2 Materials and Methods

Site description

Experimental plots were established in Uganda, Masaka district, at Kamenyamigo, Mukono Zonal Agricultural Research and Development Institute (MUZARDI) (0°18'12.1"S 31°39'56.0"E, 1242 m a.s.l.). 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). These Ferralsols are vulnerable to erosion under poor management. The site receives annual averages of about 367 and 291 mm of rainfall in the MAM and SON growing seasons, respectively (Mugume *et al.*, 2016). The experimental site had previously been under maize (*Zea mays* L.) production. 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). This was done to determine soil amendment requirements necessary for proper common bean production, especially fertilizers.

Experimental design

The experiment used a split plot design in time with three replications (blocks) done over two years (2017 and 2018). 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). Treatments included seven rates of limestone and three common bean varieties. Limestone samples were first sent to CropNuts, Nairobi 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 a rate of 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 1- September, October and November) and 2018 (Season 2 – March, April and May), the three common bean varieties used were NABE 15, NAROBAN 1 and NAROBAN 3. The beans were released by National Agricultural Research Organization (NARO) in 2010 (NABE 15) and 2016 (NAROBAN 1 and 3). NAROBAN 1 and 3 were bred and released for drought tolerance and high iron and zinc whereas NABE 15 was released for drought tolerance and yield.

Experimental site management

Prior to planting, the experimental site was deep ploughed with a tractor followed by 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 to the furrow rows by banding method and covered with a thin layer of soil to prevent seed-fertilizer contact. Fertilizers were applied at a rate of 124 kg ha⁻¹ at planting. A peat-based Mak-bio-N fixer inoculant (Makerere University, Kampala, UG) was used to inoculate seeds prior to planting in both seasons. Plot size was 7.6 m long by 3 m wide, each individual plot had four rows. Season 1 plots were planted on November 21st, 2017 and season 2 plots were planted on March 3rd, 2018. Seeds were planted in furrowed rows 50 cm apart. These 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 and covered with soil.

Weeding was done two weeks after planting, before flowering and additionally later in the season as needed. Weeding was mechanically accomplished by hand hoeing and pulling. Black bean aphids (*Aphis fabae*) were controlled using insecticide Dudu-Cyper® 5% EC (cypermethrin ((±) α-cyano-(3-phenoxyphenyl)methyl(±)-*cis-trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) early in the season 1 in 2017. Application was done at a rate of 2.5 L ha⁻¹ in 625 L H₂O ha⁻¹. A foliar wettable powder fungicide TATA MASTER® 72 (mancozeb 64% + metalaxyl 8%) was also applied at 2.5 kg ha⁻¹ in 20 L H₂O ha⁻¹. Pesticides were sprayed using a knapsack sprayer. Other pests, including bean fly (*Ophiomyia phaseoli* Tryon.), were not observed in either season.

In 2017 (Season 1), the experiment relied on natural rainfall and supplemental irrigation since the crop was planted late in the season. Hand-watering was done on December 19th and 23rd

2017 and January 2nd and 15th 2018 (on the two middle rows) at 52,794 L ha⁻¹ whereas in 2018 season 2, the experiment relied solely on natural rainfall.

Data collection

Stand counts were taken at V4 (fourth trifoliolate leaf stage) and again at R8 (full maturity) (Schwartz and Langham, 2010; NDSU, 1997) stages of development (Table 5). At V4 (fourth trifoliolate 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 from 1 m² quadrat.

Aboveground biomass was determined at harvest (R8-R9) by hand-clipping plants from 1 m² of each plot. Aboveground biomass samples were placed in a forced air oven at 60°C, dried, and weighed to a constant weight. Yield components from 1 m² at R8/R9 were determined. Pods were counted and hand threshed to remove seeds. Seeds were counted, oven dried at 60°C (NARO, Kawanda) and weighed to a constant weight. Seeds were then packaged, labelled and sent to CropNuts laboratory in Nairobi 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 treated as random elements in the model whereas lime rate and common bean variety were treated as fixed main effects. Since 2017 season 1 crop was planted off-season and therefore relied on supplemental irrigation, and yet 2018 season 2 was typically rainy, analysis was done separately by season. The PDIFF procedure was used to test for differences among means where F-tests were significant for main effects or their interactions. Differences between treatments were evaluated at a significance level of $p < 0.05$, unless

otherwise stated. Linear regression was done using PROC REG for parameters that were influenced by limestone rate.

2.3 Results and discussion

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

In soils at 0-15 cm (upper soil) and 15-30 cm (lower soil), the rate of limestone significantly affected soil pH (Table 1). Regression analysis showed that soil pH increased with increase in limestone application rates (upper soil, $r^2= 0.885$; lower soil, $r^2=0.983$) (Table 3). Changes in pH were more prominent in 0-15 cm of soil than 15-30 cm. 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 lower soil, 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 the lowest.

Agricultural lime application did not have significant effect on EC (Table 1). Provin and Pitt (2001) reported that saline soils often had an EC of 40,000 $\mu\text{S cm}^{-1}$. The EC of soils at our experimental site was between 98.7 $\mu\text{S cm}^{-1}$ in topsoil and 74.6 $\mu\text{S cm}^{-1}$ 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). The authors also added that EC mainly indicated the presence or absence of salts in the soil. Changes in EC in upper soil and lower soil were numerically different at different rates of limestone addition. Upper and lower soil EC ranges from our study were less than 1000 $\mu\text{S cm}^{-1}$ and thus the soil at the experimental site would be considered non saline after limestone was applied and thus would not negatively impact crop growth and other important soil microbial processes such as nitrogen cycling, respiration and

decomposition among others (USDA-NRCS, 2019). Such very low EC levels may also indicate low availability soil nutrients especially Mg. Optimal soil EC levels range between $1.1 \mu\text{S cm}^{-1}$ to $5.7 \mu\text{S cm}^{-1}$. The absence of significant differences in EC after lime application may be because soil EC is influenced by several soil physical and chemical factors/properties (Corwin and Lesch, 2005). Friedman (2004) characterized those factors into three categories including soil bulk and volumetric fractions (soil structure, porosity and water content), soil solution/ environmental factors (cation composition, ionic strength and temperature) and solid particle factors such as particle shape, orientation, distribution and CEC. Most of these properties except CEC were not measured in this experiment.

Limestone influenced CEC in upper ($p < 0.001$) and lower soil ($p < 0.01$) (Table 1). Regression analysis showed that upper and lower soil CEC increased with greater limestone rates (upper soil, $r^2=0.957$; lower soil, $r^2=0.836$) (Table 3). Therefore, we observed that the higher the limestone rate, the higher the CEC. The increase in CEC with increase in rate of limestone may be attributable to increase in soil pH due to liming in this study. Similarly, Edmeades (1982) also reported that ECEC increased with increasing soil pH. Such an effect was caused by the deprotonation of pH-dependent charge sites that arose from either mineral (Fe and Al oxides and hydroxides) or organic (carboxyl and phenolic) soil components (Coleman and Thomas, 1967; Gast 1977). Hendershot *et al.* (2005) also reported that CEC increased as a direct function of the amount of lime added, if the final pH of the solution remained below 7. The authors further reported a $1 \text{ cmol (+) kg}^{-1}$ increase in CEC for every $2.1 \pm 0.17 \text{ t ha}^{-1}$ of limestone added to all soils regardless of textures, organic matter or other soil properties. Similarly, Aitken *et al.* (1990) reported that 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 r^2 (0.957) values similar to Aitken *et al.* (1990), who reported that r^2 for the relationship between CEC and pH ranged from 0.81 to 0.99. However, they added that the relationship between CEC and pH became curvilinear with CEC increasing distinctly with relatively small pH increments. Wiklander (1964) explained that the increase in CEC with increased CaCO_3 rates and increased soil pH was mostly attributable to proton dissociation from coordinately bound hydroxyl and hydronium groups on hydrous oxides and clay minerals and from carboxyl and organic hydroxyl groups. Chimdi *et al.* (2012) also attributed the increment in CEC with increased lime rates to the presence of pH-dependent negative charges which can increase when soil pH increases due to applied lime. Therefore, the higher the lime rate, the higher the soil pH and the higher the CEC. Bartlett and McIntosh (1969) also 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.*, 1974).

Agricultural lime rate had no significant influence on soil OC (organic carbon) in either upper or lower soil (Table 1). Haynes and Naidu (1998) also mentioned that few studies, if any, have found a causal link between effects of lime application and soil organic matter. In our study, results may be attributable to short term (2 rainy seasons) duration of the experiment thus, our inability to see any effects of limestone on soil microbial activity which could possibly have long-term effects on soil organic carbon.

Mehlich-3 Ca was significantly affected by limestone application in both soil depths (Table 1). Overall, Ca concentration in upper and lower soils increased with incremental rates of limestone (upper soil, $r^2= 0.949$; lower soil, $r^2=0.995$) (Table 3). Plots that received the greatest

rates of limestone (19,768 kg ha⁻¹) had the greatest Ca concentration whereas plots that received 0, 1236 and 2471 kg ha⁻¹ of limestone had the lowest Ca concentration. Additionally, the Ca concentration in plots where 0, 1236 and 2471 kg ha⁻¹ of limestone were applied did not statistically differ in both upper and lower soil as did Ca concentration in plots that received 4942 and 9884 kg ha⁻¹ of limestone in lower soil. The increase in Ca concentration with increasing lime rate occurred because CaCO₃ was used for liming which was a source of additional Ca²⁺ to the soil and thus the increment in its relative percentage in the soil exchange complex. Therefore, the greater the rate of CaCO₃, the greater the calcium ions added to the soil and thus the calcium concentration in soil solution will also be increased due to CaCO₃ dissolution. Harter (2007), further explained that when limestone was added to the soil, Ca²⁺ displaced H⁺ and Al³⁺ on exchange sites, H⁺ was neutralized by OH⁻ in solution and base saturation increased as calcium substituted hydrogen ions in the soil. Chimdi *et al.* (2012) attributed the increase in exchangeable Ca and CEC when lime rates increased, to the enhancement of Ca²⁺ ion concentration and their replacement of H⁺ and Al³⁺ from the soil solution and exchange complex in the soil.

Mehlich-3 extractable magnesium in upper and lower soil was not significantly affected by application of different rates of limestone although Mg concentration was numerically different at the various limestone rates (Table 1). This is because the magnesium content of the limestone used was less than 0.5% which is quite low compared to the Ca in percentage in the limestone. Simard and Lapierre (1994) also reported that limestone had no significant effect on Mg. Additionally, Riggs *et al.* (1995) also found that calcitic limestone addition did not have any significant effect on exchangeable Mg content. However, unlike our study, the authors reported that the values of exchangeable Mg decreased after the growing season to values lower than

those recommended for agricultural crop productivity. Furthermore, those values were lower than exchangeable Mg values that existed in the soil before cropping. 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 lime. 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 significant differences were observed in Mehlich-3 soil P in upper or lower soil with different rates of limestone (Table 1). 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. This occurs when increased pH leads to hydrolysis of strengite and variscite with the release of more phosphorus ion into the soil solution, phosphorus is adsorbed onto surfaces that were previously hydrated by Al and Fe oxides and clay minerals or, if net mineralization/immobilization of soil organic P occurred through increased microbial activity in the soil. Haynes (1982) also added that if limed soils dried up 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 short term.

Application of different rates of limestone had no significant effect on Mehlich-3 K in both upper and lower soil (Table 1). 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) reported 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 in their experiment 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 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, although we observed increase in CEC with increased limestone rates, this did not affect K levels in our experiment.

Agricultural lime application rates had significant impact on Na concentration in upper soil ($p < 0.001$) and no impact on Na in subsoil (Table 1). Regression analysis showed that the greater the lime application rate, the higher the Na concentration was in upper soil ($r^2=0.938$) (Table 3). Plots that received 19,768 kg ha⁻¹ of limestone had the greatest Na and those that received 2471, 1236 and 0 had the lowest Na in upper soil. Oster *et al.* (1999) also reported that the greatest changes in Na were observed in the top 20 cm of soil. We believe Na was likely a contaminant or a constituent in the limestone applied although we did not analyze for Na content in the limestone used in this study.

Common bean stand densities, aboveground biomass, yield, yield components, and grain iron and zinc concentration for season 1, 2017

The interaction of limestone and variety was significant for yield but not significant for stand density (V4 and R8), aboveground biomass, pods m⁻², seeds m⁻², seeds per pod, seed weight and seed iron and zinc (Table 4). Stand density at V4 and V8 did not statistically differ with the different rates of limestone or amongst the different varieties. This may be attributable to proper crop management practices such as inter-row spacing (50 cm), timely weeding to avoid competition for sunlight and nutrients, and management of potential insect and disease infestations. Wide spacing was reported to facilitate cultivation (Goulden, 1975) whereas close spacing increased seedling density and could increase incidences of diseases (Heard *et al.*, 1990, Sandoval-Avila *et al.*, 1994).

Although limestone rates and their interaction with varieties had no significant effect on aboveground biomass at R8-R9, aboveground biomass differed amongst the three varieties in the experiment at $p < 0.001$ (Table 4). Varieties NABE 15 and NAROBAN 1 had the greatest aboveground biomass whereas NAROBAN 3 had the lowest. Numerically, NAROBAN 1 had the greatest amount of aboveground biomass, followed by NABE 15 and, NAROBAN 3 had the least. NABE 15 and NAROBAN 1 had 57 and 65% more aboveground biomass at R8-R9 than NAROBAN 3, respectively. The exceedingly higher aboveground biomass obtained from NAROBAN 1 may be because of the indeterminate growth habit of this variety (Table 4). Kelly *et al.* (1987) reported that determinate dry bean cultivars had below-average response to various environments compared to indeterminate cultivars under rainfed conditions. This may also be reflected in their yield.

The interaction of limestone rates and variety affected grain yield ($p < 0.05$) and grain yield was also different among the three common bean varieties ($p < 0.001$) (Table 4). Yield did not differ among the various rates of limestone applied in the experiment. Yield of varieties NABE 15 and NAROBAN 1 did not differ and was greater than NAROBAN 3 yield. NABE 15 and NAROBAN 1 had 62% and 69% more yield than NAROBAN 3, respectively. Higher grain yield may be attributable to NABE 15 being well adapted to Uganda's growing environment since it was released earlier, and NAROBAN 1 having indeterminate growth habit. 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, large 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 ones.

Agricultural lime rates had no impact on pods m^{-2} and seeds per pod (Table 4). However, pods m^{-2} and seeds m^{-2} were different among the three common bean varieties ($p < 0.001$). NAROBAN 1 had the greatest number of pods m^{-2} and seeds m^{-2} , followed by NABE 15 and, NAROBAN 3 had the least. NAROBAN 1 had 30 and 56% more pods m^{-2} as well as, 33 and 72% more seeds m^{-2} than NABE 15 and NAROBAN 3, respectively. NAROBAN 1 had 30

and 56% more pods m⁻² than NABE 15 and NAROBAN 3, respectively. These differences may also be attributable to the indeterminate growth habit of NAROBAN and its impact on these yield components. Additionally, limestone application had no significant impact on the number of seeds per pod. However, the number of seeds per pod was different among the three varieties. NAROBAN 1 and NABE 15 had the greatest number of seeds per pod and were similar whereas NAROBAN 3 had the least. NABE 15 and NAROBAN 1 had 33% and 50% more seeds per pod than NAROBAN 3, respectively. Seed weight was not impacted by limestone application rate. However, seed weight was different among bean varieties ($p < 0.01$). NABE 15 seeds had the greatest weight whereas NAROBAN 1 seeds had the least weight. NABE 15 seeds weighed 8% and 17 % more than NAROBAN 3 and NAROBAN 1 seeds, respectively. These differences or their lack thereof, may be possibly attributable to the larger seed size of NAROBAN 1 compared to NABE 15 and NAROBAN 1 whose seeds are medium sized. 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, in late stages of ontogeny, plants that originated from smaller seeds had the potential to compensate for the lower initial growth and produced final biomass similar to that from plants that emerged from larger seeds. That way, they (plants from small seeds) also ensured stable cultivar crop index and yield as much as plants from larger seeds. The authors however, reported that such large seeds did not modify the pod mass and that the earlier increased plant growth rate observed in large seeds during early development stages disappeared later in the season. They reported that although large seeds could anticipate bean growth, smaller seeded plants had the potential to offset their initial lower

growth to ensure grain yield similar to their large seeded counter parts. This may explain why the large seeded indeterminate NAROBAN 1 which numerically had greater biomass, statistically had the same yield and number of seeds per pod as NABE 15 and even lower seed weight than medium seeded NAROBAN 3 and NABE 15. Sexton *et al.* (1997) further explained that negative relationships between seed size and grain yield in common bean may be related to how the crops adapt ecologically to different domestication regions.

Seed concentration of Fe did not differ with different limestone application rates or amongst the bean varieties (Table 4). We also did not observe differences in grain zinc concentration due to limestone application rate. However, zinc concentration did differ among the three bean varieties. NAROBAN 3 seeds had the greatest zinc concentration whereas NABE 15 and NAROBAN 1 had the least. Zinc concentration in NAROBAN 3 seeds was 15% and 18% greater than that in NAROBAN 1 and NABE 15, respectively. Additionally, the zinc concentration in the latter two varieties did not differ. NAROBAN 1 zinc concentration from our study corresponded to that expected/predicted by NARO-Uganda, 31.4-34.3 ppm (Agona, 2017) although our NAROBAN 3 grain zinc concentration was slightly lower than the predicted 35-38 ppm by NARO (Agona, 2017). We do not know why the biofortified NAROBAN 3 had low zinc concentration.

Common bean stand densities, aboveground biomass, yield, yield components and, grain iron and zinc for season 2, 2018

Plant stand density at V4 and V8 differed among the three varieties but was not affected by limestone application rate (Table 5). NAROBAN 1 had the greatest plant stand density whereas NAROBAN 3 had the least at both V4 and R8 stages. At V4, the stand density of NAROBAN 1 was 10% and 13% more than NABE 15 and NAROBAN 3, respectively. At

R8, the stand density of NAROBAN 1 was 11 and 17% more than NABE 15 and NAROBAN 3, respectively. Agricultural lime rate, variety, and their interaction had significant effects on aboveground biomass at R8-R9 (Table 5). Aboveground biomass increased with increments in limestone application rates. NAROBAN 1 had 37% and 41% more aboveground biomass than NABE 15 and NAROBAN 3, respectively. This may be attributable to the NAROBAN 1 having an indeterminate growth habit as explained in Kelly *et al.* (1987).

Yield was significantly different amongst varieties ($p < 0.001$) and the interaction of varieties and limestone rates had statistical effect on yield ($p < 0.001$). Yield of variety NAROBAN 1 was 37% more than NABE 15 and NAROBAN 3. Pods and seeds per square meter were different among varieties. NAROBAN 1 had 41% and 30% more pods m^{-2} than NABE 15 and NAROBAN 1, and 37% and 35% more seeds m^{-2} , respectively. These differences in yield may be attributable to NAROBAN 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).

Seeds per pod and weight per seed were not affected by either lime treatment or variety (Table 5). Seed concentration of iron and zinc was significantly different among varieties (Table 5). NAROBAN 3 seeds contained 10% and 20% more iron than NAROBAN 1 and NABE 15, respectively. Additionally, NAROBAN 3 seeds contained 9% and 13% more zinc than NAROBAN 1 and NABE 15, respectively.

2.4 Conclusions

Weathered acidic soils in the Masaka region of Uganda call for strategies to raise soil pH to improve yield and nutritional composition of food crops, especially common bean. Our study

demonstrated that soil pH increased with greater limestone application rates along with increases in soil calcium and CEC, thus limestone application is a sure way to raise soil pH for improved bean productivity. Additionally, our results from analysis and comparison of performance of the three varieties with seven limestone application rates showed that limestone addition had no impact on yield in our two-year experiment and also demonstrated that the newly released common bean varieties, NAROBAN 1 and NAROBAN 3 yielded more and had higher grain Fe and Zn concentration, respectively compared to an older variety, NABE 15. This is attributable to breeding for biofortification of these minerals in these varieties hence rendering them superior for drought resilience, yield, and grain Fe and Zn composition. Given such findings, we would therefore recommend production of those newer varieties for better yield and to overcome Fe and Zn micronutrient deficiencies.

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2.7 References

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2.7 Tables

Table 1. Effect of different rates of limestone on soil properties at 0-15 cm and 15-30 cm for 2017 (season A) and 2018 (season B).

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

Table 2. Characteristics of limestone used in the experiment.

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

Table 3: Linear functions for limestone application rates predicting upper (0-15 cm) and lower (15-30 cm) soil pH, CEC, and Ca and Na concentrations.

Parameter	Function	r^2
Upper soil pH	$0.0002x + 5$	0.886
Upper soil CEC	$0.0011x + 12.609$	0.957
Upper soil Ca concentration (mg kg ⁻¹)	$0.3191x + 959.57$	0.949
Upper soil Na concentration (mg kg ⁻¹)	$0.0021x + 8$	0.938
Lower soil pH	$8E-05x + 5$	0.983
Lower soil CEC	$0.0005x + 11.174$	0.836
Lower soil Ca concentration (mg kg ⁻¹)	$0.1289x + 797.71$	0.995

Table 4: Stand density at V8 and R8, above ground biomass, yield and yield components and seed iron and zinc, season A, 2017.

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	32	19	411	334	32	94	3	361	69	31
Limestone Rate (kg ha ⁻¹)										
0	33	20	456	398	36	113	3	358	68	31
1236	34	20	511	455	40	134	3	346	60	29
2471	32	19	433	408	37	131	3	337	62	29
4942	34	20	483	431	42	137	3	328	63	31
9884	33	20	594	465	44	142	3	332	73	30
19768	32	19	561	523	45	148	3	338	64	33
Variety										
NABE 15	33	20	572 a [†]	510 a	40 b	138 b	3 a	371 a	60	28 b
NAROBAN 1	32	20	703 a	634 a	57 a	205 a	4 a	309 b	69	29 b
NAROBAN 3	34	19	244 b	196 b	25 c	59 c	2 b	340 ab	66	34 a
<u>Significance</u>					<i>P</i> > <i>F</i>					
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

† Values followed by different letters within a column are significantly different at $P = 0.05$ by the least square means test.

*Significant at $P = 0.05$.

**Significant at $P = 0.01$.

***Significant at $P = 0.001$; NS, not significant.

Note: NPK was applied at a rate of 124 kg ha⁻¹ in all plots with 0, 1236, 2471, 4942, 9884 and 19768 (kg ha⁻¹) rates of limestone except in control plots, therefore data for the latter was analyzed separately.

Table 5: Stand density at V8 and R8, aboveground biomass, yield and yield components and seed iron and zinc, season B, 2018.

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

[†] Values followed by different letters within a column are significantly different at $P = 0.05$ by the least square means test.

*Significant at $P = 0.05$.

**Significant at $P = 0.01$.

***Significant at $P = 0.001$; NS, not significant.

Note: NPK was applied at a rate of 124 kg ha⁻¹ in all plots with 0, 1236, 2471, 4942, 9884 and 19768 (kg ha⁻¹) rates of limestone except in control plots, therefore data for the latter was analyzed separately.

CHAPTER 3. GENOTYPE BY ENVIRONMENT EFFECTS ON YIELD AND GRAIN NUTRIENT COMPOSITION OF COMMON BEAN

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Abstract

Common bean (*Phaseolus vulgaris* L.) is a staple crop in households worldwide. Consumption is increasing due to consumer preference for protein-rich crops and greater interest in sustainable agricultural practices. Common bean is a great target for the alleviation of food insecurity and nutrition inadequacies, given its richness in protein and micronutrients including iron and zinc. We planted four diverse common bean varieties ('Montcalm'/dark red kidney, 'Taurus'/great northern, 'Eclipse'/black turtle and 'MY06326'-yellow Mayocoba) for two years in four locations (Iowa, Colorado, Michigan and Uganda). The objective of the study was to determine the effect of environment on yield, yield components and grain nutritional composition. Yield and seed weight differed across location and among varieties. Mineral composition of P, K, Mg and crude protein (CP) differed significantly among varieties. The dark red kidney bean 'Montcalm' had 18.2%, 6.9% and 4.1% greater P concentration and 12.9%, 8.6% and 4.9% more CP than black turtle bean 'Eclipse', Mayocoba bean (MY06326) and the great northern beans ('Taurus'), respectively. The great northern bean 'Taurus' contained 21.3%, 12.4% and 4.4% more Mg than dark red kidney, Mayocoba and black turtle beans, respectively. Although many soil nutrients (Mehlich-3 extractable Fe, Zn, K, P) and soil properties such as pH and organic

matter differed greatly among the four locations, we did not observe significant differences in the variety \times elemental composition across these environments. The ramification of these findings for human food and nutritional security is great since the same seed in a different environment may provide variable yield. Nutrient concentration of seed lines should be evaluated at specific sites to support human nutrition interventions.

3.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important and oldest cultivated crops worldwide. The importance of common bean in several parts of the world is evidenced in its consumption and role in many countries' economies not only as food crop to provide food and nutritional security but also as a cash crop for income generation. Common bean consumption represents 50% of all grain legumes consumed worldwide (Talukder *et al.*, 2010). It is a major protein source in many Latin American and East African countries including Brazil, Mexico, Rwanda and Uganda (Broughton *et al.*, 2003). Legume seeds, particularly common bean, have higher concentrations of iron and zinc than cereals. These nutrients are usually retained throughout harvesting and when common bean undergoes processing unlike milled grains (Wang *et al.* 2003; Beebe *et al.* 2000). Common bean is also a nitrogen fixer, highly adaptable and productive in a wide range of environments, and it can be highly profitable when planted as a cash crop (Broughton *et al.*, 2003). Common bean per capita consumption in sub-Saharan Africa increased by 1.7% per year from 1994-2008 (Akibode and Maredia, 2011). National bean research programs have invested in continuous development and release of high yielding, drought resilient, disease resistant and nutrient dense common bean varieties.

Common bean is indigenous to the Americas. It was domesticated by indigenous people during pre-Columbian times (Mensack *et al.*, 2010), then made its way from the Americas to the

rest of the world. Common bean germplasm is classified into two gene pools; the Andean (South America) and Middle American (Central and North America) (Singh *et al.* 1991).

Mesoamerican, Durango and Jalisco landraces belong to the Middle American gene pool (Blair *et al.* 2010; Mensack *et al.* 2010), have small and medium sized seeds and contribute to several commercial market classes including great northern, black beans, small red, navy, pinto and pink beans. Larger seeded commercial market classes including Peruvian, dark red kidney, white kidney and cranberry beans belong to the Andean gene pool (Blair *et al.* 2010, Mensack *et al.* 2010). Bean protein (that is, isozymes and phaseolin) and molecular marker profiles differ in the two gene pools (Blair *et al.*, 2009b). There are many questions regarding to what extent environment influences common bean yield and grain mineral composition and the implications that this may have on consumers' diets.

In legumes such as chickpea, genotype by environment interactions impact grain nutritional traits including fatty acids and tocopherols (Gül *et al.*, 2008), alongside other processing traits (milling including dehulling efficiency and splitting) (Wood *et al.*, 2008). In common bean seed iron and zinc concentration and content are influenced by both genotype and environment (Beebe, 2000; Nchimci-Msolla and Tryphone, 2010; Mukamuhirwa *et al.*, 2015). For instance, high-zinc seeds grown in high-zinc soils were reported to be more advantaged compared to high-zinc seeds grown in zinc-deficient soils (Graham *et al.*, 2001). This is because plant roots uptake soil nutrients which are then partitioned to seeds with the influence of transporters and storage reserves (Frossard *et al.*, 2000; Grusak, 2000). Graham (1984) also emphasized the role of plant genes in nutrient uptake from the soil and transport within the plant to the seed and loading traits. Some plants have the potential to modify their rhizosphere to enhance micronutrient availability and uptake by excretion of H⁺ ions or organic acids alongside

other root functions (Alloway, 2009; Zhang *et al.*, 2010; Marschner, 2012). The strategy of common bean particularly enables the crop to acquire nutrients from the soil by rhizosphere acidification, using iron reductase to reduce iron and an iron transporter for mineral cross-membrane root uptake (Marschner and Römheld, 1994; Briat and Lobreaux, 1997). Although seeds store nutrients to sustain embryo growth for the next generation, those nutrients are essentially enough to sustain root growth and thereafter, roots should have enough absorbing surface to acquire soil nutrients to supply and meet growing plant needs (Graham *et al.*, 2001). Therefore, in nutrient deficient soils, the plant has the most difficulty in storing adequate nutrients in the seeds (Graham *et al.*, 2001). Thus, low nutrient soils may yield low nutrient seeds, thereby compounding the problem of poor seedling vigor which in turn leads to poor crop establishment, more severe weed competition due to weeds having better adaptability than the crop, more crop disease susceptibility and overall lower grain yield at harvest.

Differences in yield, grain nutritional and antinutritional compound concentrations, and cooking characteristics in common bean are attributable to differences in genotype and environmental factors especially soil characteristics (Kigel, 1999). Soil chemical and physical properties including pH and organic matter were reported to have significant effect on nutrient solubility and root absorption of nutrients (Marschner, 1993; Obrador *et al.* 2003; Cakmak, 2007). For instance, a 30 to 45-fold decrease in Zn concentration in soil solution was reported for every unit increase in soil pH between 5.5 to 7.0 (Marschner, 1993). Such increases in soil pH were also associated with subsequent decreases in Zn concentration in plant tissues (Sarkar and Wyn Jones, 1982; Marschner, 1995). Therefore, the potential for crops to acquire soil nutrients and accumulate those nutrients in edible parts are likely influenced by genotype, the environment in which they are grown and the interaction of genotype with environment.

The effect of environment/soil and genotype interactions on nutritional composition of plant edible parts is often manifested through nutrient deficiencies in human diets. Widespread Zn deficiency in humans, for instance, is typical in those regions of the world where soils are Zn-deficient (Cakmak, 2008). To counter these nutritional deficiencies, plant breeding strategies such genetic biofortification for common bean grain zinc, iron and other nutrients are in place and appear to be the most sustainable and cost-effective approaches to improve grain concentrations of these nutrients. These breeding approaches are long-term and require tremendous amounts of effort and resources. The success of these breeding programs is highly dependent on the size of the soil nutrient pools and the solubility of those nutrients in the soil solution (Cakmak, 2008). Therefore, the genetic capacity of biofortified varieties to acquire, absorb and accumulate grain nutrients and attain maximum yield may not be fully realized when they are grown on poor soils.

The combination of mineral fertilizer, organic inputs and improved germplasm are therefore encouraged (“Integrated Soil Fertility Management”) (Vanlauwe *et al.*, 2010). Effects of selenium (Se) agronomic biofortification in Finland for instance, resulted in increased maize and wheat grain Se concentrations and increased intake in the human and animal populations in the country (Alfthan *et al.*, 2015). We conducted this experiment with the objective of determining the effect of environment on yield, yield components and grain nutritional composition in common bean.

3.2 Materials and methods

Experiment Locations and Site Descriptions

In 2017 and 2018, field trials were conducted in Boone, Iowa; Fort Collins, Colorado; Lakeview, Michigan and Masaka, Uganda. In Iowa, the field trials were planted at the Sorenson

farm, Iowa State University (42°00'35.7"N, 93°44'47.1"W). This site is 325 m above sea level, it is tile and ditch drained due to slow natural drainage (Hofstrand, 2010) and receives average of 974 mm of annual rainfall (US Climate data, 2019). Soil samples were collected from 0-30 cm before planting and analyzed for pH, nitrate, available P and K (Mehlich-3), and organic matter (Soil and Plant Analysis Laboratory, Iowa State University) (Table 1). The predominant soils at the Sorenson Farm were Canisteo loam (Fine-loamy, mixed, superactive, mesic Typic Hapludolls) (NRCS, 2019) (Table 1). This site was previously rotated between soybean/corn and warm-season grasses with no known production history of common beans.

In Colorado, field trials were established at the ARDEC Farm, Colorado State University (40°38'59.35"N 104°59'49.33"W). The farm is at an elevation of 1550 m above sea level and receives on average 408 mm of annual rainfall (US Climate data, 2019). Soil samples were collected before planting and analyzed (American Agricultural Laboratory, Nebraska) for pH and other nutrients to determine the appropriate fertilizer recommendations for bean production (Franzen, 2017). The predominant soils at the site were Fort Collins loam (Fine-loamy, mixed, mesic Aridic Haplustalfs) (NRCS, 2019) (Table 1). This site was previously planted with dent corn and barley in 2016 and 2015, respectively.

In Michigan, field trials were established at the Montcalm research farm, Entrican, Michigan State University (43°21'08.39"N 85°10'45.58"W). The farm is at 287 m above sea level and is not drained by tiles and ditches. This site receives an annual average of 854 mm of rainfall (Climate-data, 2019). Soil samples were collected before planting and analyzed (Soil and Plant Nutrient Laboratory, Michigan State University). The predominant soils at the site were Tekenink-Elmdale loamy sands (course-loamy, mixed, semiactive, mesic Typic Glossudalf)

(NRCS, 2019) (Table 1). This site was previously planted with corn (2015), potato (2016) and corn (2017).

In Uganda, field trials were established in the Masaka district, at the Kamenyamigo, Mukono Zonal Agricultural Research and Development Institute (MUZARDI) research station (0°18'12.78"S 31°39'56.19"E). This site is 1242 m above sea level. Soil samples were collected from 0-30 cm from each plot and analyzed for pH, nitrate, available P and K (Mehlich-3) and organic matter (Crop Nutrition Laboratory Service Ltd, CropNuts, Nairobi, Kenya) (Table 1). This was done to determine soil amendment requirements necessary for proper common bean production and fertilizer requirements. Soils around this site are 17% udults (Udox-11%, Udalfs-8% (TAXOUSA, 2014) (Table 1). The site receives an average of 367 and 291 mm of rainfall in the March-April-May (MAM) and September-October-November (SON) growing seasons, respectively (Mugume *et al.*, 2016). The experimental site was previously planted with corn.

Experimental Design

A randomized complete block design repeated was used at all four locations with four common bean varieties and three replications. The common bean varieties were 'Montcalm' (dark red kidney bean), 'MY06326' (yellow mayocoba bean), 'Taurus' (great northern bean) and 'Eclipse' (black turtle bean). Seeds at all locations were inoculated with Rhizobia at planting; an EL type inoculant (peat-based) (INTX Microbials, LLC, Kendall, IN) was used in Iowa, Colorado and Michigan and a Mak-bio-N fixer inoculant (peat-based) (Makerere University, Kampala, UG) was used in Uganda. Plots in Iowa were four rows; individual plot size was 7.6 m long by 3 m wide with 0.76 m row spacing. These were planted on May 31st and 16th 2017 and 2018, respectively. Seeds were planted at a depth of 3.2 cm using a Heavy Duty Grain Drill (HDGD) plot planter (Almaco, Nevada, IA). Plots in Colorado were also four rows and

individual plot size was 6.4 m long by 3 m wide with 0.76 m row spacing. These were planted on June 9th and May 30th, 2017 and 2018, respectively. Seeds were planted at a depth of 2.54 cm using a MaxEmerge row unit, Wintersteiger plot king (John Deere, Moline, IL). In Michigan, plots were 4 rows and individual plot size was 6 m long by 2 m wide with 0.5 m spacing. These were planted on June 13th in both 2017 and 2018. Seeds were planted at a depth of 3.8 cm using a White 6100 row unit (Great Plains, Salina, KS). Experimental plots in Uganda also had four rows and individual plot size was 7.6 m long by 3 m wide. The first season was planted on November 7th, 2017 and second season planted on March 3rd, 2018. Seeds were planted in furrowed rows 50 cm apart. These 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 and covered with soil.

Site Management

In Iowa, before planting in the spring, the site was chisel plowed followed by a disc and field cultivator. Fertilizer was applied at rates of 100 kg/acre of diammonium phosphate (DAP, 18-46-0) and approximately 79 kg/acre of potassium chloride (muriate of potash, 0-0-62) in 2017. These were applied in 2017 because soil test analysis before planting indicated inadequate amounts of those nutrients recommended for common bean production in Iowa (Mallarino *et al.*, 2013). A pre-plant application of pendimethelin (N-(1-ethylpropyl)-3, 4-dimethyl-2, 6-dinitrobenzenamine) was applied at a rate of 1.8 quarts per acre in 80 quarts H₂O acre⁻¹ in 2017 and 2018 using a modified Fimco electric pump sprayer mounted on a Stanhoist field cultivator with integrated spray boom. Additional weed management was mechanically accomplished by hand roguing. Dimethoate (0,0-dimethyl-S-[(methylcarbamoyl) methyl] phosphorodithioate)

was used to control leafhoppers (*Empoasca fabae*) early in the season in 2017. It was applied at a rate of 0.25 quarts per acre. The experiment relied solely on natural rainfall.

In Colorado, the experimental site was vertical tilled using a John Deere 2623VT (John Deere, IL), fall plowed, mulched and levelled and then mulched, bedded, cultivated and corrugated in the spring prior to planting. In 2017, 100 kg/acre of urea were applied and 3 tons per acre of manure applied. In 2018, monoammonium phosphate (MAP, 11-52-0) and urea (46-0-0) were applied at rates of 32.5 kg/acre and 114 kg/acre respectively. A pre-plant combination of pendimethelin, Eptam (S-ethyl dipropylthiocarbamate) and Outlook (Dimethenamid-P: (S)-2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-yl)-acetamide) was applied. Pendimethelin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) was applied at a rate of 1.44 quarts per acre. Eptam 7E (S-ethyl dipropylthiocarbamate) was applied a rate of 1.5 quarts per acre (3 pt/acre), and Outlook (Dimethenamid-P: (S)-2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-yl)-acetamide) applied at a rate of 0.53 quarts per acre, Dual II Magnum (S-metolachlor) applied at a rate 0.67 quarts per acre and Roundup PowerMax (glyphosate, N-(phosphonomethyl)glycine) at a rate of 0.69 quarts per acre. Additional weed management was mechanically accomplished by cultivation done with an Eversman 6 row cultivator (Eversman Mfg. Co., Denver, CO). There was a minor Mexican bean beetle (*Epilachna varivestis*) infestation but damage was minimal, and an insecticide application was not necessary. The experiment relied on both natural rainfall and irrigation. Irrigation in 2018 was done on June 15th and 25th, July 12th and 19th, and August 15th and approximately 51mm of water were applied per irrigation.

In Michigan, the experimental site was spring chisel plowed, followed by a disc and field cultivator prior planting. Prior planting, 90.7 kg of NPK (19-10-19) fertilizers were applied. Side

dressing was done using 22.7 kg of urea (46-0-0) about 43 days after planting. A pre-plant combination of Eptam (S-ethyl dipropylthiocarbamate), Sonalan (Ethalfluralin: N-ethyl-N-(2-methyl-2-propenyl)-2, 6-dinitro-4-(trifluoromethyl)benzenamine) and Dual (S-metolachlor) was applied. Eptam (S-ethyl dipropylthiocarbamate) was applied a rate of 1.25 quarts per acre in 0.12 quarts H₂O acre⁻¹, Sonolan (Ethalfluralin: N-ethyl-N-(2-methyl-2-propenyl)-2, 6-dinitro-4-(trifluoromethyl)benzenamine) was applied at a rate of 6.7 quarts per acre and Dual (S-metolachlor) at rate of 0.835 quarts per acre in 2017. Additional weed management later in the season was done using Basagran (sodium salt of bentazon' (3-{1-methyl-2,1,3-benzothiadiazin-4-(3H)-one 2,2-dioxide), applied at a rate of 0.5 quarts per acre, Reflex (Sodium salt of fomesafen 5-[2-chloro-4-(trifluoromethyl) phenoxy]-N-(methylsulfonyl)-2-Nitrobenzamide) applied at a rate of 0.5 quarts per acre and, Raptor (ammonium salt of imazamox: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1 H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid), applied at a rate of 0.125 quartz per acre. Leaf hopper (*Empoasca fabae*) and Japanese beetle (*Popillia japonica*) infestation in the plots was controlled using Asana (Esfenvalerate (S)-cyano (3-phenoxyphenyl) methyl (S)-4-chloro-alpha-(1-methylethyl) benzeneacetate). The pesticide was sprayed at a rate of 0.3 quarts per acre using a Demco RM200 sprayer. The experiment relied on both natural rainfall and irrigation. The experiment received a total of 137 mm of irrigation water through 10 applications in 2017. Plots were irrigated as needed in 2018 at an average rate of 15 mm per week.

In Uganda, the experimental site was deep ploughed with a tractor followed by fine tillage and cultivation by hand hoeing. Agricultural lime was applied 3 weeks before planting at rate of 14,826 kg/ha to allow for time to ameliorate pH (Ball, 2002) and NPK (17:17:17) fertilizer applied at planting to the furrow rows by a banding method and covered with a thin layer of soil

to prevent seed-fertilizer contact. Fertilizers were applied at a rate of 124 kg ha⁻¹ according to recommendations by NARO for pulse production on Ferralsols in Uganda (Sunday and Ocen, 2015).

Weeding was done two weeks after planting, before flowering and additionally later in the season as needed. Weeding was mechanically accomplished by hand hoeing and pulling. Black bean aphids (*Aphis fabae*) early in the season in 2017 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 a rate of 2.5 L ha⁻¹ in 625 L H₂O ha⁻¹. A foliar wettable powder fungicide TATA MASTER® 72 (mancozeb 64% + metalaxyl 8%) was applied at 2.5 kg ha⁻¹ in 20 L H₂O ha⁻¹. Pesticides were sprayed using a knapsack sprayer. Hand-watering with watering cans on the two middle rows was done on December 19th and 23rd and January 2nd and 15th 2018 for the beans planted in SON season 2017. However, in 2018 planting was done early at the beginning of the MAM rainy season and no irrigation was necessary.

Data Collection and Analysis

At full maturity (R8) stand counts were taken and beans were harvested from 1 m² quadrants per plot, oven dried overnight at 60°C, weighed and sent to the lab for chemical analysis. Concentrations of N, P, K, Na, Fe, Zn, Mn, Mg, S, and Al for samples from Iowa, Colorado and Michigan were done at the Soil and Plant Analysis Laboratory (Iowa State University) in 2017 and Agsource laboratories in Lincoln, Nebraska in 2018. Grain samples from Uganda were analyzed at CropNuts Laboratory in Nairobi. Crude protein (CP) was calculated as $N \times 6.25$.

Data were analyzed using generalized linear mixed models using PROC GLIMMIX in SAS[®] 9.4 (SAS institute Inc., Cary, NC). Varieties and locations were treated as fixed effects and years were treated as random effects. The PDIF procedure was used to test for differences among means where F-tests were significant for main effects and their interactions. Differences between main effects are evaluated at a significance level of $P \leq 0.05$, unless otherwise stated.

3.3 Results

Relationship between soil and common bean grain mineral concentrations

Soil pH, Mehlich-3 extractable P, K, Na, Mg, Al and S and TN differed across locations (Table 1). However, we did not observe statistical differences in soil OM and Mehlich-3 extractable Fe and Zn in soils from different Colorado, Iowa, Michigan and Uganda (Table 1). Grain concentrations of CP, P, K, Mg and S were significantly different among the four varieties tested (Table 2) suggesting existence of genetic variability for these traits. Grain Fe and Zn concentrations among the four common bean varieties ranged from 83 to 141 mg/kg and 38 to 52 mg/kg, respectively, whereas grain Mn, Al and S concentrations ranged between 22 to 26 mg/kg, 75 to 196 mg/kg and, 80 to 93 mg/kg, respectively. Location had no impact on common bean grain mineral composition despite the locations having a wide range of differing soil properties such as pH and nutrient pools of mineral elements (Tables 1 and 2). Soil and grain samples from Uganda were not analyzed for Al. Additionally, we did not observe any location by variety interactions for all grain mineral elements except zinc (Table 2).

Montcalm seeds contained the greatest CP and P concentrations whereas Eclipse contained the least (Table 2). Montcalm grains contained 13%, 8% and 4% more CP and, 18%, 7% and 4% more P than Eclipse, MY06326 Taurus grains, respectively. The mineral

concentration of CP and P in MY06326 and Taurus grains were similar. Grain K concentration was greatest and similar in MY06326 and Taurus grains and lowest in Eclipse (Table 2). MY06326 grain contained 13% and 7% more K than Eclipse and Montcalm grain whereas Taurus grains contained 10% and 5% more K than Eclipse and Montcalm, respectively. S concentrations ranged between 22 to 26 mg/kg, 75 to 196 mg/kg and 80 – 93 mg/kg, respectively. The grain concentration of S was greatest and similar in Montcalm and Taurus whereas S concentration was lowest in MY06326 grains. Both Montcalm and Taurus grains contained 13% and 7% more S than MY06326 and Eclipse, respectively.

Impact of environment on yield and seed weight

Stand density at maturity (R9) differed across environments and among varieties. Stand density at was higher in Iowa than the other three locations (Colorado, Michigan and Uganda) (Table 3). Common bean stand density at maturity was 54, 43 and 38% more in Iowa than Uganda, Michigan, and Colorado, respectively. Stand density was greatest in Eclipse, MY06326 and Taurus and, lowest in Montcalm. Stand density for Eclipse and Taurus was 33% more than Montcalm whereas that of MY062326 was 28% more than Montcalm at maturity. Stand density at maturity for all varieties was highest in Iowa (Figure 3). Both yield and seed weight were different across environments and among genotypes and, the interaction of yield and seed weight \times genotype were significant (Table 3, Figure 1). Yield and seed weight in Colorado and Michigan were similar and higher than in Iowa and Uganda. Yield in Colorado and Michigan was 42% and 56% more than Iowa and, 76% and 81% more than Uganda, respectively. Overall, Eclipse had the greatest yield and Montcalm had the least across all environments. Eclipse had 6, 16 and 35 % more yield than Taurus, MY06326 and Montcalm, respectively (Table 3). Eclipse, MY06326, Taurus and Montcalm yield were all greatest in Michigan and lowest in Uganda

(Figure 1). Seed weight (g/seed) was greatest in Michigan, followed by Colorado and lowest in Iowa and Uganda. Seed weight for Michigan was 24%, 44% and 49% more than common bean Colorado, Iowa and Uganda, respectively (Table 1). Montcalm had the greatest individual seed weight and Eclipse seeds had the least. Taurus and MY06326 seed weight was similar.

Montcalm seeds weighed 59% more than Eclipse seed and 27% than Taurus and MY06326 seeds (Table 1). Overall, Eclipse, MY06326, Taurus and Montcalm seeds weighed more in Michigan than the other environments in this study (Figure 2).

3.4 Discussion

In this study, grain CP concentration did not differ across locations. This is in contrast to Leleji *et al.* (1972) who reported that CP in common bean was highly influenced by environment. Differences in seed CP concentrations among varieties in our study may be related to differences in seed color. This correlation was observed in Strauta *et al.* (2013) who reported some differences in grain crude protein, especially among brown and white colored *Phaseolus coccineus* L and related them to seed color.

Average Fe concentration varied across location (61 to 150 mg/kg) and varieties (83 to 141 mg/kg), respectively, and was greater than the average Fe concentration (55 mg/kg) found by Bänziger and Long (2000). Although all locations had disparities in climate and soil mineral pools and the varieties we tested were very different, grain Fe concentration was not significantly different by location or variety. Beebe *et al.* (2000) also reported no correlation between geographic location and common bean Fe concentrations. The absence of significant differences in grain Fe levels across locations even when soils had different iron pools at planting may be attributable to the rapid and irreversible binding of Fe to soil particles immediately after

application to the soil, which prevents uptake by plants and its accumulation in grain (Hirschi, 2009).

We did not observe differences in grain zinc across locations. Common bean grain Zn concentration may not have differed across locations even with environmental differences such as soil and climate because grain Zn is dependent on its availability in the soil, as well as other chemical and physical soil properties, such as soil pH, that can reduce Zn solubility and impair root absorption (Lindsay and Schwab, 1982; Brown and Jolley, 1989; Beebe *et al.*, 2000; Cakmak, 2007). We did not observe differences in grain zinc across locations despite regional differences in soil pH (that is, 8.0 in Colorado versus 5.9-6.2 in the other locations). This is a bit unexpected based on observations by Marschner (1993) who reported that soil pH between 5.5 to 7.0 led to a 30 to 45-fold decrease in soil Zn concentration for each unit increase in soil pH, which in turn may increase the risk of Zn deficiency in plants. This was based on the observation that increasing soil pH stimulated Zn adsorption to soil constituents and reduced the desorption adsorbed Zn. Our findings were also contrary to Sarkar and Wyn Jones (1982), Lindsay (1991), and Marschner (1995) who associated an increase in soil pH to sharp decreases in soil solution Zn and Zn concentration in plant tissues. Soil moisture and soil organic matter also play a critical role in soil Zn availability for plant uptake (Obrador *et al.*, 2003; Cakmak, 2007). In our study, differences in organic matter percentages across locations did not seem to have a direct relationship with soil and grain concentration of Zn. We did not, however, measure soil moisture in our study, so the diverse climates and different levels of rainfall across our locations and the possibility of water stress (especially in Uganda) could have affected soil Zn.

Our findings were contrary to Petry *et al.* (2015) who reported that bean Fe and Zn grain concentrations strongly depended on the planting site and site factors such as soil fertilization.

The potential for varieties in our study to accumulate Fe and Zn despite the differences in the environments they were grown in may be attributable to Fe and Zn transporter proteins, which have the ability to improve the density of micronutrients in grain regardless of the availability of these nutrients in the soil (Schachtman and Barker, 1999; Eide, 2006). These proteins are involved in uptake and transport of cation micronutrients in cells. Genes that encode these Fe and Zn transporter proteins may be expressed in response to environments where Fe and Zn is deficient, therefore, countering the differences that could arise due to differences in the availability of Fe and Zn in the soil. The role that these transporter proteins play in genotypic variation for Fe/Zn deficiency tolerance or even grain Fe/Zn accumulation is still unclear (Cakmak, 2008), and warrants further investigation. Other inherent factors may also be affect grain Fe and Zn accumulation and may explain why concentrations of these nutrients were not impacted by differences in edaphic factors observed in this study. For instance, in Uauy *et al.* (2006), the potential to increase grain Fe and Zn concentrations in wheat may be attributable to the Gpc-B1 locus, which encodes a NAC transcription factor (NAM-B1) that may possibly stimulate leaf senescence and therefore remobilize Fe and Zn from flag leaves to seeds.

Studies by Rengel *et al.*, (1999) and Aciksoz *et al.* (2011) reported that application of Fe fertilizers was ineffective for improving grain Fe concentrations potentially due to inefficient Fe phloem mobility and transportation to grains, and Ramolemana (2013) reported no significant impact of soil type/location on Fe content of Morama bean seeds. Similarly, soil Zn fertilization in seven countries for wheat and rice production did not increase grain-Zn concentrations (Zou *et al.*, 2012; Phattarakul *et al.*, 2012). The ineffectiveness of fertilizer applications to raise grain Fe or Zn concentrations in these examples may because Fe and Zn concentration in edible seeds is not only dependent on the presence and provision of soil Fe to sustain plant growth (Oliver and

Gregory, 2015) but it is also controlled by several processes including root-cell uptake, root-shoot transfer, leaf tissue ability to load such nutrients into the vascular phloem and nutrient delivery to developing seed and grains via phloem sap (reviewed in Welch, 1986).

Grain P may be related to seed weight. Similar to Moraghan and Grafton (2001) who reported a positive correlation between P concentration and seed weight, we observed that large seeded variety, Montcalm, had the greatest P concentration. Although tropical soils are often P deficient usually due to Fe and Al binding effects on phosphate compounds (Alley and Vanlauwe, 2009; Tiessen, 2008) as well as P loss through erosion attributed to heavy rains (Tiessen, 2008), we did not observe differences in grain P concentrations among locations. Additionally, contrary to Tiessen (2008) and Alley and Vanlauwe (2009), soil P at the tropical experimental location in Uganda was not the lowest among locations. Differences in grain P among locations were also not observed in our study possibly because phosphorus mobility/diffusion in the soil is quite low which limits P uptake by plants aside from other factors such as soil acidity and alkalinity (Vesterager *et al.*, 2006). Genotypic variation in P use efficiency and differences in root morphology among genotypes (Fageria and da Costa, 2000; Fageria *et al.* 2010; Mourice and Tryphone, 2012) may explain the observed differences in grain P concentrations among the varieties tested. Differences in P use efficiency and root morphology may render some genotypes more adaptable to soils with varying P. Increased root growth (Vesterager *et al.*, 2006), altered root morphological and architectural characteristics (Ortiz-Monasterio *et al.*, 2007; Lynch and Brown, 2001; Shen *et al.*, 2011) increase the soil volume exploited for P acquisition, and the exudation of organic acids by roots of some genotypes for mobilization of P that may be fixed in soils (Dechassa and Schenk, 2004) all increase P acquisition. The ability to acquire more P from the soil may enable some genotypes to

accumulate more P in their grains than their inefficient counterparts even when grown in the same location (White *et al.*, 2005). Although our findings about genotype grain P concentrations were contrary to Islam *et al.* (2002) who postulated that common bean seeds from the Middle American gene pool such as, Eclipse and Taurus often contained higher phosphorus compared to seeds from the Andean gene pool such as, Montcalm and MY06326.

Grain concentration of K across locations was not significantly different although soil K was quite low in the Uganda compared to other locations. Soil K is usually reportedly low in most tropical soils (Sangakkara *et al.*, 1996). Optimum soil K for bean production in loamy soils is 111-140 mg/kg and 81-120 mg/kg for sandy, organic soils (Laboski and Peters, 2012). It is not unexpected that differences in soil K did not result in differences in grain K, because unlike other nutrients, plants have the tendency for “luxury potassium consumption” and thus absorb potassium in amounts exceeding their requirements if readily available (Parnes, 2013) and therefore even in locations with low soil K, “plant K luxury consumption” may cover soil inadequacies. Other factors such as soil aeration, moisture and temperature, among others also affect K uptake by plants (Parnes, 2013). For instance, ridge till and compaction, which are common in mechanized farming, affect soil aeration and increase K deficiency. Cool temperatures reduce the release of K into the soil solution and decrease plant metabolic processes responsible for uptake soil K (Parnes, 2013). Varietal differences in grain K among Taurus, MY06326, Montcalm and Eclipse may be attributed to genotype differences in K-use and absorption efficiency. Fageria *et al.* (2001) reported differences in common bean genotypes in relation to soil K use and noted that some genotypes were more responsive to soil K and effective at soil K. Legume species demonstrate inter and intraspecific variation in K nutrition

have been reported to impact K use efficiency when grown in nutrient deficient versus fertilized soils (Caradus, 1991; Fageria *et al.*, 2001).

Soil Mg at all locations was within optimum ranges, which may be why we did not observe significant differences in grain Mg concentrations across locations. Laboski and Peters (2012) reported 51- 250 mg/kg and 51-100 mg/kg as optimum Mg for sandy and loamy-organic soils, respectively. Differences in grain Mg among the varieties tested may be attributable to differences in how Mg is accumulated in the embryo and seed coat (Ribeiro *et al.*, 2012). Grain Mn was not different across locations. This may be because all sites had optimum soil Mn for common beans. Laboski and Peters (2012) reported 11-20 mg/kg as the optimum Mn range for plant growth in sandy and loamy soil. Mn deficiency is rare but may occur in sandy soils with $\text{pH} \geq 8$ (Manjula, 2017), which is similar to the Colorado location. In contrast to Moraghan and Grafton (2001) who reported a negative correlation between seed weight and Mn concentration, we did not observe significant differences in Mn concentration in the varieties we tested which included large to small seeded varieties.

In this study, soils with low pH contained more Al though it should be noted that we do not have soil test results from Uganda. Some authors have reported differences in seed Al concentration of common bean genotypes exposed to different rates of Al (Hirpa *et al.*, 2015), but in this study, grain Al was not significantly different across location or amongst varieties. concentrations in our study were exhibited at location with low soil pH. The lack of difference in grain Al despite the differences in soil Al across the different locations may be attributable to differences in tolerance to aluminum stress among the varieties. (Zhang *et al.*, 2007). Plant Al tolerance mechanisms such as, exudation of organic acid anions and the complexation and detoxification of Al are broadly discussed by Panda and Matsumoto (2007).

There were no differences in grain Na across locations and among varieties. This may have occurred because there was no accumulation of soluble salts at all locations. Precipitation and snow are known to flush soluble salts out of plant root zone allowing plants to thrive during the growing season. Higher soil Na was observed in Colorado, likely because this site was mostly irrigated. These results indicate that concentration of Na in the soil did affect the amount of Na in the grain harvested in Colorado or the other locations. This is expected since it has been demonstrated that beans translocate very little Na to the shoot (Gauch and Wadleigh, 1942) (and other plant parts including leaves and grains) except when sodium concentration in the growing medium is very high (Lagerwerff and Holland, 1960). A membrane or series of membranes in bean roots regulates entry or leakage of absorbed sodium into the vascular stream (Jacoby, 1964; Pearson, 1967).

In this study, grain S was not significantly influenced by location, but it was different among varieties. It has been shown in other studies that white-seeded varieties of beans often contain lower amounts of tannins (Welch *et al.* 2000) and grain S. This is further discussed in Welch and Graham, 1999; Beninger *et al.*, 2003; and Madhujith and Shahidi, 2005. However, in contrast to Welch *et al.* (2000), the white seeded variety in this study, Taurus was higher in S than two of the colored varieties especially Montcalm and Eclipse, thus indicating that varietal differences in S concentration is likely attributed to other differences like root function.

A higher stand density at maturity observed in Iowa compared to other locations could be attributable to differences in site management (weeding and pest and disease control) or genetic differences in germination rates and/or the impact of edaphic factors such as soil pathogens on seedling emergence (De Ron *et al.*, 2016). Yields observed in this study were close to or within the range of yields reported by Kandel *et al.* (2018) and Balasubramanian *et al.* (2017). Higher

yields in Michigan and Colorado may be attributable to better common bean adaptation to those environments due to long-term plant breeding efforts unlike a location like Iowa where common bean is mostly grown as alternative crop (Delate, 2013). The highest yields and seed weights were obtained in Michigan where soil P was higher than the other locations. The location also had relatively high soil K. This may have contributed to the high common bean yield and high seed weight in Michigan. In some studies, common bean yield increased when P was applied or increased (Fageria *et al.*, 2010; Gidago *et al.*, 2011; Mourice and Tryphone, 2012; Kassa *et al.*, 2014; Turuko and Mohammed, 2014). Soil P improves nodulation by facilitating early root formation and increases active nodules which in turn may generally increase productivity, weight and yield of common bean (Chekanai *et al.*, 2018). However, contrary to Ribeiro *et al.* (2011) who reported higher grain yield in common bean genotypes with high seed phosphorous content, we did not observe any relationship between genotype seed P and their yield. Additionally, contrary to Mourice and Tryphone (2012), who attributed high yield to large seeded genotypes, the smallest sided genotype-Eclipse in our study had the highest yield in our study and therefore yield may not be due to seed size. The relationship between seed size and yield in common bean remains quite unclear (Lima *et al.*, 2005). Genotype seed weights in our study were within ranges of those reported by Kandel *et al.* (2018). According to their findings, Eclipse seeds weighed between 124-216 mg as did Eclipse seeds in our study. Similarly, Taurus and Montcalm seeds weighed 334 and 501 gms/seed in their findings and 330 and 450 mg/seed in our study whereas yellow beans (327 mg/seed) in our study weighed lower than Balasubramanian *et al.*, (2016) who reported yellow bean weight ranges of 416 to 426 mg/seed.

3.5 Conclusions

In this study, yield and seed weight in common bean were influenced by environment, but we could not confirm that there is a causal relationship between seed weight, seed nutritional concentration and yield. Mesoamerican origin varieties (Eclipse and Taurus) were mostly similar to Andean origin (MY06326 and Montcalm) for seed mineral concentrations specifically the micronutrients, Fe and Zn. Regardless of differences in soil properties and climate across locations, all four varieties tested had grain Fe concentrations above 50 mg/kg, which is the average concentration for grain Fe in common bean. Because the mineral concentrations of grain Fe and Zn in common bean does not reflect how much bioavailable they are to humans, further studies will be needed to fully understand the interaction among environment, varieties and nutrient bioavailability.

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3.8 Tables and figures

Table 1. Soil properties of experimental sites in Colorado, Iowa, Michigan and Uganda after fertilization and prior to planting in 2017 and 2018.

Location	pH	OM (%)	M3Fe (mg kg ⁻¹)	M3Zn (mg kg ⁻¹)	M3P (mg kg ⁻¹)	M3K (mg kg ⁻¹)	M3Na (mg kg ⁻¹)	M3Mg (mg kg ⁻¹)	M3Mn (mg kg ⁻¹)	TN (mg kg ⁻¹)	M3Al (mg kg ⁻¹)	SO42-S (mg kg ⁻¹)
Colorado	8.0 a†	3.0	34.6	6.6	54.4 b	324.5 a	47.6 a	602.0 a	61.8 ab	1100 b	47.0 c	30.5 a
Iowa	6.2 b	3.0	76.0	3.0	15.7 b	148.9 bc	25.6 b	308.3 b	23.5 b	1500 a	827.9 b	4.4 c
Michigan	6.0 b	1.7	145.6	5.0	214 a	190.9 b	15.2 b	125.8 c	53.0 b	1000 b	949.5 a	12.0 bc
Uganda	5.9 b	2.3	123.6	3.2	24.8 b	97.6 c	20.0 b	179.0 c	129.0 a	1500 a	N/A	15.8 b
<u>Significance</u>						<i>P</i> > F						
Location	***	NS	NS	NS	***	***	***	***	**	***	***	***

†Values followed by different letters within a column are significantly different at *P* = 0.05 by the least square means test.

***Significant at *P* = 0.001.

NS, Not Significant.

Table 2. Grain mineral composition of common beans varieties (Eclipse, Montcalm, MY06326 and Taurus) grown in four locations.

Parameter	CP	Fe	Zn	P	K	Mg	Mn	Al	Na	S
	(g kg ⁻¹)	(mg kg ⁻¹)								
<u>Location</u>										
Colorado	21	150	45	4253	13696	1871	23	147	61	2405
Iowa	22	111	55	5395	13837	2083	33	25	217	2221
Michigan	20	61	38	4901	14143	1773	19	174	56	1921
Uganda	27	65	30	4395	12998	1902	21	N/A	12	2342
<u>Variety</u>										
Eclipse	21 b†	141	40	4181 b	12648 b	2016 ab	26	196	86	2181 ab
Montcalm	24 a	83	52	5110 a	13432 ab	1658 c	22	107	86	2341 a
MY06326	22 ab	79	38	4750 ab	14473 a	1846 bc	24	82	93	2028 b
Taurus	23 ab	84	39	4899 ab	14125 a	2108 a	23	75	80	2338 a
<u>Significance</u>							<i>P</i> > <i>F</i>			
Location (L)	.08	NS								
Variety (V)	*	NS	NS	*	**	***	NS	NS	NS	**
L × V	NS	NS	*	NS						

† Values followed by different letters within a column are significantly different at *P* = 0.05 by the least square means test.

*Significant at *P* = 0.05.

**Significant at *P* = 0.01.

***Significant at *P* = 0.001; NS, not significant.

Table 3. Stand density at R9, yield and seed weight differences across environments and among genotypes.

Parameter	Stand density R9 (no. m⁻²)	Yield (kg ha⁻¹)	Seed weight (mg seed⁻¹)
<u>Location</u>			
Colorado	23 b†	3374 a	343 b
Iowa	37 a	1951 b	257 c
Michigan	21 b	4402 a	456 a
Uganda	17 b	802 b	233 c
<u>Variety</u>			
Eclipse	27 a	3066 a	183 c
Montcalm	18 b	1989 c	450 a
MY06326	25 a	2590 b	327 b
Taurus	27 a	2883 ab	330 b
<u>Significance</u>		<i>P</i> > F	
Location (L)	***	***	***
Variety (V)	***	***	***
L × V	NS	*	***

† Values followed by different letters within a column are significantly different at *P* = 0.05 by the least square means test.

*Significant at *P* = 0.05.

**Significant at *P* = 0.01.

***Significant at *P* = 0.001; NS, not significant.

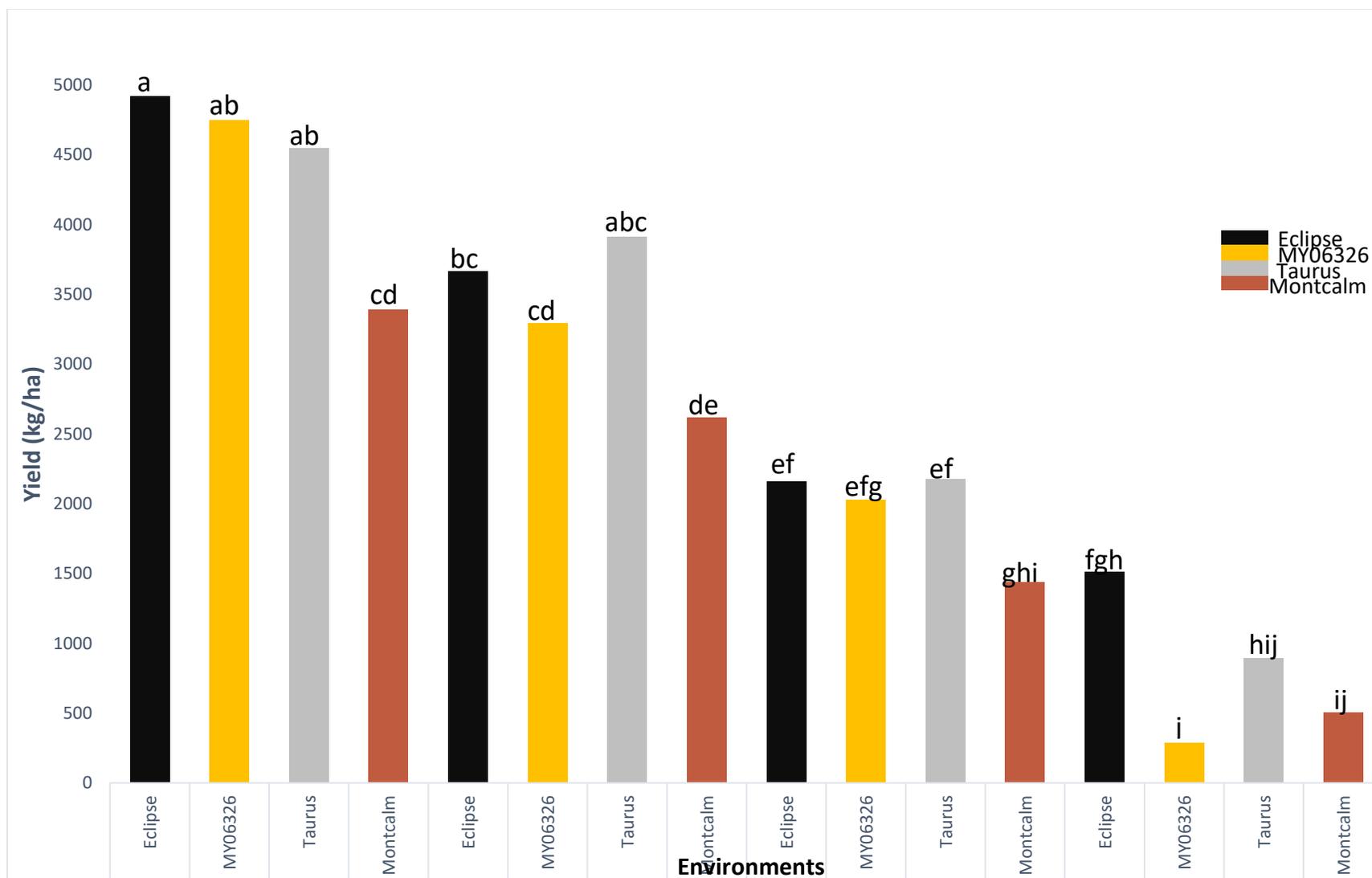


Figure 1. Common bean yield (kg/ha) of four genotypes (Eclipse, MY06326, Taurus, Montcalm) grown in Michigan, Colorado, Iowa and Uganda

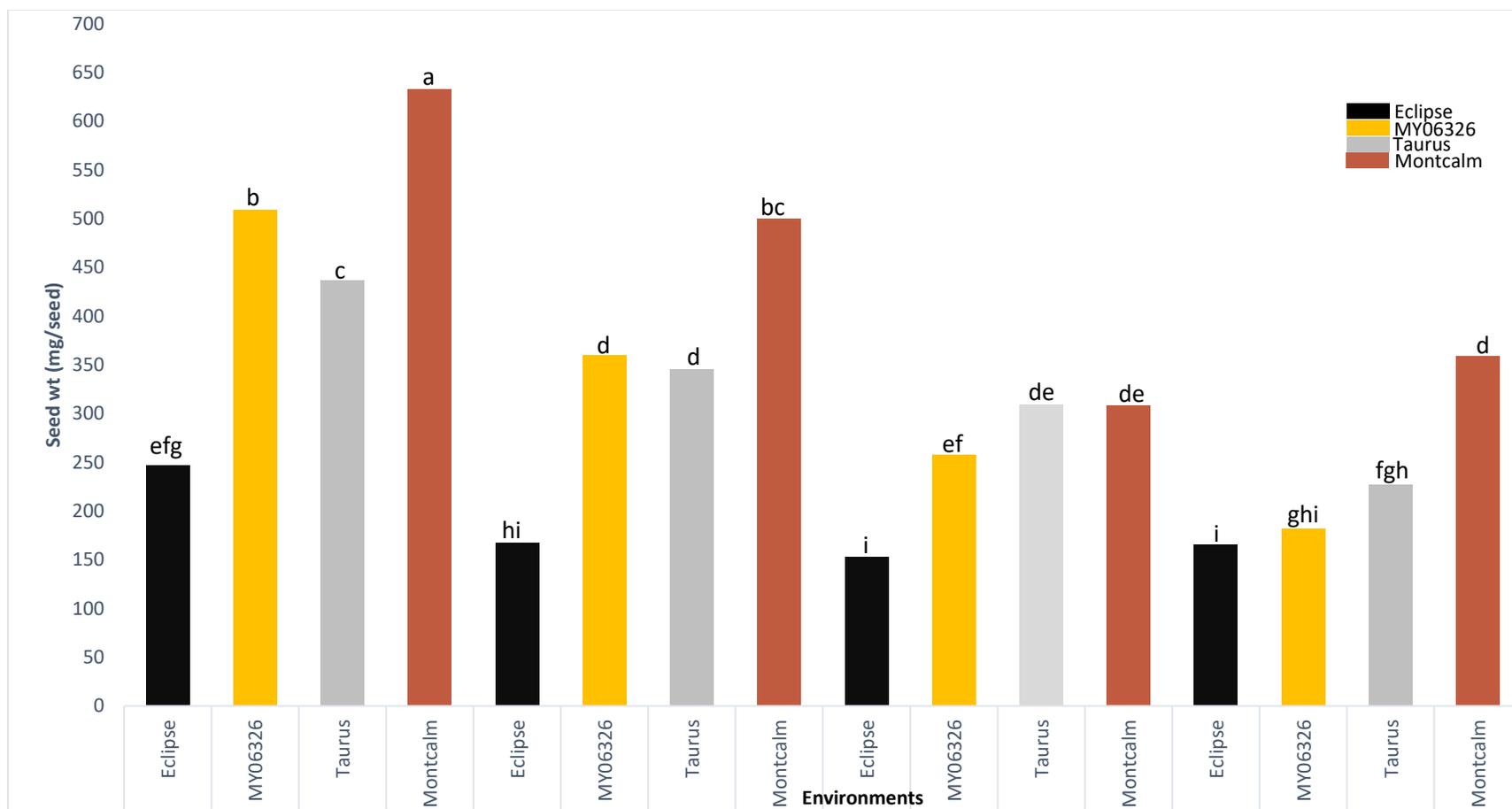


Figure 2. Common bean seed weight (mg/seed) of four genotypes (Eclipse, MY06326, Taurus, Montcalm) grown in Michigan, Colorado, Iowa and Uganda

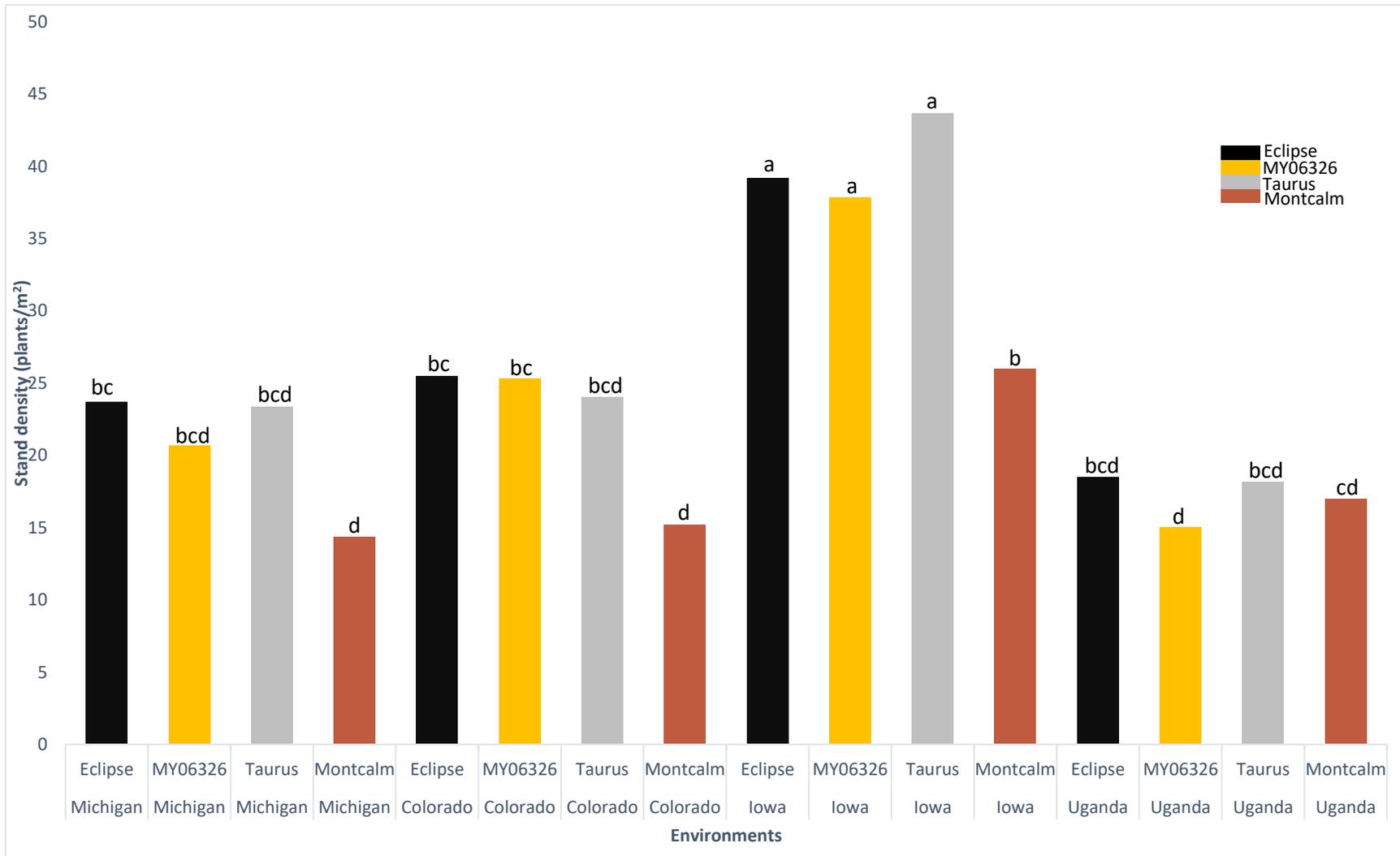


Figure 3. Common bean stand density at maturity (plants/m²) of four genotypes (Eclipse, MY06326, Taurus, Montcalm) grown in Michigan, Colorado, Iowa and Uganda

CHAPTER 4. GENERAL CONCLUSIONS

Agriculture is the main source of food for the growing population of the world. Therefore, in the past decades, emphasis was placed on the production paradigm that focused more on increasing yield to overcome starvation. However, it has been found that ensuring enough food supply is not the only need and that food systems should strive to meet nutritional needs of the population in order to overcome hidden hunger. Hidden hunger due to micro nutrient deficiencies such as iron, zinc and vitamin A not only negatively impacts individual's wellbeing but also has lifelong impacts on cognitive development, leads to stunting and even death in children and, affects overall productivity of the workforce and leads to the vicious cycle of poverty especially in developing countries. By improving the quality of food produced by agricultural food systems through nutrient sensitive agriculture, such gaps between agriculture, nutrition and health may be closed. Efforts such as limestone application to overcome soil acidity and an understanding of environment \times genotype interactions to better connect agronomic and genetic biofortification provide a pathway to produce more nutrient dense foods to ensure food and nutritional security. There is also need for additional studies on how to ensure bioavailability of nutrients from food to humans.