RESPONSE TO SOIL pH, VARIABILITY AND HERITABILITY OF COMMON BEAN (*Phaseolus vulguris* L) GENOTYPE FOR IRON AND ZINC CONCENTRATION

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EXTENDED ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is the most important food legume in the world. In Tanzania the common bean crop is cultivated for home consumption as well as for cash income. The objectives of this study were to determine the effect of soil pH on levels of iron and zinc in twenty five common bean genotypes and to determine heritability of iron and zinc using the progeny of crosses between low and high micronutrients (Fe and Zn). An incubation experiment was performed in screen house to adjust soil pH to levels of 5.5, 6.5 and 7.5 using hydrated lime $(Ca(OH)_2)$ for four weeks. The experimental design followed a randomized complete block design in a split plot arrangement with three replications per treatment. Two common bean seeds were sown at 5 cm in each pot and grow for three months before harvesting for analysis of iron and zinc in the laboratory. Concentration of iron and zinc in leaves and seeds were adversely affected by soil pH. The result demonstrated that soil pH can affect absorption of micronutrients directly or indirectly by affecting the nutrients availability of common beans genotypes. Hence assessing variability is fundamental to identify the most important traits in common bean improvement program. Four crosses (Nua 11 × Zawadi, Nua 11x Pesa, Nua 17 x Zawadi and Nua 17 x Pesa) using diverse parents with varied levels of grain iron and zinc concentrations were made to study heritability for iron and zinc. Broad sense heritability observed in Nua 11 x Zawadi was (56%) and Nua 11 x Pesa was (76%) for concentration of iron while Nua 17 x Zawadi was (57%) and Nua 17 x Pesa was (59%) for concentration of zinc. Narrow sense heritability observed in Nua 11 x Zawadi was (65%) and Nua 11 x Pesa was (71%) for concentration of iron while Nua 17 x Zawadi was (79%) and Nua 17 x Pesa was (63%) for concentration of iron and zinc. This study demonstrates, there were increase of concentration of iron and zinc in common bean genotypes at soil pH levels 6.5 and 5.5 that have potential for

improvement of micronutrients in common beans and heritability of zinc and iron are moderate to high indicating that these traits can be improved by breeders.

DECLARATION

I, Kinguye Masamaki Magomere do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution

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DEDICATION

I wish to dedicate this work to my mother Paulina Mtimba

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LIST OF ABBREVIATIONS

%	Percent
˚C	Degree of Celsius
CEC	Cation Exchange Capacity
CIAT	International Center for Tropical Agriculture
DNMRT	Duncan's New Multiple Range Test
FAO	Food and Agriculture Organization
GM	Geometric Mean
Ha	Hectare
K	Potassium
Kg	Kilogram
N	Nitrogen
NSPP	Number of Seed Per Pod
P	Phosphorus
PCA	Principal Components Analysis
pH	potential hydrogen concentration
ppm	Part Per Million
PPP	Pod number Per Plant
RCBD	Randomized Complete Block Design
S.E	Standard Error
SUA	Sokoine University of Agriculture
TARI	Tanzania Agricultural Research Institute
ANOVA	Analysis of Variance
B1	Progeny of backcross of F1 with parent 1
B2	Progeny of backcross of F1 with parent 2

Ca	Calcium
C.V.	Coefficient of Variation
EMS	Error Mean Sum of square
et al.	and other workers
F1	First filial generation
F2	Second filial generation
Fe	Iron
G	Gram
GA	Genetic Advance
GAM	Genetic Advance as a percentage of Mean
ha^{-1}	Per hectare
h^2b or $h^2(b)$	Heritability in broad sense
h^2n or $h^2(n)$	Heritability in narrow sense
K	Potassium

CHAPTER ONE

1.0 INTRODUCTION, JUSTIFICATION AND OBJECTIVES

1.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume in the world (Mekonnen *et al.,* 2014). It represents 50% of the grain legumes consumed worldwide (Talukder *et al*., 2010). In Tanzania, the common bean crop is cultivated for home consumption as well as for cash income (Hillocks *et al.* 2006). Common beans have high mineral concentration in grains (Tryphone and Nchimbi, 2010). It is a valuable source of protein, vitamins and nutrients such as calcium, potassium, phosphorus, iron, copper, zinc, and magnesium (Ribeiro *et al.,* 2008). The mineral contents in bean grains can vary largely depending on the varieties and on environmental factors, such as soil acidity (Braz, 2010). The availability of common bean cultivars with high nutritional qualities, along with important agronomic traits, would contribute to the sustainability of the common bean value chain (Ribeiro *et al.,* 2008).

Soil acidity may affect all stages of growth in common beans and specifically the legume-rhizobium symbiosis, from strain survival in soil and on the seed, to root-hair infection, nodule initiation and nitrogen fixation (Bambara and Ndakidemi, 2010). Higher concentrations and contents of hydrogen ion, aluminium and manganese in acidic soils are known to be the major causes of poor plant growth due to their toxicity effects to plants and microorganisms such as N fixing bacteria (Bambara and Ndakidemi, 2010). It is estimated that in tropical South America alone, 85% of the soils are acidic, and approximately 850 million ha of such land area is underutilized (Fageria and Baligar 2001). Soil pH levels in Tanzania range from 4 to 10 (Mlingano Agricultural Research Institute Report, 2006). Both extremes pose some limitations to crop production. Areas

such as Magadu (Ultisol), Mlingano (Oxisol), Nkundi (Ultisol) and Sasanda (Andisol) are of acidic soils. They have poor inherent fertility and are acidic (Semoka *et al.,* 2005). Soil acidity is ameliorated by applying lime or other acid-neutralizing materials such sugar factory lime, basic slag or wood ash to increases calcium   concentrations and ionic strength in the soil solution, thus improving in soil structure and hydraulic conductivity (Bambara and Ndakidemi, 2010).

Genetic differences have been reported for seed Zinc and Iron concentrations among genotypes and landraces (Moraghan and Grafton, 2002). To begin a breeding program aiming at obtaining cultivars with higher nutritional quality, it is important to have information on genetic control of the traits involved. Breeding crop plants for higher micronutrient concentration, an approach termed bio fortification, has become an active goal of plant breeding programs in the developing world at both the international and national agricultural research centres (Bouis *et al*., 2011). To obtain a cultivar with all these properties, the best strategy is to assess the nutritional composition of the elite lines of breeding programs, which already have favourable agronomic traits (Martins *et al*., 2016). However, one of the current focus of common bean breeding programs is to increase the iron and zinc content; therefore, more genetic variability studies are required to estimate genetic and phenotypic parameters and the genetic associations between traits (Martins *et al*., 2016).

Zinc and Iron minerals are crucial to human well-being and an adequate supply of these nutrients helps to prevent iron deficiency, anaemia and zinc deficiency, the two prevalent health concerns of the developing world (Blair *et al.* 2009). Iron is essential for the formation of haemoglobin and Fe deficiency causes anaemia. Zn is necessary for the sexual maturation, fertility and reproduction. Zn deficiency causes growth retardation

and delayed sexual maturation (Ribeiro *et al*., 2008). Iron and zinc deficiencies affect about 40% and 33%, respectively, of the people in the world (The World Bank, 2007). The rates of iron deficiencies among pre-school aged children and pregnant women in Tanzania can reach up to 72% and 58%, respectively (Ministry of Health and Social Welfare (2008). The prevalence of zinc deficiency in Tanzania has been found to be as high as 70% among children aged 6 months to 5 years (Veenemans *et al*., 2011).

1.2 Justification

The common bean (*Phaseolus vulgaris* L.) is the most important food legume in the world (Mekonnen *et al.,* 2014). In Tanzania, the common bean crop is cultivated for home consumption as well as for cash income (Hillocks *et al.* 2006). The national average yield of common beans which ranges from 0.72 to 1.10 tones/ha, is far below potential yields of 1.5 to 3 tones/ ha recommended by agricultural research using improved varieties (Ronner and Giller, 2013). Among the main reason for the low yield obtained by most smallholder farmers is soil acidity (Wortmann *et al.,* 1998). A build-up of soil acidity is a threat to agriculture productivity, especially if strong acidifying fertilizers are used. It has been observed that application of fertilizer has already contributed to the acidification of some soil in Tanzanis (Breman *et al.,* 2008). Long-term chemical fertilization severely affected the biological processes of the soil and influence soil acidification. Under increasing population pressure, acid soils are now rapidly being brought into cultivation in many parts of Africa, including Tanzania. Some of the major bean production areas such as the Usambara and Uluguru Hills have acid soils with pH <5.5, which limit crop productivity (Wortmann *et al.,* 1998). Plants grown in acid soils can experience a variety of stresses including aluminium (Al), hydrogen (H), and/or manganese (Mn) toxicity, as well as deficiencies of calcium (Ca) and magnesium (Mg) (Brady and Weil, 2002). Acidic soil affects macronutrient

availability because the H^{+} ions take up space on the negative charges on the soil surface displacing macronutrients such as nitrogen, phosphorus, potassium, sulphur, calcium, and also micronutrients such as manganese and molybdenum may be unavailable, or only available in insufficient quantities. This pH limits the uptake of macronutrient by making them unavailable to the plant.

In breeding, heritability values are helpful in predicting the expected progress to be achieved through selection process (Zulfahmi *et al*., 2016). The genetic variability of common bean genotypes exists for most minerals, including iron and zinc (Blair *et al.* 2009). The International centre for Tropical Agriculture (CIAT) has developed some common bean lines in Uganda which are of high iron and zinc contents. These genotypes have been introduced in Tanzania. The genetic variability for iron and zinc and the heritability levels of these genotypes are not known when grown in Tanzania. Information about the genetic variability for iron and zinc of bean lines helps in decision making by identifying genes that can add value to genetic resources for breeding purposes. The knowledge of genetic diversity patterns increases the efficiency for conservation, utilization and genetic improvement of common beans (Talukder *et al*., 2010). Knowledge of genetic diversity in a crop species is fundamental to its improvement (Talukder *et al*., 2010). The success of any crop improvement program depends not only on the amount of genetic variation present in the genotypes but also on how they will be inherited by the progeny. There was a need therefore to conduct an experiment in order to determine the genetic variability and heritability levels for iron and zinc contents of these genotypes.

1.3 Objectives

1.3.1 Overall objectives

To assess performance of the levels of zinc and iron concentration in common bean genotypes

1.3.2 Specific objectives

The specific objectives of this study were;

i. To determine the influence of soil pH on levels of iron and zinc in twenty five common bean genotypes

ii. To determine heritability effects of iron and Zinc content in the common bean genotypes

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of zinc and iron in the body

Iron and zinc are the two most abundant trace minerals in the human body, with 3 to 4 g of iron and 1.5 to 2.5 g of zinc present in the average adult (Wood and Ronnenberg 2006). Zinc is essential for adequate growth and for resistance to gastroenteric and respiratory infections, especially in children whereas iron is an important component of hemoglobin, the substance in red blood cells that carries oxygen from the lungs to transport it throughout the body. Hemoglobin represents about two-thirds of the body's iron. Iron has other important functions, too. It is also necessary for maintaining healthy cells, skin, hair, and nails. Zinc is an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement (Hambidge, 2000). Zinc is involved in numerous aspects of cellular metabolism (Hambidge, 2000). It is required for the catalytic activity of approximately 100 enzymes and plays a role in immunity function, protein synthesis wound healing, DNA synthesis, and cell division. Zinc also supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste and smell. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Hambidge, 2000).

2.2 Genetic variation of iron and zinc concentration in beans

Beans exhibit sufficient genetic variability in iron concentration, which is the basic requirement for bio fortification. Several studies have reported the presence of genetic variation, indicating the possibility of selecting lines with higher content (Araújo *et al.,* 2003). The recent research on common bean has reported genetic diversity in

concentration of Fe and Zn among adopted genotypes in Tanzania (Tryphone and Nchimbi, 2010). Studies on mineral concentration in the grain of common bean have confirmed that broad genetic variability exists for most minerals, including iron and zinc (Tryphone and Nchimbi, 2010). The initial goal of the Harvest Plus bean bio fortification initiative was to use selective plant breeding strategies to produce bean varieties with at least 80% more iron than found in conventional beans (Hurrell *et al*., 2015). Zn and Fe concentrations in different bean varieties are highly associated (Guzman-Maldonado *et al.* 2003). Therefore, selecting for high-iron bean varieties will also tend to select for high-zinc varieties. Evaluation of the bean core collection revealed a range of 21 to 54 mg/kg in zinc content, with an average value of 35 mg/kg. According to the literature, the iron content in bean seeds ranges from 40.0 mg/ kg to 84.0 mg /kg (Blair *et al.* 2009). According to Beebe *et al*., (2000) there are sufficient genetic variability exists to improve iron content by about 80% and zinc content by about 50. Therefore, genetic variability of common beans genotypes exists for most minerals, including iron and zinc. Selecting for high-iron bean varieties will also tend to select for high-zinc varieties.

2.3 Iron and Zinc concentration

Common bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption worldwide and an important source of microelements, especially Fe and Zn. Bean bio fortification breeding programs develop new varieties with high levels of Fe and Zn targeted for countries with human micronutrient deficiencies. Bio fortification efforts have relied on phenotypic selection of raw seed mineral concentrations in advanced generations. Bean breeding desires stable genotypes with good agronomic performance across all environmental conditions. A study of recombinant inbreed navy bean population showed that the Zn content varied from 32.3 to 17.5 mg /kg and the Fe

content from 86.9 to 63.5 mg/ kg (Islam *et al*., 2002). In another report, Achom (2013) showed that, mean seed Fe content present in the accession EC500745 (58.23 mg/kg) was the higher among all the accessions followed by IC101264 (51.43 mg/kg) and IC319827 (51.33 mg/kg). Two of the popular released varieties Jwala (dark brown) and Arka Komal (light brown) also showed a fair amount of seed Fe content with mean concentration of 49.90 mg/kg and 44.76 mg/kg (Achom, 2013). Similarly, Arka Anoop (white seed coat) and all the normal brown seed coat colour accessions (EC115962, EC304657, IC319825 and EC512812), showed mean seed Fe content ranging from 41.6 mg/kg to 44.6 mg/kg (Achom, 2013). The other accessions with cream seed coat (IC262749 and EC541908) and light brown seed coat (EC540797, EC500641, IC342273) showed mean seed Fe content ranging from 33.33 to 39.33 mg/kg (Achom, 2013).

The grand mean seed Zn content was found to be 22.19 mg/kg and was significantly ($p=0.05$) higher in released variety Jwala (29.13 mg/kg) followed by the black seed coat accessions EC500745 (28.83 mg/kg), IC101264 (27.50 mg/kg) and dark brown EC530819 (26.10 mg/kg) (Achom, 2013). The seed Zn content was found to be fairly higher in all the black seed coat accessions (24.60 mg/kg to 28.83 mg/kg) followed by dark brown seed coat accession (11.47 mg/kg to 19.97 mg/kg) (Achom, 2013). Golam *et al.* (2011) showed 29 US grown CIAT breeding genotypes with variability of Fe content as 80.9 - 112.9 mg /Kg and Zn content of 30.90- 64.60 mg /Kg. Thus show that, selecting for a higher Fe level in bean seeds will also tend to select for increased Zn levels in the seeds.

2.4 Soil pH

Soil pH is a measure of acidity (hydrogen ion concentration) in the soil. The pH values range from 0 to 14; 0 is most acidic, 7 is neutral, and 14 is most basic. Soil pH values in Tanzania range from 4 to 10. Both extremes pose some limitations to crop production (Mlingano Agricultural Research Institute, 2006). Extremes of soil pH release substances such as H^+ and Al^{3+} from soils in amounts that can be toxic to plants. Acid soils may dissolve toxic amounts of metals (such as aluminum and manganese). Alkaline soils may accumulate salts and sodium carbonates in toxic concentrations that can alter soil structure, thereby making it difficult for roots to grow. Stunted root systems have trouble taking up adequate water and nutrients. Toxic metals in acid soils, subsoil nutrient depletion, and subsoil clay pans also stunt root growth. (Mlingano Agricultural Research Institute, 2006). Slightly acidic soils (pH of 6.5) are considered most favourable for overall nutrient uptake. Such soils are also optimal for nitrogen-fixing legumes and nitrogen-fixing soil bacteria. Some plants are adapted to acidic or basic soils due to natural selection of species in these conditions. Potatoes grow well in soils with pH <5.5 while cotton, garden pea, and many grasses grow well in alkaline soil (>7.5). (Mlingano Agricultural Research Institute, 2006). Soil pH also affects the soil in other ways. For example, soil microbe activity; particularly nitrogen-fixing bacteria may be reduced in acid soil (Mlingano agricultural research institute, 2006).Soil acidity is managed by adding lime (carbonates of calcium and magnesium) (Mlingano agricultural research institute, 2006).

2.5 Effect of soil pH on plant growth

Plants grown in acid soils can experience a variety of stresses including aluminium (Al), hydrogen (H), manganese (Mn) toxicity and/or iron (Fe) toxicity, as well as deficiencies of calcium (Ca) and magnesium (Mg). Aluminium toxicity is the most widespread problem in acid soils (Brady and Weil, 2002). Bacteria that change and release nitrogen from organic matter and some fertilizers operate best in the pH range of 5.5 to 7.0 making this the optimum pH range. Plant nutrients leach from the soil much faster at pH values below 5.5 than from soils within the 5.5 to 7.0 range. In some mineral soils, aluminum can be dissolved at pH levels below 5.0 becoming toxic to plant growth. Soil pH may also affect the availability of plant nutrients (Miller, 2013). Nutrients are most available to plants in the optimum 5.5 to 7.0 range. Soil pH can also affect the structure of the soil, especially in clay soils. In the optimum range clay soils are granular and easy to work with. However, if the soil is either extremely acid or alkaline, clay soils tend to become sticky and hard to cultivate (Kidd and Proctor, 2001). In the root, the initial effect of Al^{3+} is the inhibition of the expansion of the cells of the rhizodermis, leading to their rupture; thereafter it is known to interfere with many physiological processes including the uptake and transport of calcium and other essential nutrients, cell division, cell wall formation, and enzyme activity. Proton (H^+ ion) stress can also limit plant growth. A high proton activity in the external growth medium overcomes the capacity of the cell to maintain the cytoplasmic pH and growth shuts down (Kidd and Proctor, 2001). In soils with a high content of manganese-containing minerals, Mn toxicity can become a problem at pH 5.6 and lower.

2.6 Deficiencies of micronutrients in human body

Deficiencies of micronutrients often coexist and have independent as well as interacting (Pedraza and Rocha 2016). Iron deficiency is one of the most common nutrient

deficiencies in the world, affecting more than 25% of people worldwide. Iron is an essential mineral critical for motor and cognitive development (Cantez *et al.,* 2015). Children and pregnant women are especially vulnerable to the consequences of iron deficiency (Prevel *et al.,* 2016). Low hemoglobin concentration (anemia) affects 43% of children 5 years of age and 38% of pregnant women globally. Anemia during pregnancy increases the risk of maternal and perinatal mortality and low birth weight (Prevel *et al.,* 2016).

Global population is at risk for zinc deficiency due to dietary inadequacy, though up to 30% of people are at risk in some regions of the world. Zinc deficiency causes alterations in immune response that probably contribute to increased susceptibility to infections, such as those that cause diarrhea, especially in children (Cantez *et al.,* 2015).

2.7 The major characteristics of landraces

A landrace is a local variety of a domesticated plant species which has developed large adaptation to the natural and cultural environment in which it lives. It differs from a cultivar which has been selectively bred to conform to a particular standard of characteristics. Landrace populations are often variable in appearance, but they can be identified by their appearance and have a certain genetic similarity (Asfaw, 2000). Landraces have continuity with improved varieties. According to Maxted (2006) the major characteristics of landraces include (i) high levels of genetic diversity within populations, characterized by a limited range of variation between individuals, with distinctive traits that make the landrace identifiable; (ii) adaptation to soil and climate conditions typical of the region, combined with resistance to common pests; (iii) edible parts that are valued by local people, normally shaping and being shaped by the local cuisine; and (iv) modest but stable yield, conferring food security to the local community

under normal environmental variation. The relatively high level of genetic variation of landraces is one of the advantages that these can have over improved varieties. Although yields may not be high, the stability of landraces in face of adverse conditions is typically high. As a result, new pests or diseases may affect some, but not all the individuals in the population (Negri, 2003).

Primary landrace: a crop that has developed its unique characteristics through repeated in situ grower selection and that has never been subjected to formal plant breeding. These can be divided into autochthonous (a crop that is grown in the original location where it developed its unique characteristics through grower selection (Gao, 2003); its genetic and socio-economic characteristics are associated specifically with this location) and allochthonous (an introduced crop that is locally adapted but that has developed its unique characteristics through grower selection in another region) (Gao, 2003).

Secondary landrace: a crop that has been developed in the formal plant breeding sector but which is now maintained through repeated in situ grower selection and seed saving, which is likely to be genetically distinct from the original bred material (Brush,1995).

2.8 Mechanism of iron and zinc inheritance in common bean

Breeders use the term 'heritability' to express that portion of a quantitative trait that is under genetic control. Breeding can increase the bioavailability of mineral concentrations in the edible portions of plants (White and Broadley, 2005) thereby providing enhanced nutritional quality, without additional food costs. In the wide sense, heritability is important to find out the effect of additive genes that can be transferred to their progeny (Bello, 2012). Iron and zinc concentration are characteristics of quantitative inheritance in both Mesoamerican and Andean common bean. Most studies

have indicated multigenic inheritance of micronutrient traits. The proportionate quantity of Fe and Zn present in seeds is a complex quantitative trait and governed by many major genes(QTLs) involving between 7 and 11 loci (White and Broadley, 2005). Meanwhile, separate QTL for each mineral alone were identified on B4, B6, and B8 for iron and on B3, B6, and B9 for zinc (Blair *et al.,* 2009). Other QTL were identified on linkage groups B3, B6, B7, and B9 for zinc and B4, B6, B7, and B8 for iron (Guzman-Maldonado *et al.,* 2003) Other studies using lines of different genetic origins have also shown evidence of a relationship between iron concentration and zinc concentration (Blair *et al.,* 2009); however, additional studies found no evidence of a relationship between iron concentration and zinc concentration (Blair *et al.,* 2009). Some studies reported positive intermediate correlations between iron concentration and zinc concentration (Cichy *et al.,* 2005), but these were phenotypic and not genetic correlations. Most studies indicated that the inheritance of iron concentration and zinc concentration is quantitative (Blair *et al.,* 2009) although another study identified monogenic inheritance for zinc concentration (Cichy *et al.,* 2005).

2.9 Methods of estimates of heritability

Heritability is the most important genetic parameter on which different breeding strategy depends. The knowledge of heritability is a prerequisite for the formulation of breeding plans on scientific lines. There is need to know the heritability of different characters which are used for selection of parents for future breeding programme (Nyquist *et al.,* 2003). Here different methods of estimation of heritability such as Regression method, Half-sib correlation method, Full-sib correlation method and method using Isogenic line (Warner, 1952).

2.10 Advantages of Common Bean for Mineral Bio fortification

Bio fortification is the process of improving staple crops for mineral or vitamin content as a way to address malnutrition in developing countries. Biofortification can be achieved through plant breeding and offers a cost-effective and sustainable approach to fighting micronutrient malnutrition. Nutritional quality in common beans has been found to be higher than in cereals, with large amounts of minerals and vitamins accumulated in the seeds (Sperotto and Ricachenevsky, 2017). Bio fortification a of minerals also target to supply adequate of iron and zinc which help to prevent iron deficiency anaemia and zinc deficiency, to prevalent health concerns of the developing world (Sperotto and Ricachenevsky, 2017).

The main goal of mineral bio fortification have been to increase the concentration of iron or zinc in certain major cereals and legumes. Bio fortified foods are more easily integrated into the livelihoods and diets of the poor.   Bio fortification is an agricultural intervention targeted to rural areas where more than seventy-five percent of the poor in developing countries live, and where access to supplements, fortified foods and other urban-based interventions are limited (Blair, 2013). Furthermore, once developed, bio fortified crops can be adapted to similar agro ecological zones, or improved, at relatively low additional cost. Bio fortification may well prove to be a sustainable long-term approach for providing millions of poor people in developing countries with at least a part of their micronutrient requirements (Blair, 2013).

 A component of a strategy that includes dietary diversification, supplementation and commercial fortification, significant progress could be made in reducing hidden hunger globally. Unlike the continual financial outlays required for supplementation and commercial fortification programs, an upfront investment in plant breeding yields

micronutrient-rich bio fortified planting material for farmers to grow at virtually zero marginal cost (Broughton *et al.,* 2003). Once developed, nutritionally improved crops can be evaluated and adapted to new environments and geographies, multiplying the benefits of the initial investment. Bio fortified crops are also a feasible means of reaching rural populations who may have limited access to diverse diets or other micronutrient interventions (Blair, 2013). Bio fortification puts a solution in the hands of farmers, combining the micronutrient trait with other agronomic and consumption traits that farmers prefer (Broughton *et al.,* 2003).

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CHAPTER THREE

3.0 To determine the effect of soil pH on levels of iron and zinc in twenty common bean genotypes

Abstract

A study was carried out to determine the effect of soil pH on levels of iron and zinc in twenty five common bean genotypes. Plastics cups trial was carried out in the screen house to determine the actual amount of quick lime Ca (OH) $_2$ is required to reach a targeted soil pH level. A soil incubation experiment was performed before conducting the pot culture experiment to attain the standard curve. The experimental design followed a randomized complete block design in a split plot arrangement with 3 replications per treatment. In each pot, 4 Kg soil from Magadu was amended with Ca (OH) $_2$ at four incremental rates (0, 0.4, 3.5, and 10 g) to obtain the target soil pH levels. After mixing the soil with Ca (OH) $_2$ the samples were incubated at 25 $^{\circ}$C. The mixtures were pulverized every 5 days to mix the Ca (OH) $_2$ with the soil. The soils were then moistened with distilled water every day to maintain moisture at 60% of field capacity and placed under a polyethylene cover containing a hole. After 4 weeks, soil pH was measured and common beans lines were surface sterilized with 10% hydrogen peroxides (H_2O_2) for 10 min, washed with running tap water, distilled water, and then two seeds were sown at 5 cm depth in each pot. Analysis of Variance (ANOVA) was performed for all data collected include days to 50 % flowering, days to 85 % maturity, number of pods per plant, number of seed per pods, leaf iron concentration, seed iron concentration, leaf zinc concentration, and seed zinc concentrations were analysed by using the GenStat statistical package 15th edition at p $\leq$ 0.05. The result demonstrated that soil pH can affect absorption of micronutrients directly or indirectly by affecting the nutrients

availability of common beans genotypes. At low soil pH of 5.3 ability to uptake the zinc and iron in both leaves and seeds was low compared with optimal soil pH of 6.5.

3.1 Introduction

Soil pH is a measure of the concentration of hydrogen ions held to soil particles and organic matter (Miller, 2013). Soil pH is very important because it directly affects soil nutrient availability (Fageria, 2002). Aluminum toxicity and hydrogen toxicity are the primary limitation to agricultural production on acid soils. Aluminum toxicity is recognized as a major constraint to crop productivity in acidic soils. It limits plant growth and development, and the subsequent performance of economically important crops in various parts of the world (Legesse *et al.,* 2016). A critical effect of excess soluble Al is the slowing or stopping of root growth (McCauley *et al.,* 2017). Aluminum inhibits absorption of nutrients by plant roots, especially Ca, Mg, Fe and Mo. It also limits availability of P in the soil in addition to promoting Mn and H^+ toxicity (Legesse *et al.,* 2016). Plant roots can only absorb nutrients after they have been transformed into certain ionic forms. Only within certain pH ranges can sufficient amounts of these nutrients be transformed into these ionic forms (McCauley *et al.,* 2017).

Extreme pH values decrease the availability of most nutrients. As pH rises, micronutrients precipitate as insoluble minerals, which cannot be taken up by plants. These nutrients are not lost, but rather precipitated into solid minerals, and become unavailable to plant roots. Low pH reduces the availability of the macro- and secondary nutrients, while high pH reduces the availability of most micronutrients. Microbial activity may also be reduced or changed. If a micronutrient deficiency is observed in an acidic soil, it is probably related to lower concentrations and the leached nature of the soil. Adjusting soil pH to recommended levels can increase the availability of important

nutrients (McCauley *et al.,* 2017). Plants usually grow well at pH values above 5.5. Soil pH of 6.5 is usually considered optimum for nutrient availability (McCauley *et al,* 2017). The toxic effects of acids in the soil can be overcome through appropriate soil amendment measures such as application of lime (Legesse *et al.,* 2016). However, to be effective, the application of lime must be repeated over seasons. In addition, most smallholder farmers growing the crop in the tropics and subtropics cannot afford to apply lime which is costly and labor-intensive (Legesse *et al.,* 2016).

Genetic diversity is also important because it can strongly influence the long-term viability of plant populations, and their ability to adapt to changing climatic and environmental conditions (Manjarrez-Sandoval *et al.* 1997). It is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic (environmental) conditions, and enables change in the genetic composition to cope with changes in the environment. It provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits (yield potential and large seed, etc.) and breeders preferred traits (pest and disease resistance and photosensitivity, etc.). In agriculture, natural genetic variability has been exploited within crop species to meet subsistence food requirement, and now it is being focused to surplus food for growing populations (Moraghan and Grafton, 2002). Therefore the objective of this study is to determine the effect of soil pH on twenty five common bean genotypes.

3.2 Materials and Methods

3.2.1 Location

The screen house experiment was carried out at Sokoine University of Agriculture (SUA) which is found in the Morogoro region, Tanzania (Figure 1). SUA is located at 6°

45' S latitude and 37° 40' E longitudes at an altitude of 547 masl. The climate of the area is characterized by a bimodal rainfall pattern, with short season rains occurring in November/December for some years and long season rains in February to May. Rainfall varies from 1200 mm in the highland plateaus to 600 m in lowlands. The average annual temperature in the region's highlands is 18°C but reaches 30°C in the lowland.

Figure 1: A map showing study area

3.2.2 Plant materials

Genotypes used for the determination of the effect of soil pH on levels of iron and zinc were twenty five namely; NUA 9, NUA 11, NUA 13, NUA 15, NUA 16, NUA 17, NUA 18, NUA 19, NUA 23, NUA 30, NUA 31, NUA 39, NUA 40, NUA 48, NUA 57, NUA 59, NUA 64, NUA 66, NUA 67, NUA 79 from CIAT, Uganda and ZAWADI, MSHINDI, ROJO, PESA, SUA 90 from SUA bean programme were used as check materials. The Fe and Zn concentration of these genotypes are given in (Table 1).

Table 1: Iron and Zinc concentration of 25 bean genotypes used in this study

Genotypes	Source of seeds	Concentration of Fe in seed (mg/Kg)	Concentration of Zn in seed (mg/Kg)
NUA 9	CIAT Uganda	50.7	29.5
NUA 11	CIAT Uganda	49.3	27.3
NUA 13	CIAT Uganda	49.2	28.3
NUA 15	CIAT Uganda	56.9	33.4
NUA 16	CIAT Uganda	56.1	32.3
NUA 17	CIAT Uganda	53.9	31.7
NUA 18	CIAT Uganda	59.4	31.6
NUA 19	CIAT Uganda	59.8	33.0
NUA 23	CIAT Uganda	70.0	37.0
NUA 30	CIAT Uganda	50.9	30.1
NUA 31	CIAT Uganda	58.4	31.8
NUA 39	CIAT Uganda	50.4	30.7
NUA 40	CIAT Uganda	62.7	32.2
NUA 48	CIAT Uganda	54.5	25.5
NUA 57	CIAT Uganda	71.9	31.6
NUA 59	CIAT Uganda	69.7	29.8
NUA 64	CIAT Uganda	55.7	28.4
NUA 66	CIAT Uganda	47.2	33.2
NUA 67	CIAT Uganda	67.7	34.5
NUA 79	CIAT Uganda	72.2	37.4
ZAWADI	Morogoro	70.0	22.0
MSHINDI	Morogoro	59.6	27.7
ROJO	Morogoro	75.0	39.0
PESA	Morogoro	50.0	27.0
SUA 90	Morogoro	36.6	23.9

3.3 Methods

3.3.1 Soil sampling and analysis

Bulk soil samples were taken at a depth of 0 - 20 cm at Magadu area. Composite soil samples constituted ten sub-samples randomly collected from an area covering 1.0 ha. Sub-samples were thoroughly mixed, air dried and ground to pass through a 2.0 mm mesh. All samples were bulked and composited and a kilogram composite sample was taken for analysing physical and chemical properties of the soil. All soil samples were analysed for soil pH, cation exchange capacity (CEC), exchangeable bases (Ca, K, Mg and Na), micronutrients (Fe and Zn), organic carbon (OC) and available phosphorus. Soil textural classes were determined using the USDA textural class triangle (USDA, 1975).

Soil pH was determined in water at a soil: water ratio of 1:2.5 suspension using pH meter (MacLean, 1982). Available P was extracted using the Bray 1 method (Bray and Kurtz, 1945) and quantified was developed by the ascorbic acid colourimetric method of Murphy and Riley (1962). Exchangeable calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry whereas K and Na were extracted using ammonium acetate and analysed by flame emission spectrophotometry. Cation exchange capacity (CEC) was determined by ammonium acetate saturation method at pH 7.0 (Chapman, 1973). Organic carbon was determined by the Walkley-Black wet combustion method (Tan, 1996) and total N was determined using the Kjeldahl method. The DTPA extractable Fe and Zn were determined by atomic absorption spectrophotometry (Lindsay and Norvell, 1978).

3.3.2 Incubation experiment to obtain the target soil pH

Plastics cups trial was carried out in the screen house to determine the actual amount of quick lime Ca (OH) $_2$ required to reach a targeted soil pH level. A soil incubation experiment was performed before conducting the pot culture experiment to attain the standard curve. The composite soil sample was air dried ground and passed with 2 mm sieve and then 0.5 kg soil was placed in 6 plastic cups replicated 3 times and mixed with six different treatments in a greenhouse. Six equivalent rates of quick lime 0, 2.5, 5, 10, 15 and 20 tons per hectare in terms of Ca (OH) $_2$ were separately applied to obtain a standard curve. The soils were then moistened with distilled water, to 60 % field capacity and placed under a polyethylene cover containing a hole and in each five days the soil were pulverized. After 4 weeks, soil pH was measured. The relationships between soil pH and the amounts of Ca (OH) $_2$ were established in a standard curve and amount Ca (OH) $_2$ required for pot culture experiment was calibrated.

Table 2: Rates of lime equivalent to calcium hydroxides

Amount of lime applied in plastic cup g/Kg			pH obtained after 4 weeks of incubating the soil sample			Average pH in 3 reps
Rep 1	Rep 2	Rep 3	pH Rep1	pH Rep 2	pH Rep 3	pH
0	0	0	5.31	5.28	5.37	5.3
0.625	0.625	0.625	6.03	6.17	6.25	6.2
1.25	1.25	1.25	7.04	7.09	7.0	7.0
2.5	2.5	2.5	7.51	7.49	7.51	7.5
3.75	3.37	3.37	7.73	7.71	7.77	7.7
5	5	5	7.89	8.01	7.88	7.9

The formula used to calculate amount of lime applied in plastic cup g/kg is

$$\text{Lime requirement } \frac{kg\ Ca(OH)_2}{hectare} = \frac{mg\ Ca(OH)_2}{g\ soil} \times \frac{10^3\ g\ soil}{kg\ soil} \times \frac{2 \times 10^6\ kg\ soil}{hectare} \times \frac{1\ kg\ Ca(OH)_2}{10^6\ mg\ Ca(OH}$$

Lime requirement is usually expresses in kg or metric tons per hectare. There is approximately 2×10^6 kg of soil in the plow layer of one hectare.

3.3.3 Standard calibration curve (Calcium hydroxides (Ca (OH) $_2$) vs pH)

Calcium hydroxide (Ca (OH) $_2$) is divalent, yielding two moles of hydroxide ions which were responsible for increasing the pH of the soil. Calcium hydroxides (Ca (OH)$_2$ neutralized the acid, by turning the H^+ into water molecules; therefore increases the soil pH in the soil. Soil pH was determined in 1:2.5 (w/v) soils to water (H_2O) suspension ratio using a glass electrode attached to a digital pH meter and curve showing relationship between amounts of Calcium hydroxides used and were established. From the curve the amount of quick lime (Ca (OH) $_2$) required for pot culture experiment to obtain the targeted pH of 5.3, 5.5, 6.5, 7.5 were obtained as 0, 0.2, and 0.8 and 2.5g respectively.

Figure 2: Graph showing pH Curve of Calcium Hydroxide Solutions at 25° C

3.3.4 Treatments, experimental design and pot culture

The treatments consisted of 25 genotypes and soil pH at four levels. There were four target soil pH levels ranging from 5.3 to 7.5 (i.e. 5.3, 5.5, 6.5, 7.5). The experimental design followed a randomized complete block design in a split plot arrangement with 3 replications per treatment. In each pot, 4 Kg soil from Magadu was amended with Ca $(OH)_2$ at four incremental rates (0, 0.2, 0.8, and 2.5 g) to obtain the target soil pH levels. After mixing the soil with Ca $(OH)_2$ the samples were incubated at 25 ^{o}C. The mixtures were pulverized every 5 days to mix the Ca $(OH)_2$ with the soil. The soils were then moistened with distilled water every day, to 60% field capacity and placed under a polyethylene cover containing a hole. After 4 weeks, soil pH was measured and common beans lines were surface sterilized with 10% H_2O_2 for 10 min, washed with running tap water, distilled water, and then two seeds were sown at 5 cm depth in each pot.

3.3.5 Plant sampling

At early flowering (10% flowering of the whole plant), trifoliate leaves were sampled randomly from 10 plants per row in a plot for all soil pH levels. Leaf samples were put into paper bags, clearly labelled, oven dried at 70 to 100 ^{o}C and then grounded to fine powder using a motor and pestle to pass through a 0.5 mm sieve for Fe and Zn analyses. After physiological maturity, seeds were harvested from each pot in all soil pH levels and put into paper bags and then air dried. Then, seeds were grounded using a sample mill. The powder obtained was used for determination of Fe and Zn in the seeds.

3.3.6 Plant and seeds analysis

The samples were put into four replicates for iron and zinc analysis. One gram of ground plant leaves and seeds were weighed in separately digestion tubes. Then, 5 ml of 68% nitric acid was added into each tube and the mixture left to stand overnight. The

digestion tubes were then placed in the digestion block and the temperature set at 125°C for one hour before being cooled. After cooling, 5 ml of 30% hydrogen peroxide (H_2O_2) was added into each tube and heated at about 70°C on digestion block until the reaction stopped. After cooling, 5 ml of 30% H_2O_2 were again added and heated at 70°C. The samples were repeated until the digest became colorless. The temperature was increased to 180°C and continued digesting to almost dryness and then left to cool. Ten ml of 10% nitric acid was added and the dissolved digest was transferred to a 50 ml volumetric flask. The flask was then filled to the mark with distilled water and the contents were mixed. The solution was then ready for determination of iron and zinc by using atomic absorption spectroscopy (AOAC, 1995).

3.3.7 Data collection

Number of days to 50% flowering was measured as days after planting to the time coinciding with the initiation of developmental stage when 50% of the plants had one or more flowers. Days to 80% maturity were measured as days after planting to the time coinciding with the initiation of developmental stage when 85% of the plants had reached maturity. Pods per plant were recorded as an average from five plants picked at random, and the mean counted as pods per plant and number of seeds per pod. Leaf iron concentration, seed iron concentration, leaf zinc concentration, and seed zinc concentrations were determined by analysis of leaves and seeds in the laboratory as described in section 3.3.6

3.3.8 Statistical analysis

Analysis of Variance (ANOVA) was performed for all data including days to 50 % flowering, days to 85 % maturity, number of pods per plant, number of seed per pods, leaf iron concentration, seed iron concentration, leaf zinc concentration, and seed zinc

concentrations were analysed by using the GenStat statistical package 15th edition at p = 0.05.Significant means were separated using Turkey method of mean separation.

3.3.9 Estimation of Simple Correlation Coefficients

The data for 50 % flowering, days to 85 % maturity, number of pods per plant, number of seed per pods, leaf iron concentration, seed iron concentration, leaf zinc concentration, and seed zinc concentrations traits were utilized for the computation of correlation coefficients between grain iron and zinc concentrations with other traits for all the genotypes. The formulae suggested by Snedecor and Cochran (1967) were utilized for the computation of correlation coefficients.

r(xy) = <u>Cov (xy)</u>
      $\sqrt{}$ (Var x). (Var y)

Where,

r(xy) = Correlation between x and y

Cov (xy) = Covariance for traits x and y

Var (x) = Variance for x

Var (y) = Variance for y

r = Correlation coefficient

xy = Two independent variables

To test the significance of correlation coefficients, the estimated values were compared with the table values of correlation coefficients (Fisher and Yates, 1967) at 5% levels of significance with (n-2) degrees of freedom, where, „n‟ is the total number of observations used.

3.10 Results

3.10.1 Pre-cropping Soil Fertility Status and pH Curve

Results of pre-sowing soil analysis showed that soils of the experimental sites were Sandy clay loam in texture with a pH of 5.3. The soil is strongly acidic with medium levels of organic matter, total nitrogen and available phosphorus, respectively. Exchangeable K, and Mg in the soil were high and Ca was medium; whereas exchangeable Na was low and Cation Exchange Capacity (CEC) in the soil was high. The micronutrients such Mn, Zn and Fe were high. The physical and chemical properties of the experimental soil are shown in Table 3.

Table 3: Physical-chemical properties of the experimental soil

Soil parameter	Values	Remark (London 1991)
pH in water	5.3	Strongly acid
Cationic Exchange Capacity (CEC)	26.4	High
Organic Carbon (% C)	3.21	medium
Organic matter (% OM)	1.51	Medium
Nitrogen (%)	0.50	Medium
Phosphorous (mg kg^{-1})	18.09	Medium
Extractable K (Cmol(+) kg^{-1})	0.91	High
Extractable Na (Cmol(+) kg^{-1})	0.19	Low
Extractable Mg (Cmol(+) kg^{-1})	3.33	High
Extractable Ca (Cmol(+) kg^{-1})	7.28	Medium
DTPA Extractable micronutrients (mg kg^{-1})		
Fe	64.96	High
Zn	4.81	High
Mn	6.31	High
Particle size analysis (PSA)		
%Clay	31.96	
%silt	8.94	
%Sand	54.5	
Textural class	Sandy clay loam (USDA, 1975)	

3.10.2 Effects of genotypes on Zinc, Iron concentration and yield components

Results indicate that there were highly significant ($p < 0.05$) differences among genotypes in concentration of zinc in leaves (Table 4). The highest concentration of zinc in leaf was 50.6 mg/Kg observed in Nua 17 and lowest concentration of zinc in leaf was 24.7 mg/Kg observed in Zawadi as shown in Table 4.

Concentration of zinc in seeds were highly significant ($p < 0.05$) differences among genotypes (Table 4). The highest concentration of zinc in seeds was 40.9 mg/Kg observed in Nua 17 and lowest concentration of zinc in seeds was 24.1 mg/Kg observed SUA 90 (Table 4).

Concentration of iron in leaves were highly significant ($p < 0.05$) differences among genotypes (Table 4). The highest concentration of iron in leaves was 373.3 mg/Kg observed in SUA 90 and lowest concentration of iron in leaves was 141.7 mg/Kg observed in ROJO (Table 4).

Table 4: Effects of Genotypes on different variables measured (mg/Kg) at P <.05

Genotypes	C zinc in leaf	C zinc in seed	C iron in leaf	C iron in seed	Days to 50% flowering	Days to 85% maturity	No pods/ plant	No of seeds/p ods
NUA 9	47.0 d-e	30.8 a-f	210.5 c-i	76.6 a-c	28 a-c	68 f	7a	3a
NUA 11	46.6 d-e	27.3 a-d	220.3 e-i	118.28 d	31 d-e	68 f	7a	3a
NUA 13	46.2 d-e	31.3 a-g	175.5 a-e	78.8 a-c	27 a	63 a	8a	3a
NUA 15	42.0 b-e	34.8 c-h	223.3 e-i	68.2 a-c	29 a-c	67 ef	7a	2a
NUA 16	41.7 b-e	35.5 d-h	219.8 d-i	61.8 a	28 a-c	63 a	7a	3a
NUA 17	50.6 e	40.9 h	198.4 a-g	71.1 a-c	29 b-c	74 j	7a	3a
NUA 18	38.8 a-e	34.2 c-h	247.9 f-i	80.6 a-c	29 a-c	67 ef	7a	2a
NUA 19	49.1 d-e	33.8 c-h	159.9 a-e	89.7 b-c	28 a-b	66 d	6a	3a
NUA 23	48.8 de	34.9 c-h	155.6 a-d	87.7 a-c	34 f	66 d	7a	2a
NUA 30	36.7 a-e	35.3 d-h	264.8 g-j	68.2 a-c	31 d-e	67 f	7a	3a
NUA 31	33.6 a-d	32.4 a-h	142.1 a-b	73.5 a-c	33 e-f	71 i	8a	2a
NUA 39	33.8 a-d	39.2 f-h	134.1a	74.6 a-c	37 b-c	70 h	7a	3a
NUA 40	34.8 a-e	36.8 e-h	148.7 a-c	92.0 c-d	37 b-c	73 j	8a	2a
NUA 48	37.6 a-e	34.0 c-h	244.4 f-i	71.9 a-c	31 d-e	67 ef	8a	3a
NUA 57	28.6 a-c	34.7 c-h	316.9 j-l	88.9 b-c	32 e	70 h	8a	3a
NUA 59	44.1 c-e	29.9 a-f	184.3 a-f	84.6 a-c	33 e	67 ef	8a	3a
NUA 64	27.3 a-b	32.9 b-h	226.5 e-i	78.7 a-c	29 a-c	65 c	8a	2a
NUA 66	37.4 a-e	38.1 e-h	204.9 b-h	64.7 a-b	30 c-d	69 g	7a	3a
NUA 67	38.7 a-e	39.4 g-h	220.7d-i	84.5 a-c	28 a-c	66 d	7a	2a
NUA 79	46.8 d-e	38.2 f-h	247.9 f-i	93.9 c-d	29 b-c	67 de	8a	3a
SUA 90	40.7 a-e	24.1 a	373.3 i	75.7 abc	31 de	64 b	7a	2a
MSHINDI	26.9 a-b	24.9 a-b	274.9 i-k	72.8 a-c	27 a	63 a	7a	2a
PESA	32.8 a-d	26.4 a-c	340 k-l	74.2 a-c	28 a-c	67 ef	7a	3a
ROJO	26.1 a-b	32.3 a-g	141.7 a-b	73.1 a-c	29 b-c	69 g	8a	3a
ZAWADI	24.7 a	27.4 a-d	271.7 h-j	79.6 a-c	29 b-c	67 ef	7a	3a
G. MEAN	**38.5**	**33.2**	**221.9**	**79.3**	**30.3**	**67.4**	**7.3**	**2.6**
S.D	**7.9**	**4.6**	**62.4**	**11.7**	**2.8**	**2.8**	**0.6**	**0.5**
S.E.M	**1.6**	**0.9**	**12.5**	**2.3**	**0.6**	**0.6**	**0.1**	**0.1**
CV (%)	**20.52**	**13.86**	**28.12**	**14.75**	**9.24**	**4.15**	**8.22**	**19.23**
P value	**< .001**	**<.001**	**<.001**	**<. .001**	**<.001**	**<.001**	**0.97 1**	**0.915**

Means with the same letters in a column are not significantly different at 5 % level of significance by the Turkey test

Where c.zinc= concentration of zinc, c.iron= concentration of iron

Further indicated no significant ($p < 0.05$) differences among genotypes in concentration of iron in seeds (Table 4). The highest concentration of iron in seeds was 118.28 mg/Kg, observed in Nua 11 and lowest concentration of iron in seeds was 61.8 mg/Kg, observed in Nua 16 (Table 4).

Number of days to 50% flowering were highly significant ($p < 0.05$) differences among genotypes (Table 4). Longest time to reach flowering was recorded 37 days for Nua 40 and Nua 39. Shortest time to reach 50% flowering was recorded 27 days for Mshindi (Table 4).

Number days to 85% maturity were highly significant ($p < 0.05$) differences among genotypes (Table 4). Early maturity was recorded 63 days for Mshindi and Nua 13 and late maturity was recorded 74 days for Nua 17 (Table 4).

The number of pod per seeds were not significantly ($p < 0.05$) different among the genotypes (Table 4). The lowest number of pods recorded 6 pods for Nua 19 and highest number of pods recorded was 8 pods for Nua 13, Nua 31, Nua 40, Nua 48, Nua 57 Nua 59, Nua 64, Nua 79 and Rojo (Table 4).

The number of seeds per pod was not significantly ($p < 0.05$) different among the genotypes (Table 4). High of number of Seed per pod was 3 seeds per pod observed in Nua 13 and Nua 40 and Nua 57. Lowest numbers of seeds per plant was 2 recorded in Nua 66, Nua 23, Mshindi and Pesa (Table 4).

3.10.3 Effects of pH on Zinc, Iron and yield components

Concentration of zinc in leaves were highly significant ($p < 0.05$) differences at pH level 5.3 while at pH levels 5.5 and 7.5 were significant and non-significant differences at pH level 6.5 (Table 5). The overall mean values for high concentration of zinc in leaf

was 42.7 mg/Kg at pH 5.5 and the overall mean values for low concentration of zinc in leaf were 33.7 mg/Kg at pH 5.3 (Table 6).

Concentration of zinc in seeds were highly significant (p < 0.05) differences at pH 5.3, 5.5 and 6.5 while at pH 7.5 were significant (Table 5). The overall mean values for high concentration of zinc in leaf were 37.3 mg/Kg at pH 5.5 and the overall mean values for low concentration of zinc in leaf were 29.7 mg/Kg at pH 5.3 (Table 6).

Table 5: Mean square of pH on Zinc, Iron and yield components

PARAMETER	pH 5.3	pH 5.5	pH 6.5	pH 7.5
Conc. of Zinc in leaf	211.56**	221.8 *	278.4ns	171.97*
Conc. of Zinc in seed	137.03**	82.79**	105.47**	67.62*
Conc. of Iron in leaf	13380**	11559**	12801**	12515.**
Conc. of Iron in seed	335.5ns	572.1ns	485.3ns	650.4**
Days to 50% flowering	23.336**	22.980**	20.861**	21.486**
Days to 85% maturity	26.4200**	26.8022**	21.9244**	21.9244**
No. pods/ plant	1.692ns	4.063ns	2.247ns	2.74ns
No. seeds/ pod	0.542ns	1.163ns	0.913ns	0.5978ns

** = highly significant, * = significant, ns = non-significant at p = 0.05

Concentration of iron in leaves were highly significant (p < 0.05) differences in all pH levels (5.3, 5.5, 6.5 and 7.5) (Table 5). The overall mean values for high concentration of iron in leaf were 243.3 mg/Kg at pH 6.5 and the overall mean values for low concentration of zinc in leaf were 197.7 mg/Kg at pH 5.3 (Table 6).

Concentration of iron in seeds indicated no significant (p < 0.05) differences 5.3, 5.5 and 6.5 except at pH 7.5 which were highly significant (Table 5). The overall mean values for high concentration of iron in seeds were 90.9 mg/Kg at pH 6.5 and the overall mean values for low concentration of zinc in leaf were 71.5 mg/Kg at pH 5.3(Table 6).

Number of days to 50% flowering were highly significant ($p < 0.05$) differences at pH levels 5.3, 5.5, 6.5 and 7.5 (Table 5). Longest time to reach 50% flowering recorded was 31 days at pH levels of 5.3 and shortest times to reach flowering was recorded 29 days at pH levels of 6.5 and 7.5. Early flowering was observed at high pH levels of 6.5 and 7.5 while late flowering was observed at pH levels 5.3 (Table 6).

Number of days to 85% maturity were highly significant ($p < 0.05$) differences at pH levels 5.3, 5.5, 6.5 and 7.5 (Table 5). Longest time to reach maturity was 69 days at pH levels of 5.3 and iron concentration in seeds 5.5. Shortest time to reach flowering was 66 days at pH levels of 6.5 and 7.5. Early maturity was observed at high pH levels of 6.5 and 7.5 while late maturity was observed at pH levels 5.3 and 5.5 (Table 6).

Number of pods per plant indicated no significant ($p < 0.05$) differences in all pH levels 5.3, 5.5, 6.5 and 7.5 (Table 5). Overall mean for high number of pods per plant were 8 pods at pH 6.5 and 7.5 while the overall mean values for low number of pods per plant were 6 pods at pH 5.3 and 5 (Table 6).

Number of seeds per pods also indicated no significant ($p < 0.05$) differences at pH levels 5.3, 5.5, 6.5 and 7.5 (Table 5). Overall mean for high number of seeds per pods were 3 seeds at pH 6.5 and 7.5 while the overall mean values for low number of seeds per pods were 2 seeds at pH 5.3 and 5.5 (Table 6).

Table 6: Effects of pH levels on different variables measured at P <.05

pH	C.zinc in leaf	C.zinc in seed	C.iron in leaf	C.iron in seed	50% flowering	85% maturity	pods/plant	seed/pods
pH 5.3	33.7 a	30.7 a	197.7 a	71.5 a	31 b	69 b	7 a	2a
pH 5.5	42.7 b	37.3 b	239.4 b	78.8 a	31 b	69 b	6a	2a
pH 6.5	42.3 b	35.2 b	243.3 b	90.9 b	29 a	66 a	8a	3b
pH 7.5	35.4 b	29.7 a	207.3 a	76.1a	29 a	66 a	8a	3b
GRAND MEAN	38.53	33.23	221.93	79.33	30	67.5	7.25	2.5
S.D	4.64	3.62	22.83	8.28	1.15	1.73	0.96	0.58
S.E	2.32	1.81	11.41	4.14	0.58	0.87	0.48	0.29
CV%	12.04	10.89	10.29	10.44	3.83	2.56	13.24	23.2

Means with the same letters in a column are not significantly different at 5 % level of significance by the Turkey test
Where c.zinc= concentration of zinc, c.iron= concentration of iron

3.10.4 Effects of genotypes x pH interaction on Zinc, Iron and yield components

Table 7: Analysis of variance for the different variables evaluated for the common at P <.05

Variables	Source	Df	*ss*	Ms	F value	P value
Days to 50 % Flowering	Genotype x pH	72	21.22	0.295	0.18	0.985
Number of Days to 85 % Maturity	Genotype x pH	72	91.68	1.2733	6.82	<.001
Number of Pods per Plant	Genotype x pH	72	226.987	3.153	0.81	0.852
Number of Seed per Pod	Genotype x pH	72	52.84	0.7339	0.74	0.933
Concentrations of Zinc in leaf (mg/Kg)	Genotype x pH	72	2900.5	40.3	0.33	0.978
Concentrations of Zinc in seed (mg/Kg)	Genotype x pH	72	3257.86	45.25	1.33	0.062
Concentrations of iron in seeds (mg/Kg)	Genotype x pH	72	10036.5	139.4	0.44	0.996
Concentrations of iron in leaf (mg/Kg	Genotype x pH	72	85420	1186	0.58	0.986

3.10.4.1 Concentration of Zinc in leaf (mg/Kg)

Concentration of zinc in leaf were non-significant ($p < 0.05$) differences among genotypes x pH levels interaction (Table 7). The highest concentration of zinc in leaf was 59.92 mg/Kg observed in NUA 16 at pH levels 5.5 and lowest concentration of zinc in leaf was 20.5 mg/Kg observed in NUA 64 at pH levels 5.3. The overall mean values for high concentration of zinc in leaf were 42.67 mg/Kg at pH 5.5 (Table 8).

3.10.4.2 Concentration of Zinc in seed (mg/Kg)

Concentration of zinc in seeds indicated no significant ($p < 0.05$) differences among genotypes x pH levels interaction as displayed in Table 7. The highest concentration of zinc in seed were 52.18 mg/Kg observed in NUA 17 at pH levels 5.3 and lowest concentration of zinc in seed was 18.3 mg/Kg, observed in PESA at pH levels 5.3 as

displayed in Table 7. The overall mean values for high concentration of zinc in seed were 37.31 mg/Kg at pH 5.5 (Table 8).

Table 8: Concentrations of zinc in leaf and seeds (mg/Kg) of common bean genotypes grown under different pH levels at SUA in screen house at P <.05

Genotypes	Conc. of Zn in leaf				Conc. of Zn in Seed			
	pH 5.3	pH 5.5	pH 6.5	pH 7.5	pH 5.3	pH 5.5	pH 6.5	pH 7.5
NUA 9	43.7 ab	55.9 ab	49.4 ab	46.8 ab	24.8 a-g	35.5 a-k	37.5 a-k	25.6 a-g
NUA 11	45.9 ab	50.9 ab	52.5 ab	37.1 ab	26.4 a-h	28.9 a-i	30.7 a-j	23.1 a-f
NUA 13	42.3 ab	47.8 ab	54.3 ab	40.6 ab	27.4 a-i	32.8 a-k	31.8 a-k	30.1 a-i
NUA 15	42.0 ab	45.5 ab	40.9 ab	39.2 ab	30.9 a-i	38.7 c-k	38.5 b-k	34.4 a-k
NUA 16	44.2 ab	59.9 ab	54.5 ab	43.9 ab	29.4 a-i	42.2 f-k	37.7 a-k	32.9 a-k
NUA 17	33.7 ab	44.5 b	52.7 ab	35.8 ab	52.2 k	39.0 c-k	39.3 c-k	33.1 a-k
NUA 18	33.4 ab	43.1 ab	45.5 ab	33.3 ab	28.2 a-i	34.7 a-k	41.4 e-k	32.5 a-k
NUA 19	41.5 ab	51.9 ab	54.0 ab	39.9 ab	30.6 a-j	38.5 b-k	34.5 a-k	32.2 a-k
NUA 23	38.0 ab	49.3 ab	59.0 ab	48.6 ab	31.6 a-j	37.4 a-k	37.1 a-k	33.3 a-k
NUA 30	30.6 ab	43.7 ab	39.6 ab	32.6 ab	33.3 a-k	37.1 a-k	37.2 a-k	33.7 a-k
NUA 31	31.8 ab	34.5 ab	38.6 ab	30.3 ab	31.2 a-j	33.7 a-k	33.8 a-k	31.1 a-j
NUA 39	25.7 ab	36.9 ab	38.7 ab	33.1 ab	37.5 a-k	46.9 ijk	38.1 a-k	34.0 a-k
NUA 40	25.2 ab	43.2 ab	37.2 ab	33.5 ab	32.7 a-k	43.7 g-k	36.7 a-k	34.1 a-k
NUA 48	28.3 ab	47.7 ab	40.6 ab	33.9 ab	36.4 a-k	33.9 a-k	34.1 a-k	31.7 a-j
NUA 57	25.3 ab	31.6 ab	31.2 ab	26.1 ab	32.1 a-k	37.7 a-k	35.0 a-k	33.9 a-k
NUA 59	44.9 ab	47.2 ab	46.3 ab	37.9 ab	25.9 a-h	39.1c-k	29.4 a-i	25.2 a-g
NUA 64	20.5 a	33.2 ab	31.4 ab	24.1 ab	31.8 a-j	33.8 a-k	35.4 a-k	30.5 a-j
NUA 66	30.8 ab	46.2 ab	37.1 ab	35.4 ab	38.2 a-k	39.6 c-k	40.7 d-k	33.8 a-k
NUA 67	31.2 ab	45.8 ab	42.4 ab	35.6 ab	37.6 a-k	47.0 ijk	37.9 a-k	34.9 a-k
NUA 79	45.3 ab	46.5 ab	54.3 ab	50.3 ab	32.4 a-k	45.8 hijk	50.5 jk	26.8 a-h
SUA 90	41.2 ab	37.8 ab	41.3 ab	42.4 ab	27.9 a-i	25.1 a-g	25 a-g	18.5 ab
Mshindi	22.7 ab	29.4 ab	28.2 ab	27.1 ab	22.2 a-d	37.0 a-k	20.38 abc	21.3 a-e
PESA	26.3 ab	39.4 ab	35.3 ab	30.3 ab	18.3 a	32.8 a-k	30.1 a-i	24.5 a-g
ROJO	26.9 ab	28.4 ab	26.2 ab	22.9 ab1	29.7 a-i	37.8 a-k	37.1 a-k	24.6 a-g
ZAWADI	21.9 ab	26.5ab	26.7 ab	23.6 ab	20.9 a-f	33.6 a-k	27.1 a-i	26.9 a-i
G. MEAN	33.7	42.7	42.3	35.4	30.8	37.3	35.1	29.7
S.D	11.2	12.2	14.8	10.9	7.8	7.1	7.6	6.9
S.E.M	1.3	1.4	1.7	1.3	0.9	0.8	0.8	0.8
CV (%)	33.2	28.6	34.9	31.1	25.6	19.0	21.7	23.3

Means with the same letters in a column are not significantly different at 5 % level of significance by the Turkey
Where c.zinc= concentration of zinc, c.iron= concentration of iron

3.10.4.3 Concentration of iron in leaf (mg/Kg)

There were non-significant ($p < 0.05$) differences among genotypes x pH levels interaction in the concentration of iron in leaves as displayed in Table 7. The highest concentration of iron in leaf was 390.8 mg/Kg, observed in SUA 90 Nua at pH levels 5.3, and lowest concentration of iron in leaf was 109 mg/Kg, observed in NUA 40 at pH levels 5.3. The overall mean values for high concentration of iron in leaf was 239.4 mg/Kg at pH 5.5 as displayed in Table 9.

3.10.4.4 Concentration of iron in seed (mg/Kg)

Concentration of iron in seeds indicated highly significant ($p < 0.05$) differences among genotypes x pH levels interaction as displayed in Table 7. The highest concentration of iron in seed was 151 mg/Kg observed in NUA 11 at pH levels 5.3 and lowest concentration of iron in seed were 51.4 mg/Kg, observed in NUA 16 at pH levels 7.5. The overall mean values for high concentration of iron in seed was 90.9 mg/Kg at pH 6.5 respectively as displayed in Table 9.

Table 9: Concentrations of zinc and iron in leaf (mg/Kg) of common bean genotypes grown under different pH levels at SUA in screen house

Genotypes	Conc of Fe leaves				Conc of Fe Seeds			
	pH 5.3	pH 5.5	pH 6.5	pH 7.5	pH 5.3	pH 5.5	pH 6.5	pH 7.5
NUA 9	165.2 a-f	202.1 a-i	247.3 a-n	227.4 a-l	77.9 abc	67.4 ab	89.1 abc	71.9 abc
NUA 11	175.7 a-f	235.7 a-n	238.5 a-n	231.5 a-m	151.0 abc	127.7 bc	132.1c	127.5 bc
NUA 13	172.2 a-f	179.0 a-f	193.3 a-i	157.6 a-f	67 ab	70.8 abc	96.4 abc	80.0 abc
NUA 15	210.2 a-k	277.4 a-n	277.7 a-n	226.4 a-h	66.7 ab	64.2 ab	76.1abc	65.9 ab
NUA 16	135.6 a-n	223.3 a-n	229.1 a-k	205.9 a-f	66.7 ab	64.8 ab	64.5 ab	51.4 a
NUA 17	237.6 a-e	245.9 a-l	215.3 a-m	180.4 a-i	53.3 a	80.2 abc	86.8 abc	64.1 ab
NUA 18	149.2 a-k	176.4 c-n	172.7 c-n	141.5 a-l	75.9 abc	79.7 abc	89.8 abc	77.0 abc
NUA 19	217.4 a-f	236.9 a-f	252.4 a-f	186.4 a-f	82.4 abc	98.6 abc	101.4 abc	76.2 abc
NUA 23	238.2 abc	284.4 a-g	272.5 a-f	263.9 a-f	83.8 abc	84.4 abc	96.0 abc	86.6 abc
NUA 30	123.2 a-n	181.4 d-n	170.6 b-n	147.3 a-n	59.4 a	68.4abc	77.6 abc	69.4 abc
NUA 31	157.2 a-d	169.2 a-f	144.9 a-f	123.5 ab	64.2 ab	77.2 abc	84.3 abc	68.3 abc
NUA 39	127.6 a	161.0 a-f	162.7 a-f	117.1 a	66.4 ab	69.0 abc	88.5 abc	74.7 abc
NUA 40	109.0 a-f	142.6 a-f	172.2 a-f	112.6 abc	78.4 abc	94.8 abc	100.8 abc	94.1 abc
NUA 48	224.6 a-l	261.6 a-n	257.0 a-n	234.5 a-n	66.9 ab	67.5 ab	81.9 abc	71.3 abc
NUA 57	280.9 c-n	343.9 h-n	346.1 i-n	296.7 f-n	75.9 abc	85.2 abc	106.4 abc	88.3 abc
NUA 59	196.5 a-f	241.5 a-i	259.6 a-k	208.2 a-f	82.5 abc	86.7 abc	95.6 abc	73.7 abc
NUA 64	151.3 a-i	200.8 a-n	216.1 a-n	169.1 a-j	87 a	80.8 abc	83.7 abc	63.4 abc
NUA 66	244.0 a-f	265.5 a-n	264.5 a-n	215.3 a-g	52.5 a	65.3 ab	82.5 abc	58.6 abc
NUA 67	204.5 a-i	234.2 a-n	240.0 a-n	203.9 a-i	86.8 abc	82 abc	88.7 abc	80.5 abc
NUA 79	153.7 a-n	233.8 a-n	249.1 a-n	183.2 a-k	84 abc	86.9 abc	106.6 abc	94.8 abc
SUA 90	349.4 i-n	390.8 n	385.6 mn	367.5 k-n	66.7 ab	74.8 abc	92.1 abc	70.0 abc
MSHINDI	113.0 a-n	173.7 d-n	147.8 e-n	132.2 c-n	70.0 abc	75.8 abc	86.8 abc	72.4 abc
PESA	366.1 j-n	339.3 g-n	379.5 l-n	275.2 b-n	66 ab	73.8 abc	86.4 abc	70.0 abc
ROJO	246.6 a	284.6 a-f	289.6 a-f	278.8 a-e	66 ab	65.7 ab	88.6 abc	72.0 abc
ZAWADI	193.9 a-i	298.8 f-n	297.2 f-n	296.8 f-n	56.1 abc	78.6 abc	91.2 abc	78.5 abc
G.MEAN	197.7	239.4	243.3	207.3	74.1	78.8	90.9	76.1
S.D	72.2	72.7	75.9	74.5	18.3	20.5	19.2	20.2
S.E.M	8.3	8.4	8.8	8.6	2.1	2.4	2.2	2.3
CV (%)	36.5	30.4	31.2	35.9	25.6	25.9	21.1	26.5

Means with the same letters in a column are not significantly different at 5 % level of significance by the Turkey test

3.10.4.5 Days to 50 % Flowering

Number of days to 50 % maturity indicated no significant (p < 0.05) differences among genotypes x pH levels interaction as displayed in Table 7. The numbers of days to 50% flowering for all genotypes were lowered as the pH levels increases. High   mean of 31.2 was observed at pH level 5.3. The higher mean values showed that the genotypes took long time to reach flowering. Longest time to reach flowering was recorded 39 days for NUA 31 at pH levels of 5.3. Lower mean value of 25 was recorded at pH levels 7.5 in MSHINDI as displayed in Table 10.

3.10.4.6 Number of Days to 85 % Maturity

Number of days to 85 % maturity were highly significant (p < 0.05) differences among genotypes x pH levels interaction as displayed in Table 7. The numbers of days to 85% maturity for all genotypes were lowered as the pH levels increased. High   overall mean of 68.6 was observed at pH level 5.3. The higher mean values showed that the genotypes took long time to reach maturity. Longest time to reach maturity was recorded 75 days for NUA 17 at pH levels of 5.3 and 5.5. Lower   overall mean value of 66.3 was recorded at pH levels 6.5 and 7.5 as displayed in Table 10.

Table 10: Days to 50% flowering and days to 85% maturity of bean genotypes grown in screen house

Genotypes	Days to 50% Flowering				Days to 85% maturity			
	pH 5.3	pH 5.5	pH 6.5	pH 7.5	pH5.3	pH 5.5	pH 6.5	pH7.5
NUA 9	29a-hi	29 a-i	28 a-f	28 a-e	68 de	68 de	67 cd	67 cd
NUA 11	32 f-o	32 f-o	30 b-k	30 b-k	68 de	68 de	67 cd	67 cd
NUA 13	28 a-f	28 a-f	26 ab	26 ab	64 b	64 b	62 a	62 a
NUA 15	29 a-j	29 a-j	28 a-f	28 a-g	68 de	68 de	66c	66c
NUA 16	29 a-h	29 a-h	27 a-d	28 a-e	64 b	64 b	62 a	62 a
NUA 17	30 b-k	30 b-k	28 a-g	28 a-e	75 i	75 i	71 h	71 h
NUA 18	30 b-k	30 b-k	28 a-e	28 a-e	68 de	68 de	65 c	65 c
NUA 19	32 a-h	32 a-h	30 abc	30 abc	68 de	68 de	64b	64 b
NUA 23	35 m-q	35 m-q	33 j-p	33 i-o	72 h	72 h	70 fg	70 fg
NUA 30	29 g-o	29 e-n	27 c-k	27 c-k	68 de	68 de	67 cd	67 cd
NUA 31	39 k-p	38 k-p	36 e-m	36 e-m	72 h	72 h	70 fg	70 fg
NUA 39	37 pq	37 pq	36 n-q	36 n-q	71 gh	71 gh	68 de	67 de
NUA 40	34 q	34 q	31 opq	31 opq	74 i	74 i	72 h	72 h
NUA 48	32 g-o	34 g-o	31 c-k	30 b-k	72 de	72 de	68 c	68 c
NUA 57	33 h-o	33 h-o	31 d-l	31 d-l	68 h	68 h	66 de	66 de
NUA 59	33 i-o	33 i-o	32 e-n	32 e-n	68 de	68 de	67 c	67 c
NUA 64	29 a-j	30 b-k	28 a-e	28 a-e	69c	68 c	67 b	67 b
NUA 66	29 c-k	29 c-k	28 a-h	28 a-h	69 ef	69 ef	64 de	64 de
NUA 67	30 a-h	30 a-i	28 a-e	28 a-e	68 cd	68 cd	66 c	66 c
NUA 79	31 c-k	30 b-k	29 a-e	29 a-f	66 ef	66 ef	64b	64b
SUA 90	32 g-o	32 g-o	30 c-k	30 c-k	64 b	64 b	63 b	63 b
MSHINDI	29 a-h	28 a-f	27abc	25a	65 b	64 b	62 a	62 a
PESA	29 a-i	29 a-i	28 a-e	28 a-e	68 de	68 de	66 c	66 c
ROJO	30 b-k	30 b-k	29 a-j	28 a-i	67 fg	69 fg	67 cd	67 cd
ZAWADI	30 b-k	30 b-k	29 a-h	29 a-f	68 de	68 de	64 c	64 c
G.MEAN	31.2	31.1	29.4	29.3	68.6	68.5	66.3	66.3
S.D	2.9	2.9	2.8	2.7	3.0	3.0	2.7	2.7
S.E.M	0.34	0.34	0.31	0.32	0.34	0.34	0.31	0.31
CV (%)	9.54	9.53	9.36	9.53	4.30	4.30	4.04	4.04

Means with the same letters in a column are not significantly different at 5 % level of significance by the Turkey

3.10.4.7 Number of pod per Plant

Number of pod per plant were non-significant ($p < 0.05$) differences among genotypes x pH levels interaction as displayed in Table 7. Numbers of pods per plant for all genotypes were increased as the pH levels increases. The highest Number of pod per plant was 10 pods, observed in NUA 13, NUA 40 and NUA 79 at pH levels 7.5, NUA 31 at pH levels 6.5. The lowest Number of pod per Plant was 4 pods observed in MSHINDI at pH levels 5.5. The overall mean values for high Number of pod per Plant was 8.4 mg/Kg at pH 7.5 as displayed in Table 11.

3.10.4.8 Number of Seed per Pod

Number of Seed per Pod indicated no significant ($p < 0.05$) differences among genotypes x pH levels interaction as displayed in Table 7. Numbers of Seed per Pod for all genotypes were increased as the pH levels increases. The highest Number of Seed per Pod was 4 seeds, observed in NUA 13 at pH levels 7.5, NUA, 40 at pH levels 7.5 NUA 57 at pH levels 6.5 and NUA 79 at pH levels 7.5 and lowest Number of Seed per Pod was 1 seeds observed in NUA 66 at pH levels 5.5, MSHINDI pH levels 5.5, PESA pH levels 5.3 and NUA 30 pH levels 6.5. The overall mean values for high Number of Seed per Pod was 3.1 mg/Kg at pH 7.5 as displayed in Table 11.

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Table 11: Number of pods per plant and seeds per pod of common bean genotypes grown under different pH levels at SUA in screen house

Genotypes	Number of pods per plant				Number of seed per pods			
	pH 5.3	pH 5.5	pH 6.5	pH 7.5	pH 5.3	pH 5.5	pH 6.5	pH 7.5
NUA 9	7a	6a	8a	9a	2 ab	2 ab	3 ab	3 ab
NUA 11	7a	7a	7a	9a	2 ab	2 ab	2 ab	3 ab
NUA 13	7a	6a	8a	10a	3 ab	3 ab	3 ab	4 b
NUA 15	6a	6a	9a	8a	2 ab	2 ab	3 ab	3 ab
NUA 16	8a	6a	8a	8a	2 ab	2 ab	3 ab	3 ab
NUA 17	7a	7a	8a	8a	3 ab	3 ab	3 ab	3 ab
NUA 18	7a	9a	6a	7a	2 ab	3 ab	2 ab	3 ab
NUA 19	7a	4a	7a	7a	3 ab	2 ab	2 ab	3 ab
NUA 23	7a	6a	7a	7a	2 ab	2 ab	3 ab	3 ab
NUA 30	7a	6a	6a	6a	2 ab	2 ab	1 ab	3 ab
NUA 31	8a	4a	10a	9a	3 ab	2 ab	3 ab	3 ab
NUA 39	5a	6a	7a	8a	2 ab	2 ab	3 ab	3 ab
NUA 40	6a	7a	8a	10a	3 ab	2 ab	3 ab	4 ab
NUA 48	8a	7a	9a	7a	2 ab	3 ab	2 ab	2 ab
NUA 57	6a	6a	8a	9a	2 ab	3 ab	4 ab	3 ab
NUA 59	7a	7a	9a	8a	2 ab	3 ab	3 ab	3 ab
NUA 64	6a	8a	7a	9a	2 ab	3 ab	2 ab	3 ab
NUA 66	6a	5a	7a	8a	2 ab	1 ab	3 ab	3 ab
NUA 67	6a	6a	7a	7a	2 ab	2 ab	3 ab	3 ab
NUA 79	7a	6a	8a	10a	2 ab	2 ab	3 ab	4 ab
SUA 90	6a	7a	8a	9a	2 ab	3 ab	3 ab	3 ab
MSHINDI	8a	4a	8a	8a	3a	1 ab	2 ab	3 ab
PESA	5a	6a	9a	9a	1 ab	2 ab	3 ab	3 ab
ROJO	8a	6a	8a	9a	3 ab	2 ab	3 ab	3 ab
ZAWADI	6a	7a	8a	8a	3 ab	2 ab	3 ab	3 ab
GRAND MEAN	6.7	6.2	7.8	8.4	2.3	2.2	2.8	3.1
S.D	0.9	1.2	0.9	1.1	0.9	0.6	0.6	0.4
S.E.M	0.21	0.27	0.21	0.18	0.11	0.13	0.12	0.09
CV (%)	26.9	37.7	22.9	18.6	39.1	27.2	37.3	26.1

Means with the same letters in a column are not significantly different at 5 % level of significance by the Turkey test.

3.10.4.9 Correlation Analysis among Variables under pH levels 5.5 and 6.5

Concentration of iron in leaves were significantly and positively correlated with number of days to 50% flowering (r = 0.262**, 0.274**) under pH levels 5.5 and 6.5 respectively. Concentration of iron in leaves were significantly and positively correlated with number of days to 85 % maturity (r = 0.259**) under pH levels 6.5. Also concentration of iron in leaves were highly significantly and positively correlated with number of days to 85 % maturity (r = 0.340**) under pH levels 5.5. Further indicated that there were significant and positive correlations at p ≤ 0.05 between zinc concentration and number of seeds per pods under soil pH levels 6.5 (r = 0.270**) as shown in Table 12. There were significant and positively correlations at p ≤ 0.05 for number of days to 85% maturity and concentration of zinc in seed under pH levels 5.5 (r = 0.231**) as shown in Table 12.

Table 12: pH level 5.5 (Above diagonal) and pH level 6.5 (Below diagonal) correlation coefficients of different character combinations at P <.05

CHARACTER COMBINATION	50 % DF	85% DM	pod/plant	seed /pod	C.Fe seeds	C.Fe leaves	C. Zn seeds	C. Zn leaves
50 % DF	1	0.563***	0.023*	0.031*	0.114*	0.262**	0.157*	0.005*
85% DM	0.620***	1	0.017*	0.009*	0.143*	0.340***	0.231**	0.106*
pod/plant	0.046*	0.055*	1	0.799***	0.099*	0.114*	0.036*	0.054*
seed /pod	0.092*	0.088*	0.704***	1	0.069*	0.078*	0.126*	0.106*
C.Fe seeds	0.137*	0.091*	0.093*	0.071*	1	0.130*	0.181*	0.002*
C.Fe leaves	0.274**	0.259**	0.017*	0.066*	0.209*	1	0.181*	0.218*
C. Zn seeds	0.045*	0.166*	0.116*	0.093*	0.078*	0.209*	1	0.051*
C. Zn leaves	0.057*	0.001*	0.136*	0.270**	0.123*	0.025*	0.123*	1

** = highly significant, * = significant, ns = non-significant at 0.05,

3.11 DISCUSSION

3.11.1 Concentration of zinc in leaf and seeds (mg/Kg)

Soil pH is known to control the availability and uptake of micronutrients from soil so it is a quite important factor to be observed. At pH 5.3, concentration of zinc in seed and leaves was low due high content of free Fe, and Mn ions which caused adsorption of Zn to non-exchangeable form on their hydrated oxides surface and make it unavailable to plant. The results are in agreement with Phogat *et al.,* (1994) who reported that low Zn contents in most of the soils probably is due to high content of free Fe, Al and Mn ions which caused adsorption of Zn to non-exchangeable form on their hydrated oxides surface. The results also were similar to the findings of Hafeez *et al.,* (2013) who reported that insoluble Zn compounds formed are likely to be with Mn and Fe hydroxides from the breakdown of oxides and adsorption on carbonates, specifically magnesium carbonate. Under the submerged conditions for rice cultivation, Zn is transformed into amorphous sesquioxide precipitates or franklinite; $ZnFe_2O_4$ (Hafeez *et al.,* 2013). Also uptake of zinc ion was reduced in acidic soil due to reduction in loading of polyvalent cations in the apoplasm of root cortical cells. The results were similar to the findings of Marschner, (1995) who reported that in acid soils, there is a reduction in loading of polyvalent cations in the apoplasm of root cortical cells, not only Ca^{2+} but also Mg^{2+}, Zn^{2+} and Mn^{2+}.

At pH 5.5 and 6.5 concentration of zinc in leaves was high for examples NUA 16 at pH 5.5 and NUA 23 pH 6.5, had high concentration of zinc in leaves. At pH 5.5 and 6.5, solubility of zinc increased and the dominant form of Zn^{++} was taken up by genotypes. The results were similar to the findings of McCauley *et al.,* (2017) who reported that the nitrogen (N), potassium, calcium, magnesium and sulfur are more available within soil pH 6.5 to 8, while boron (B), copper, iron, manganese, nickel (Ni), and zinc are more

available within soil pH 5 to 7. The results are also in agreement with Rengel (2015) who did the similar work and reported that increasing soil pH, especially above 6.5, results in decreased extractability and plant availability of soil Zn and iron.

At pH 7.5, concentrations of zinc in both leaves and seeds was low because hydroxides and carbonates present in soil led to adsorption of Zn on their surface or precipitation of Zn as Zn hydroxide or Zn carbonate, which reduce Zn availability in soil and hence limiting uptake by the plants. The results were similar to the findings of Hafeez *et al.,* (2013) who reported that the lower availability of Zn under alkaline conditions is attributed to the precipitation of Zn as $Zn(OH)_2$ or $ZnCO_3$.

Genotypes responded differently on concentration of zinc in seeds and leaves. Differences in Zn concentrations found in each genotype in leaves and seeds, suggest that there are differences in the uptake and partitioning of nutrients in common bean genotypes. The results were similar to the findings of Tryphone and Nchimbi, (2010) who reported that differences in iron and zinc concentration found in each genotype for both seeds and leaves were due to their differences in uptake capacity and partitioning of nutrients in the different parts of plant. Some genotypes in this experiment showed tolerance to low pH (5.3) and high pH (7.5). For examples NUA 17 and NUA 79 at pH levels 5.3 and 7.5 respectively showed high concentrations of zinc in seed i.e both in acidic soil and alkaline soil. This demonstrates the superior performance of the genotype when grown on both acidic and alkaline soil and thus they might have genes for tolerance of both kinds of stresses i.e the acidic and alkaline condition. It is therefore important for breedes to exploit genetic potential of common bean and develop acidic and alkalinity tolerant and high yielding genotypes.

Genotypes x pH levels interaction did not have significant effects on both zinc in leaf and seeds. Soil pH levels in both seeds and leaves contributed more mean square than genotype and interaction. This indicates that concentration of zinc in seeds and leaves were influenced by soil pH factors than genotype and interaction of two factors.

Zinc concentrations in leaves in all soil pH were higher than zinc concentrations in seeds. The higher accumulation of zinc in leaves and stem than in seeds is related to their functions in plant metabolism. Zinc used in the formation of chlorophyll and some carbohydrates, conversion of starches to sugars and its presence in plant tissue helps the plant to withstand cold temperatures. The results are in agreement with Fernandes *et al.* (2013) who reported that, the amounts of micronutrients accumulated in the vegetative part, i.e., in the leaves and stem, were higher than the amounts in the reproductive structures.

3.11.2 Concentration of iron in leaf and seeds (mg/Kg)

The differences in concentration of iron in leaves and seeds found in each genotype was due to the soil pH levels. At pH 5.3, concentration of iron in seed and leaves was low due manganese competing with Fe uptake from the soil. This was rendering both nutrients unavailable for plant uptake due to decreased root Fe concentration and uptake. Zinc deficiency prevents transfer of Fe from root to shoot in zinc deficiency conditions. The results are in agreement with Rengel and Romheld (2000) who reported that zinc deficiency leads to iron (Fe) deficiency, due to restricted or interfering of transfer of Fe from root to shoot in zinc deficiency conditions. The results are in agreement with Mortvedt *et al.* (1991) who reported that the antagonistic interaction between Fe and Mn was probably due to the reduction of Mn concentration by dilution effect, reduction in root to shoot ratio, reduced Mn uptake, or toxic concentration of Fe in plant tissue. The

results were also similar to the findings of Moosavi and Ronaghi (2010) who stated that soil Fe applications decreased root Mn concentration of dry bean by 17 % due to the dilution effect.

At pH 5.5 and 6.5 concentration of iron in seeds and leaves was high because of availability of nutrients which support growth and development of shoot and roots of the plant. At these pHs levels solubility of Iron increased and the dominant ferric (Fe^{3+}) form was converted to a ferrous (Fe^{2+}) form in the soil, and was then absorbed by plants. The results are in agreement with Rout and Sahoo, (2015) who reported that, insoluble ferric (Fe^{3+}) form was reduced and converted to ferrous form in the soil. Then ferrous was absorbed by plants and translocation into plant tissue.

At pH 7.5 concentration of iron in seed and leaves were low indicating that Iron predominantly existed as Fe^{+3} chelates in the soil, and could not absorb under this form because were less available in the soil. The results are in agreement with finding of Rengel (2015) who did the similar work and reported that increasing soil pH, especially above 6.5, resulted in decreased extractability and plant availability of soil Zn and iron.

Fe concentrations genotypes were responded differently in seeds and leaves in common bean genotypes. Differences in Fe concentrations found in each genotype in leaves and seeds, suggest that there is a variety difference in the uptake and partitioning of nutrients in common bean genotypes due their genetic makeup. The results were similar to the findings of Tryphone and Nchimbi, (2010) reported that differences in iron and zinc concentration found in each genotype for both seeds and leaves were due to their different in uptake capacity and partitioning of nutrients in the different parts of plant. Some genotypes such NUA 11 in this experiment showed tolerance in low pH (5.3) and had showed high concentrations of iron in seed in low pH. This demonstrates the

superior performance of the genotype when grown on acidic thus it might have tolerant genes for acidic condition.

Genotypes x pH levels interaction did not have significant effects in both iron in leaf and seeds. Soil pH levels of iron in leave contributed more than genotype and interaction. This indicates that concentration of iron in leaves was influenced by soil pH factors than genotype and interaction. Genotype of iron in seed contributed more mean square than pH and interaction. This indicates that concentration of iron in seed was influenced by genotype than pH and interaction.

Iron concentrations in leaves in all soil pH were higher than iron concentrations in seeds. The higher accumulation of iron in leaves and stem is due to their different distribution and partitioning of nutrients in the different parts of plant tissue. The results are in agreement with Adalton *et al.,* 2013 who reported that higher concentration of Fe and Zn in the in leaves than in grains in the current study may be attributed to the role of leaves as the source of assimilates which is accumulated in grains as the sink.

3.11.3 Days to 50% flowering and 85% maturity

Days to 50% flowering and 85% to maturity were affected when genotypes grew under low soil pH. At soil pH 5.3 and 5.5, there were significantly reduction in the overall growth of the common bean genotypes. These results suggest that H^+ and Al^{3+} toxicity in these genotypes distorted plant tissue and restricted cell division, and other metabolic process in the plant hence slowing plant growth. The result of this study agrees with that of Rout *et al.* (2001) who reported that low soil pH led to late maturity as a result of Al-toxicity.

At pH 6.5 and 7.5 genotypes grew well because of availability of macronutrients influenced by these pHs as results growth of common bean genotypes hence early flowering and maturity examples Nua 13 and Mshindi. This result was in agreement with the study by Legesse *et al.* (2016) who reported that lime application generally improved growth and dry matter partitioning of the genotypes possibly through decreasing the toxic effect of aluminum and improving the availability of nutrients for uptake by the roots of the common bean plants.

Leaf areas of both genotypes were adversely affected by low soil pH. These changes were accompanied by a reduction in chlorophyll content and photosynthesis rate and abnormal chloroplast structure. Proper plant growth is attained when the rate of photosynthesis is greater than that of respiration because the respiration consumes the photosynthetic products. Since photosynthesis is slowed, growth is slowed automatically. This result was in agreement with the study by Wang *et al.* (2006) who stated that soil acidity led to stunted leaf growth. The result of this study agrees also with that of Rout *et al.* (2001) who reported that low soil pH led to late leaf maturity as a result of Al-toxicity.

Genotypes x pH levels interaction did not have significant differences on 50% days to flowering. This showed that flowering was more influenced by genotype than soil pH and interaction. This result was in agreement with the study by Rutaihwa *et al.* (2004) who reported that a lack of genotype x environment interaction suggests that maturity was controlled more by genetics than by the pH. There was highly significant interaction between genotype x soil pH interaction ($p < 0.05$) in the number of days required 85% to reach maturity. Presence of interaction between genotype x soil pH showed that maturity was more influenced by soil pH than genotype.

3.11.4 Number of Pods per Plant and Number of Seed per Pod

Number of pods per plant and number of seed per pod were non-significant however there was slight variation among genotypes at pH 5.3 and 5.5. The variation among genotypes was due to the fact that soil pH less than 5.5 doesn't support availability of macronutrients such as calcium, magnesium, and phosphorus for genotype uptake. As result soil acidity tend to react quickly with calcium (Ca) to form less soluble compounds and became less available for absorption by common beans genotypes. Similar finding were also recorded by Fageria *et al.,* (2008) who reported that, liming effectively increased grain yield, the dry mass of the shoot and the number of pods and grains per plant in common beans.

Number of pods per plant and number of seed per pod were non-significant however there was slightly variation among genotypes at pH 6.5 and 7.5. The variation among genotypes was probably due to the effect of the calcium concentration in the nutrient solution. Solubility of calcium concentration and other nutrients increases at these pH levels and hence formation of pod and seeds in common beans. The results are in agreement with Dominguez *et al.* (2014) who did the similar work and reported that; the high calcium concentration in the nutrient solution increased the mean value for number of pods and of grains per plant, number grains per pod and grain yield.

Genotypes grown at low pH showed poor growth and yield. Increase in soil acidity was observed to have a deleterious effect on the root growth and the overall growth and development of the common bean genotypes. This is because genotypes are sensitive to acidic soil conditions hence poor formation of number of pods per plant and numbers of Seed per pods. Increases in soil acidity have a harmful effect on the root growth and the overall growth and development of the common bean genotypes. Soil acidity reduce

root growth, reduce nutrient availability to plant, affect crop protectant activity and thus, result in poor plant growth and reduction in the agronomic yield such as formation of pods and seeds. In agreement with this result Favaro *et al.* (2007) who reported that, number of green pods on snap bean plants increase when grown in high calcium concentration in the nutrient solution at 10 to 12 days after anthesis of flowers.

There was no significance difference between genotype x pH interaction for number of pods per plant and number of seeds per pods. Mean square of soil pH in number of pods per plant and number of seeds per pods contributes more than genotypes and interaction factors. This showed that number of pods per plant and numbers of seeds per pods were more influenced by soil pH than genotype and interaction.

3.11.5 Correlation among Variables

The positive correlations between concentration of zinc and iron in leaves with number of days to 50% flowering, number of days to 85 % maturity, number of pods per plants and number of seeds per pods indicated that improvement for one of the trait could lead to significant parallel increase of concentration of iron and zinc in leaves. This suggested the possibility of developing cultivars with high zinc and iron concentrations booth in leaves and seeds and also with other traits. This means that it is possible to increase both yield and Fe and Zn concentrations in seeds in the same genotypes. This result disagree with results of Phuke *et al.* (2017) who found negative correlation between grain micronutrient with 50% flowering in a study on sorghum.

The positive correlations between concentration of zinc and iron in seeds with number of days to 50% flowering, number of days to 85 % maturity, number of pods per plants, number of seeds per pods, suggest that it is possible to develop cultivars with high zinc

and iron concentrations in seed together with other useful traits. Also it indicates that indirect selections to increase the concentration of zinc and iron in seed can be a recommendable strategy.

Concentration of zinc in leaves was positively correlated with concentration of iron in leaves and concentration of zinc in seeds was positively correlated to iron in seeds. This implies that the amount of zinc and iron in leaves can be reflected in seeds. Our result are in agreement with the study by Tryphone and Nchimbi Msolla (2010) who reported that a significant positive correlation between grain Fe and Zn concentration and leaf Fe and Zn concentration suggests that genetic factors for increasing Fe and Zn are co-segregating with genetic factors for increasing Zn.

3.12 CONCLUSION AND RECOMMENDATIONS

3.12.1 Conclusions

Soil pH affects absorption of micronutrients directly or indirectly by affecting the nutrients availability of common beans genotypes. At low soil pH of 5.3 the concentration of zinc and iron in both leaves and seeds was low compared with concentrations observed at optimal soil pH of 6.5. However, some genotypes such as NUA 11 and NUA 17 showed high performance in absorption of zinc and iron at pH 5.3. Therefore, selecting and growing common bean genotypes that are tolerant to low pH, such as as NUA 11 and NUA 17 genotypes, could lead to increased production of the crop in the area where soil acidity is a serious threat to enhancing bean production. Also some genotypes showed high ability in absorption zinc and iron at pH greater than 6.5 which are optimal conditions for most micronutrients in the soil. NUA 79 at pH 7.5 demonstrates the superior performance when grown under alkaline soil condition. However, most of genotypes perform well under pH levels of 5.5 and 6.5. Also selecting

and growing common bean genotypes that are tolerant to high pH, such as NUA 79 genotypes, could lead to increased production of the crop in the area, and where soil alkaline is a serious threat to enhancing bean production.

3.12.2 Recommendations

The following have been recommended

i. Studies should be conducted to determine the morphological and chemical characteristics possessed by the tolerant genotypes in acidic and alkaline soil condition.

ii. Screening large germplasm and breeding programs for common bean genotypes should be conducted to develop cultivars that are tolerant to soil acidity and alkalinity with potential and quality grain on such acid and alkaline soils in the future.

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CHAPTER FOUR

4.0 Heritability characteristics of Fe and Zn nutrient concentration

Abstract

A study was carried out to determine heritability characteristics of zinc and iron in common bean. Genotypes namely, NUA 11 and NUA 17 which were used as donor parents for high zinc and iron while ZAWADI and PESA were used as recipient parent. Crosses were made with each donor and recipient parent to obtain four different types of crosses. Part of the F1 seed from each cross was sown in the screen house to produce F2 seeds and also backcrossed to both parents. Heritability was estimate using backcross method for high broad sense heritability and narrow-sense heritability. Broad sense heritability was reported in all crosses. Broad sense heritability observed in NUA 11 x Zawadi was (56%), NUA 11 x Pesa was (76%) while NUA 17 x Zawadi was (57%) and NUA 17 x Pesa was (59%). High narrow sense heritability was reported in all crosses. Narrow sense heritability observed in NUA 11 x Zawadi was (65%), NUA 11 x Pesa was (71%) while NUA 17 x Zawadi was (79%) and NUA 17 x Pesa was (63%). Genetic advance as per cent of mean was expressed in NUA 17 x Zawadi was (35%) followed by NUA 11 x Pesa was (12.30%), NUA 17 x Peas was (3.5%) and NUA 11 x Zawadi was (1%). This study demonstrates that there is a potential for improvement of concentration of zinc and iron in common bean genotypes and developing them using those two donor parents. Therefore, in order to select a superior genotype on the basis of its phenotypic performance, heritability of traits could be efficiently utilized.

4.1 Introduction

The process of genetic inheritance which involves the transmission of characteristics or a quality from parents to offspring allows farmers and breeders to improve food security by increasing both yields and the nutritive qualities of crop varieties (Nyquist, *et al,.* 2003). It is a basic principle of genetics and explains how characteristics are passed from one generation to the next. Inheritance of a character differs markedly in its expression amongst individuals of a species (i.e. variation in that species is discontinuous). Inheritance is dependent upon several to many genes at different loci, each gene contributing a small effect to the phenotypic expression of the character. Characters controlled by a number of genes with small effects that may be measured in metric units are said to be quantitative characters and their inheritance is referred to as quantitative inheritance (Falconer and Mackay 1996).

Heritability is the proportion of this total variation between individuals in a given population due to genetic variation. This number can range from 0 (no genetic contribution) to 1 (all differences on a trait reflect genetic variation) (Nyquist, *et al.,* 2003). Heritability reflects the fact that all individuals in any species of living things differ in many ways among each other. The variation (differences) among individuals within a species depends on both genetic and environmental differences. Heritability is a statistic used in the fields of breeding and genetics that estimates the degree of variation in a phenotypic trait in a population that is due to genetic variation between individuals in that population (Nyquist, *et al.,* 2003). Heritability concerns how much variation in traits is caused by variation in genes. Heritability tells us if this variation occurs because people have different genes or because they live in different environments. Heritability is used to partition observable phenotypic variation between individuals into genetic and environmental components. Heritability is an important concept in quantitative genetics,

particularly in selective breeding . Estimates of heritability use statistical analyses to help to identify the causes of differences between individuals (Nyquist, *et al.,* 2003). Since heritability is concerned with variance, it is necessary an account of the differences between individuals in a population. Heritability measures the relative importance of hereditary and environmental influences on the development of a specific quantitative trait Heritability is useful for characteristics that are partly affected by genetic differences and partly affected by environmental differences. In the field of quantitative genetics, the concept of heritability is used to partition observable phenotypic variation between individuals into genetic and environmental components (Falconer and Mackay 1996). Estimation of heritability in populations depends on the partitioning of observed variation into components that reflect unobserved genetic and environmental factors.

4.2 Materials and methods

4.2.1 Location

The screen house experiment was carried out at Sokoine University of Agriculture (SUA) which is found in the Morogoro region, Tanzania (Figure 1). SUA is located at 6° 45' S latitude and 37° 40' E longitudes at an altitude of 547 masl. The climate of the area is characterized by a bimodal rainfall pattern, with short period rains occurring in November/December for some years and long period rains in February to May. Rainfall varies from 1200 mm in the highland plateaus to 600 m in lowlands. The average annual temperature in the region's highlands is 18°C but reaches 30°C in the lowland.

4.2.2 Materials

Plant materials were selected based on screening of common beans genotypes done in objective one under pH 5.3 (Table 9). Therefore, NUA 11 and NUA 17 were selected as donor parents because of high concentration of iron and zinc respectively while Zawadi

and Pesa were selected as recipient parents because of low concentration of iron and zinc.

4.2.3 Methods

4.2.3.1 Planting of parental lines for hybridization

Parental lines were planted in the screen house located at Sokoine University of agriculture (SUA). Six pots were used per variety. Three seeds were sown per pot. Thinning was done two weeks after planting. Watering was done once a day throughout the growth of the plants so as to make sure that there are flowers for crossing for longer time (Figure 3). Recipient as well as donor parents were planted in a staggered mode at an interval of seven days so as flowering of the two parents match. Donor lines (NUA 11 and NUA 17) were planted first because of their late flowering at an interval of seven days before planting recipient (Zawadi and Pesa) parents. Crosses were made between genotypes with higher iron and zinc concentration in seeds (NUA 11 and NUA 17) and genotypes with lower concentration of iron and zinc female parents.

Figure 3: Plants ready for making crosses

4.2.3.2 Soil sampling and analysis

Soil samples were collected from the Magadu site at the depth of 0-20 cm using an auger. Ten soil samples were taken from each arm of the shaped pattern. All samples were bulked and composited and a one kilogram composite sample was taken for analysing physical and chemical properties of the soil. The samples were air-dried, disaggregated and sieved through a 2 mm sieve and analysed (Thompson and Banerjee, 1991). All soil samples were analysed for soil pH, cation exchange capacity (CEC), exchangeable bases (Ca, K, Mg and Na), micronutrients (Fe and Zn), organic carbon (OC) and available phosphorus. Soil textural classes were determined using the USDA textural class triangle (USDA, 1975). Soil pH was determined in water at a soil: water ratio of 1:2.5 suspension using pH meter (MacLean, 1982). Available P was extracted using the Bray 1 method (Bray and Kurtz, 1945) and quantified by the ascorbic acid calorimetric of Murphy and Riley (1962). Exchangeable calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry whereas K and Na were extracted using ammonium acetate and analysed by flame spectrophotometry. Cation exchange capacity (CEC) was determined with ammonium acetate saturation method at pH 7.0 (Chapman, 1973). Organic carbon was determined by the Walkley-Black wet combustion method (Tan, 1996) and total N was determined using the Kjeldahl method. The DTPA extractable Fe and Zn were determined by atomic absorption spectrophotometry (Lindsay and Norvell, 1978). The soil was mixed thoroughly with basic nutrients (rates in mg/kg soil) nitrogen 40 (as sulphate of ammonia), potassium 10 (as potassium chloride), and zinc 10 (zinc sulphate)

4.2.3.3 Hybridization

Emasculation and pollination was done either early in the morning or evening as described by Tumwesigye (1988). The procedure of crossing involved emasculation of the female flowers (Zawadi and Pesa) line and transfer of pollen from donor flowers

(NUA 11 and NUA 17) to the stigma of emasculated bean plants. Both rubbing and hooking methods were used. Pollination was performed by rubbing the pollinated stigma of the male flower to the female flower. Crosses were as follows: - NAU 11 x Zawadi, NUA 11 x Pesa, NUA 17 x Zawadi, and NUA 17 x Pesa to get F1 populations (figure 4). Hooking technique was done by removing the pollinated stigma of donor parent (Nua 11 and NUA 17) by means of forceps and hooking it against recipient parent (Zawadi and Pesa) flower. Forceps were sterilized by dipping in alcohol to avoid contamination with pollen or other pathogenic organisms from one flower to another. The pollinated flowers were covered with small pieces of cello-tape to avoid desiccation of flower (figure 4). Then a cotton thread and a tag labelled with the pedigree of the cross were tied loosely on the flower stalk. At maturity, the pods were harvested together with their identification tags. These were sun-dried and threshed to give F1 seed. Thereafter, seeds were bulked into four replicates for iron and zinc analysis at SUA Laboratory. Part of the F1 seed from each cross was sown in the screen house to produce F2 seeds and also backcrossed to both parents.
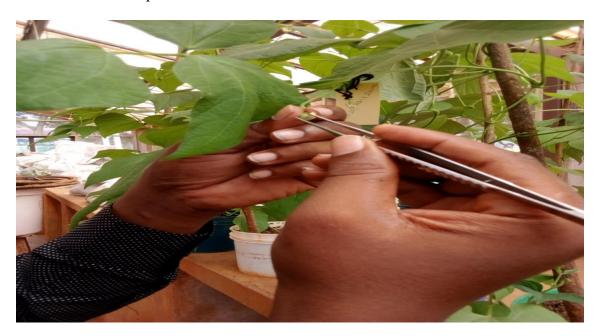

Figure 4: Hybridization process in screen house

4.2.3.4 Grain micronutrients analysis

At maturity, the pods of F1, F2 and backcrosses plants were harvested, sun-dried and threshed to give F1 and F2 seeds. Thereafter, seeds were put into four replicates for iron and zinc analysis at SUA Laboratory. A gram of ground seed was weighed in digestion tubes. Then, 5 ml of 68% nitric acid was added into each tube and the mixture left to stand overnight. The digestion tubes were then placed in the digestion block and the temperature set at 125°C for one hour before being cooled. After cooling, 5 ml of 30% hydrogen peroxide (H_2O_2) was added into each tube and heated at about 70°C on digestion block until the reaction stopped. After cooling, 5 ml of 30% H_2O_2 were again added and heated at 70°C. The samples were repeated until the digest was colorless. The temperature was increased to 180°C and continued digesting to almost dryness and then left to cool. Ten ml of 10% nitric acid was added and the dissolved digest was transferred to a 50 ml volumetric flask. The flask was then filled to the mark with distilled water and then mixed. The solution was therefore ready for determination of iron and zinc as per AAS method (AOAC, 1995).

4.2.4 Data collection

Data collected were seed iron concentration, seed zinc concentration after analysis of seeds in the laboratory for F1, F2 and BC lines.

4.2.5 Statistical and genetic analyses

Genstat statistical package was used to compute means, variance, standard deviation and standard error of variation between variables. Mean performance of the crosses and parents were obtained based on the general analysis of variance model in GenStat 15th edition. The genetic parameters were estimated from the variances of the parents' (P1

and P2) and the F1, F2, BC1 and BC2 generations based on seeds generation for each hybrid combination.

4.2.5.1 Estimation of heritability (broad sense, narrow sense) and genetic advance.

The genetic parameters were estimated from the variances of the parents (P1 and P2) and the F1, F2, BC1 and BC2 generations based on concentration of zinc and iron for each population. The broad-sense heritability and the narrow-sense heritability were estimated with the backcross method (WARNER, 1952), by the following expressions

4.2.5.1.1 Broad-sense heritability

The broad-sense heritability was estimated by the following expressions suggested by (WARNER, 1952),

$$h^2_a = \sigma^2_G / \sigma^2_P$$

4.2.5.1.2 Narrow-sense heritability

Narrow-sense heritability was estimated by the following expressions suggested by (WARNER, 1952),

$$h^2_r = \sigma^2_A / \sigma^2_P$$

Where

σ^2_G refers to the genotypic variance, estimated by:

$$\sigma^2_G = \sigma^2_P - \sigma^2_E$$

Where

σ^2_E represents the environmental variance in F_2 estimated by:

$$\sigma^2_E = (\sigma 2F1 + \sigma^2_{PI} + \sigma^2_{P2})/3)$$

σ^2_P is the phenotypic variance $\sigma^2_P = \sigma^2_{F2}$

And

$\sigma^2 A$ is the additive variance, estimated by:

$\sigma2A = 2\sigma^2 F2 - (\sigma^2 BC1 + \sigma^2 BC2)$

The range of heritability estimates were categorized as follows as suggested by Johnson *et al.,* (1955): Low: 0-30%, Medium: > 30-60%, High: > 60%.

4.2.5.1.3 Genetic advance

The extent of genetic advance expected by selecting certain proportion of the superior progeny was calculated by using the following formula suggested by Robinson *et al.,* (1947)

Genetic advance (GA) = $k.\sigma_{p.}h^2$

Where:

K = Intensity of selection 5% (k= 2.06)

σp = Phenotypic standard deviation

h^2 = Heritability in narrow sense

4.2.5.1.4 Genetic advance expressed as percentage over mean (GAM)

Genetic advance percentage (GAM) = GA / X * 100

Where:

GA= Genetic advance

X= General mean character

The GAM was categorized as suggested by Johanson *et al.*(1955) as: 0-10% = Low, 11-20% = Moderate, >20% and above, High

4.3 Results

4.3.1 Pre-cropping Soil Fertility Status and pH Curve

Results of pre-sowing soil analysis showed that soils used for experimental sites were Sandy clay loam in texture with a pH of 5.1.The soil is strongly acidic with medium contents of organic matter, total nitrogen and available phosphorus respectively. Exchangeable K, and Mg in the soil were high and Ca was medium; whereas exchangeable Na was low and Cation Exchange Capacity (CEC) in the soil was high. The micronutrients such Mn, Zn and Fe were high. The physical and chemical properties of the experimental soil are shown in Table 13.

Table 13: Physical-chemical properties of the experimental soil

Soil parameter	Values	Remark (London, *et al.,* 1991)
pH in water	5.1	Strongly acid
Cationic Exchange Capacity (CEC)	6.4	Low
Organic Carbon (% C)	3.21	High
Organic matter (% OM)	4.31	High
Nitrogen (%)	0.50	Medium
Phosphorous (mg/kg^{-1})	9.33	low
Extractable K (Cmol(+) kg^{-1})	0.91	High
Extractable Na (Cmol(+) kg^{-1})	0.19	Low
Extractable Mg (Cmol(+) kg^{-1})	2.33	High
Extractable Ca (Cmol(+) kg^{-1})	2.28	High
DTPA Extractable micronutrients (mg kg^{-1})		
Fe	54.96	High
Zn	4.08	High
Mn	6.31	High
Particle size analysis (PSA)		
%Clay	31.96	
%Silt	8.94	
%Sand	54.5	
Textural class	Sandy clay loam (USDA, 1975)	

4.3.2 Heritability and components of variation

The estimates of heritability, genetic advance, components of variance, have been presented in as displayed in Table 13. Broad sense heritability was reported in all crosses. Broad sense heritability observed in NUA 11 x Zawadi was (0.56), NUA 11 x Pesa was (0.76) while NUA17 x Zawadi was (0.57) and NUA 17 x Pesa was (0.59). High narrow sense heritability was reported in all crosses. Narrow sense heritability observed in Nua 11 x Zawadi was (0.65), NUA 11 x Pesa was (0.71) while NUA 17 x Zawadi was (0.79) and NUA 17 x Pesa was (0.63). Genetic advance as per cent of mean was expressed in NUA 17 x Zawadi was (35%) followed by NUA 11 x Pesa was (12.30%), NUA 17 x Peas was (3.5%) and NUA 11 x Zawadi was (1%). The GAM was categorized as suggested by Johanson *et al.* (1955) as: 0-10% = Low, 11-20% = Moderate, >20% and above is High as displayed in Table 14.

Table 14: Genetic parameters and components of variation in four crosses of common bean genotypes

Genetic parameters	Iron concentration		Zinc concentration	
	NUA 11 x Zawadi	NUA 11 x Pesa	NUA 17 x Zawadi	NUA 17 x Pesa
Additive variance	0.31	0.68	0.53	0.61
Environment variance	0.21	0.23	0.29	0.4
Phenotypic variance	0.48	0.96	0.67	0.97
Genotypic variance	0.27	0.73	0.38	0.57
Broad sense heritability	0.56	0.76	0.57	0.59
Narrow sense heritability	0.65	0.71	0.79	0.63
Genetic advance as % Genetic advance	1%	12.30%	35%	3.5%
	0.93	1.43	13.32	1.28

4.3.3 Concentration of iron in grain

In the population of NUA 11x Zawadi, heritability in broad sense was moderate (0.56) for concentration of iron in grain. Heritability in narrow sense was high (0.65) for concentration of iron in grain. Genetic advance was 0.93 for concentration of iron in grain. Genetic advance as per cent mean was low (1) for concentration of iron in grain as displayed in Table 14.

In the population of NUA 11x Pesa, heritability in broad sense was higher (0.76) for concentration of iron in grain. Heritability in narrow sense was high (0.71) for concentration of iron in grain. Genetic advance was 1.43 for concentration of iron in grain. Genetic advance as per cent mean was low (12.30%) for concentration of iron in grain as displayed in Table 14.

4.3.4 Concentration of Zinc in grain

In the population of NUA 17x Zawadi heritability in broad sense was moderate (0.57) for concentration of zinc in grain. Heritability in narrow sense was high (0.79) for concentration of iron in grain. Genetic advance was 13.32 for concentration of iron in grain. Genetic advance as per cent mean was high (35%) for concentration of iron in grain as displayed in Table 14.

In the population of NUA 17 x Pesa, heritability in broad sense was low (0.59) for concentration of iron in grain. Heritability in narrow sense was high (0.63) for concentration of iron in grain. Genetic advance was 1.28 for concentration of iron in grain. Genetic advance as per cent mean was low (3.5%) for concentration of iron in grain. as displayed in Table 14.

4.4 DISCUSSION

4.4.1 Heritability and genetic advance

Broad sense heritability was moderate while narrow sense heritability was high for the concentration of iron in the crossing of NUA 11 x Zawadi with low genetic advance as percentage. In crossing of NUA 11 x Pesa, both broad sense heritability and narrow sense heritability were high with moderate genetic advance as percentage. These results indicate that the additive gene effect plays an important role for concentration of iron. There is contribution equally to the production of qualitative phenotype. Early generation selection would therefore be successful. Improvement will be achieved through selection because of the environmental effect has less effect on genotypes in early generation selection. It also indicating important role genetic variance, hence, direct selection for these traits could bear desirable results. This result was in agreement with the study by Panse (1957) who stated that high heritability coupled with high genetic advance indicates the additive gene effects while high heritability coupled with low genetic advance indicates the non-additive gene effects for control of the particular character.

High narrow sense heritability and moderate broad sense heritability were observed for crossing of NUA 17 x Zawadi and NUA 17 x pesa for concentration of Zinc in grain. Genetic advance as percentage for the crossing NUA 17 x Zawadi while for NUA 17 x pesa was low. A high heritability means that most of the variation that is observed in the present population is caused by variation in genotypes. It means that, in the current population, the phenotype of an individual is a good predictor of the genotype. Results indicate that the trait has high ability of transferring gene and additive gene effect plays an important role for concentration of zinc. Improvement can be achieved through selection because of the environmental effect has less effect on genotypes. This is in

agreement with the findings by Mollasadeghi *et al*. (2012) also reported high estimates of heritability for days to heading (84%) and thousand kernel weight (89%). Also the results are in agreement with the findings by Sarvamangala, *et al*. (2018) who reported that high heritability (>60%) with moderate genetic advance (11-12%) were recorded for the characters like number of primary branches at 50 DAS, plant spread (E-W) at 50 DAS, plant spread (N-S) at 25 DAS, pod length, pod flesh thickness, number of seeds per plant, number of clusters per plant, number of pods per cluster, dry matter content of leaves and stem. Hence, the traits are highly heritable; selection based on these traits would improve the characters.

4.5 CONCLUSION AND RECOMMENDATIONS

4.5.1 Conclusion

This study demonstrates that the evaluated common bean germplasm has potential for improvement of concentration of zinc and iron in common bean genotypes. Therefore in order to select a superior genotype on the basis of its phenotypic performance, heritability of traits could be efficiently utilized. Heritability was relatively greater proportion for both broad sense heritability and narrow sense heritability. Heritabiltiy in broad sense was (56%), for iron concentration in NUA 11 x Zawadi, (76%) for iron concentration in NUA 11 x Pesa while for zinc concentration was (57%) in NUA 17 x Zawadi and (59%) in NUA 17 x Pesa. Heritabiltiy in narrow sense was (65%), for iron concentration in NUA 17 x Zawadi, (71%) for iron concentration in NUA 17 x Pesa while for zinc concentration was (79%) in NUA 17 x Zawadi and (63%) in NUA 17 x Pesa. High heritability and genetic advance in these crosses might be due to additive gene action. Hence, genetic components of these traits are important and selection based on these traits is effective. Therefore, direct selection of these genotypes can be fulfilling.

4.5.2 Recommendations

    i. The identified suitable parents and crosses should enhance selection of breeding strategies for high genetic gain in the development of high micronutrients varieties in future common bean breeding programmes.

ii.   Genotypes with high zinc and iron should be used for improvement of preferred bean genotypes with low concentration of these micronutrients

CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Soil pH affects absorption of micronutrients directly or indirectly by affecting the nutrients availability to common beans genotypes. However, the increase in the absorption of micronutrients was observed in pH 5.5 and 6.5 than pH 5.3 and 7.5. The increase in the absorption of micronutrients was also observed in Nua 11, Nua 79 and Nua 17 genotypes for all soil pH levels. Most genotypes show high ability in absorption of zinc and iron at pH of 5.5 and 6.5. Therefore pH of 5.5 and 6.5 can be an optimal condition for absorption of zinc and iron in these genotypes. Nua 79 show superior performance of uptake of nutrients in high pH soil. Nua 11 and Nua 17 genotypes show superior performance of uptake of nutrients in low soil pH. These genotypes could be used in breeding programs to develop common bean varieties for profitable production of the crop on acidic and alkalinity soils.

Heritability in all crosses was relatively greater proportion for both broad sense heritability and narrow sense heritability. Broad sense heritability observed in Nua 11 x Zawadi was (56%) and Nua 11 x Pesa was (76%) for concentration of iron while Nua 17 x Zawadi was (57%) and Nua 17 x Pesa was (59%) for concentration of zinc . Narrow sense heritability observed in Nua 11 x Zawadi was (65%) and Nua 11 x Pesa was (71%) for concentration of iron while Nua 17 x Zawadi was (79%) and Nua 17 x Pesa was (63%)for concentration of and zinc. Hence, the traits are highly heritable; selection based on these traits would improve the concentration of iron and zinc in common bean.

5.2 Recommendations

The following are recommended for future studies

1) The QTL Analysis should also be conducted for future study in order to understand the genetic point of view of the genotypes with respect to acidic and alkalinity stress tolerance.

2) The superiority of the acidic and alkalinity tolerant genotypes should be harnessed into local varieties that are performing better as they are not acidic and alkalinity tolerance at all.

3) To increase breeding programmes of acidic tolerant crop for micronutrients in order to overcame the problem of acidification of the soil caused by the use of acid based fertilizer such as ammonium sulphate.

4.6 References

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