

**IRON AND ZINC GENETIC BIOFORTIFICATION OF YELLOW
COMMON BEAN (*Phaseolus vulgaris* L.) GENOTYPES IN TANZANIA**

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**A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor
of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and
Technology**

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ABSTRACT

Iron and zinc deficiencies are the global leading micronutrient deficiencies particularly in developing countries such as Tanzania. Iron deficiency in humans causes anemia, whereas zinc deficiency leads to compromised immunity, decreased growth rate, and mental retardation. This study was conducted in the Northern, Eastern, and Southern Highlands of Tanzania from 2018 to 2020 to address the iron and zinc deficiencies in humans using genetic biofortification. The approach involved screening common bean genotypes for iron and zinc contents and the factors (such as phytic acid, and ferritin) that inhibit and or enhance their availability in the human gut. The genotypes with high seed iron and zinc contents were used in the genetic biofortification of the consumers' preferred yellow bean varieties. Field experiments (involving 99 common bean genotypes) were conducted at TARI-Selian, SUA, and TARI-Uyole to screen common bean genotypes for seed minerals and yield. The genotypes were planted following alpha lattice design in three replications each contained five blocks with 20 plots. Data were recorded for days to 75% flowering, number of pods/plant, number of seeds/pod, 100 seed weight, and seed yield. Furthermore, the contents of seed iron, zinc, phosphorus, magnesium, manganese, ferritin, phytic acid, and phytic acid to mineral molar ratios were determined. Results showed that there were highly significant ($P \leq 0.001$) effects between bean genotypes, environments, and genotype by environment interaction on seed yield, yield related traits, seed iron, and zinc contents. The highest and stable bean genotypes for seed iron and zinc contents and seed yield were identified. High phosphorus, magnesium, manganese, and low phytic acid and phytic acid to mineral molar ratio bean genotypes were also identified. Furthermore, the study developed F2 bean crosses with a 12.5 - 146.4 % increase in seed iron content and a 1.0 - 53.1 % increase in zinc content. Seventeen of the developed F2 crosses had a high seed iron content ≥ 70 mg/kg and seed zinc content ≥ 30 mg/kg, these include BF01, BF04, BF05, BF06, BF08, BF10, BF13, BF16, BF22, BF24, BF25, BF27, BF29, BF31, BF32, BF33, and BF35.

DECLARATION

I, Mashamba Philipo do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination by The Nelson Mandela African Institution of Science and Technology a dissertation entitled “Iron and Zinc Genetic Biofortification of Yellow Common Bean (*Phaseolus vulgaris* L.) Genotypes in Tanzania” and recommend for examination in fulfillment of the requirements for the degree of Doctor of Philosophy of Life Sciences (Sustainable Agriculture) of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	Atomic Absorption Spectrometry
AEA	Average Environmental Axis
ALP	Alkaline Phosphatase
AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of Variance
APS	Ammonium Persulfate
ASV	AMMI Stability Value
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CV	Coefficient of Variation
DALY	Disability-Adjusted Life Years Lost
DNA	Deoxyribonucleic Acid
DNMRT	Duncan's New Multiple Range Test
DSBs	Double-Stranded Breaks
ECL	Electrogenerated Chemiluminescence
F1	First Filial Generation
F2	Second Filial Generation
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GFDE	Global Fortification Data Exchange
GGE	Genotype and Genotype by Environment Interaction
GMC	Genetically Modified Crops
GN	Genotype Number
GSI	Genotype Stability Index
GWAS	Genome Wide Association Study
HR	Homologous Recombination
ICP-OES	Coupled Plasma-Optical Emission Spectroscopy
IPCA	Interaction Principal Component Axis
IRT	Iron-Regulated Transporter
IZiNCG	International Zinc Nutrition Consultative Group
kDa	kiloDaltons
LSD	Least Significant Differences of Means (5% Level)
MAFC	Ministry of Agriculture, Food and Cooperatives

MoHCDEC	Ministry of Health, Community Development, Gender, Elderly and Children
NAS	Nicotianamine Synthase
NBS	National Bureau of Statistics
NHEJ	No Homologous End Joining
NIH	National Institutes of Health
NM-AIST	Nelson Mandela African Institution of Science and Technology
OECD	Organization for Economic Co-Operative and Development
PA	Phytic Acid
PC	Principal Component
ppm	Parts Per Million
P-value	Statistical F Probability
QTL	Quantitative Trait Loci
RASV	Rank of Genotype Based on AMMI Stability Value
RDA	Recommended Dietary Allowance
RIL	Recombinant Inbred Line
RY	Rank of Genotype Based on Seed Yield or Mineral Content
RYSI	Rank of the Genotype Based on Yield Stability Index
SDG	Sustainable Development Goal
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SNP	Single-Nucleotide Polymorphism
SSR	Simple-Sequence Repeats
SUA	Sokoine University of Agriculture
TALENs	Transcription Activator-Like Effector Nucleases
TARI	Tanzania Agricultural Research Institute
TBS	Tris Buffered Saline
TCA	Trichloroacetic Acid
TEMED	Tetramethylethylenediamine
URT	United Republic of Tanzania
WHO	World Health Organization
YLD	Years Lost Due to Disability
YSI	Yield Stability Index
ZFNs	Zinc-Finger Nucleases

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Common bean (*Phaseolus vulgaris* L.) is mainly a self-pollinating crop and a diploid genotype with 11 chromosome pairs (Singh *et al.*, 2014). The crop is grown for home consumption and source of income. Common bean is an important source of protein, fiber, folate, thiamin and minerals particularly iron and zinc (Sperotto & Ricachenevsky, 2017). The protein content is 20-28%, energy 32% and fiber 56% of dry seed weight (Ugen *et al.*, 2009). The content of thiamin in common bean ranges from 3.9 to 11.4 ppm with folate ranging from 0.2 to 5.8 ppm of seed dry weight (Organisation for Economic Co-operation and Development, 2015). The global average of cultivated common bean seed iron content is 30 - 110 ppm and zinc 25 - 60 ppm of dry weight (Blair, 2013), while in wild type iron concentration is reported to reach 280 ppm (Guzman-Maldonado *et al.*, 2000). Based on high nutritional contents and other agronomic importance of common bean, the production and consumption of this crop has been increasing in Tanzania and sub-Saharan Africa at large (Food and Agriculture Organization Corporate Statistical Database [FAOSTAT], 2018).

Common bean ranks number five in terms of production among staple food crops and is the most grown and consumed grain legume in Tanzania (Ministry of Agriculture [MoA], 2019). The area under common bean production is 1 118 406 hectares resulting in the production of 1 158 039 tonnes countrywide (FAOSTAT, 2016). Common bean consumption per capita per year in the country is reported to range from 13.02 Kg to 50.45 Kg (Msolla & Fivawo, 2009; FAOSTAT, 2016). However, despite this consumption level, iron and zinc deficiencies continue to be a public health problem in the country (Balarajan *et al.*, 2011). Most common bean and other staple food crop varieties consumed have high levels of phytic acid (a potent inhibitor of iron and zinc absorption in human gut) and low seed iron and zinc contents (Petry *et al.*, 2013). This leading to low iron and zinc bioavailability in human gut (La Frano *et al.*, 2014). Common beans having reasonable high seed iron and zinc contents compared to other staple food crops is a good source of nutritional iron and zinc to humans particularly in developing countries such as Tanzania (Grusak & DellaPenna, 1999; Ahmad *et al.*, 2015; Bouis & Saltzman, 2017). Iron and zinc are important in both plants and animals including humans. In human beings about 60 - 70 % of iron is used in hemoglobin to carry oxygen

around the body and in production of red blood cells (Gupta, 2014). Insufficient intake of iron by humans leads to iron deficiency, which retards the growth and cognitive ability of children, lowers resistance to infectious diseases, and reduces the physical work capacity and productivity of adults (Osendarp & Eilander, 2011; World Health Organisation [WHO], 2014). In plants, iron is important for plant growth, playing a key role in respiration, photosynthesis, DNA synthesis, nitrogen assimilation and other metabolic processes (Rout & Sahoo, 2015; Márquez-Quiroz *et al.*, 2015). Insufficient iron uptake by plants leads to chlorotic, which reduces photosynthesis, respiration and thus yield and nutritional quality of edible parts (Grillet *et al.*, 2014). Iron stored in seeds is used up during seed germination before the seedling can take up iron from the soil (Connorton *et al.*, 2017).

Zinc plays an important role in the human body's immune system, cell division, cell growth, wound healing, carbohydrate metabolism, reproduction and smell and taste senses (Lokuruka, 2012). Zinc deficiency occurs when food intake or supplements cannot meet body demand, due to poor absorption, increased zinc loss and high body system zinc utilization (Ahmad *et al.*, 2015). Zinc deficiency leads to reduced body immune response, slow wound healing, infertility and reduced growth and development (Plum *et al.*, 2010). In plants, zinc is an important constituent of enzymes involved in carbohydrate, proteins and lipid metabolism, it is also involved in auxin synthesis, pollen formation and regulation of genes controlling environmental stress tolerance (Hafeez *et al.*, 2013; Chattha *et al.*, 2017). Insufficient supply of zinc in plants results in spikelet sterility, chlorosis and reduced growth and tolerance to environmental stress (Broadley *et al.*, 2007; Sharma *et al.*, 2013).

The primary cause of the micronutrient deficiency in humans is poor nutrition because the main source of the metal ions, such as iron and zinc is through dietary intake (WHO, 2014). In developing countries, such as Tanzania most of the populations depends on staple food crops with low grain iron and zinc contents (Garcia-Casal *et al.*, 2017). Approaches to alleviate iron and zinc malnutrition have focused on pharmaceutical supplementation, food chemical fortification, and dietary diversification, all of which have been facing lots of challenges during implementations in developing countries where deficiency is most prevalent. A complementary intervention approach is to develop high iron and zinc-containing varieties of staple food crops such as common bean through genetic biofortification. Biofortification is the cost-effective approach in alleviating iron and zinc

deficiency and can easily reach low income household residing in remote areas of the developing countries like Tanzania.

Common bean like other plants store iron in ferric state (Fe^{3+}) within the proteins called ferritin and mobilized upon cellular demand (Zielińska, 2015). The concentration of Ferritin in common bean seeds is estimated to be 50 - 70 mg/kg of seed dry weight, depending on species and varieties (Hoppler *et al.*, 2014; Zielińska, 2015). Up to 42 % of total seed iron in common bean is bound to ferritin depending on species and varieties and it is readily available in the human gastrointestinal for absorption (Lukac *et al.*, 2009; Cvitanich *et al.*, 2010). Bioavailability of divalent positively charged ions, such as iron and zinc from grains and other plant edible parts are negatively influenced by phytic acid (phytate; myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate) which reduces absorption of these minerals in the human gastrointestinal tract (Petry *et al.*, 2013). In common bean, phytic acid (PA) ranges from 0.4 g/100 to 2.6 g/100 g of seed dry weight. The inhibition ability of phytic acid on iron and zinc absorption depends on PA content and PA to mineral (Fe, Zn, Mg, Mn) molar ratios (Giuberti *et al.*, 2019). Thus PA to mineral molar ratios predicts the bioavailability of the minerals in human gut, the lower the PA to mineral molar ratio, the higher the bioavailability of the mineral in human gastrointestinal tract and vice versa (Golam *et al.*, 2011). Therefore, this study aimed at understanding the levels of minerals including iron and zinc and determines phytic acid and ferritin contents among common bean varieties grown in Tanzania and improves seed iron and zinc contents of the widely consumed yellow common bean varieties for increased intake of these minerals in the country.

1.2 Statement of the problem

The widely grown and consumed yellow common bean varieties in Tanzania have low to medium range of seed iron and zinc contents ≤ 45.0 and ≤ 23.3 mg/kg, respectively (Tryphone & Nchimbi-Msolla, 2010; Bucheyeki & Mmbaga, 2013), compared to the recommended high seed iron ≥ 70.0 mg/kg and zinc ≥ 30.0 mg/kg for common beans (Kimani & Warsame, 2019). Seed iron content of common bean genotypes grown in Tanzania as obtained in a screen house experiment ranged from 23.63 to 105.50 mg/kg, whereas zinc content ranged from 19.00 to 56.13 mg/kg (Tryphone & Nchimbi-Msolla, 2010), highlighting a high variation among the genotypes and possibility of improving these traits. Having reasonably high minerals and proteins contents, common bean is an important staple food crop for nutrition security in Tanzania and accounts for the fourth largest area of

cultivation in Tanzania next to maize (MoA, 2019). Nutrition security defined as equitable access to essential nutrients is as important as food security to attaining the United Nations 2015 Sustainable Development Goal 2 (SDG 2), which is about zero hunger, through achieving food security, improve nutrition and promote sustainable agriculture.

Lower iron and zinc levels in plant-based food can have serious negative impact on humans leading to iron deficiency and zinc deficiency respectively (Petry *et al.*, 2015; Lassi *et al.*, 2020). Iron deficiency is the leading cause of “Years lost due to disability (YLD)” in sub-Saharan African countries such as Tanzania (WHO, 2018). About 41% of children under age of 5 years and 35% women aged 15 – 49 years have iron deficiency anemia in Tanzania (National Bureau of Statistics (NBS) [Tanzania] and ICF (Macro, 2011). Zinc deficiency ranks number three, after iron and vitamin A among the micronutrient deficiencies (WHO, 2013). In Tanzania about 37.5 % of the population is at risk of insufficient zinc intake, this puts the country in the group of high zinc deficiency countries globally (Ministry for Health and Social Welfare [MOH], 2014). Due to its wide consumption across different age groups, gender and social status, common bean can be an important source for increasing essential nutrients (including iron and zinc) intake in Tanzania. The bioavailability of seed iron and zinc from plant to humans is negatively influenced by the level of phytic acid, as PA forms insoluble complexes with divalent ions such as iron and zinc which are not digested and absorbed in the human gut. Improving seed iron and zinc content and reducing phytic acid content of widely consumed staple food offers a viable strategy for combating iron and zinc deficiencies in Tanzania. To date there is limited information on levels of phytic acid, ferritin and minerals such as iron and zinc of the common bean genotypes grown in different agro-ecological zones of Tanzania. Thus, there was a need to investigate the levels of these seed quality traits and improve iron and zinc content of the yellow common bean varieties grown in Tanzania for improved iron and zinc intake of common bean consumers and reducing the impact of hidden hunger in Tanzania. In Tanzania, there are several landraces grown mainly in some specific agro-ecological zones and some varieties released many years back. Due to the fact that, different agro-ecological zones differs in environmental conditions and climate change. There is a strong need of assessing the performance of different bean genotypes in different environments and identify a few with superior stabilities in yield and yield components across agro-ecologies for use in plant breeding programs targeting bean varietal development and release.

1.3 Rationale of the study

This study aimed at generating information on the influence of different agro-ecological zones of Tanzania on the levels of seed yield, iron and zinc among common bean genotypes, in order to identify those with high and stable seed yield, iron and or zinc across the agro-ecological zones. The genotypes identified with high and stable seed iron and zinc content were used to improve the contents of seed iron and zinc in the widely grown and consumed yellow common bean varieties (Kigoma and Njano Uyole) in Tanzania. Furthermore, the levels of magnesium, phosphorus, manganese, phytic acid and ferritin for some selected common bean genotypes were determined. Development of yellow common bean varieties with increased concentration of iron and zinc in seeds through plant breeding so that when consumed regularly they can generate measurable improvement in iron and zinc nutritional status in humans is of much importance in alleviating iron and zinc deficiencies in the country. Understanding the levels of iron and zinc forms the basis for iron and zinc genetic biofortification of common beans via conventional and marker assisted breeding.

Having knowledge in levels of phytic acid among common bean varieties grown in Tanzania is important, as this highly negatively charged complex compound naturally found in plant seeds have not been studied in common beans in Tanzania. The increase in seed iron while relatively reducing phytic acid levels of common bean seeds during biofortification results into increased iron bioavailability in human gut when biofortified bean varieties are consumed. Determination of phytic acid will enable understanding of the phytic acid to mineral molar ratios for estimation of the negative effect of phytic acid on common bean seeds iron, zinc and other divalent minerals bioavailability in human guts. The higher the phytic acid to mineral molar ratio in common bean seeds, the low bioavailability of the mineral from the bean consumed seeds in human gut while the lower the phytic acid to mineral molar ratio, the higher bioavailability of the mineral in the human gut.

1.4 Objectives

1.4.1 General objective

The overall objective of this study was to evaluate seed iron and zinc concentration of the common bean genotypes and increase their contents in seeds of the widely consumed yellow common bean varieties for improved nutrition security in Tanzania.

1.4.2 Specific objectives

- (i) To determine yield and yield components of common bean genotypes in three bean growing agro-ecological zones of Tanzania.
- (ii) To determine levels of iron and zinc among common bean genotypes in three bean growing agro-ecological zones of Tanzania.
- (iii) To determine levels of phytic acid, P, Mg and, Mn among common bean varieties grown in Tanzania.
- (iv) To determine the levels of ferritin protein using Western Blot analysis.
- (v) To develop F2 population of common bean crosses by crossing widely consumed yellow bean varieties with a high and stable seed iron and zinc content cultivar selected in objective (ii).

1.5 Hypothesis

This study was governed by the null hypothesis that there is no variation in seed iron, zinc, phytic acid, and other minerals in common bean genotypes grown in Tanzania and that seed iron and zinc contents cannot be increased in yellow beans. The alternative hypothesis was that, there are variations in seed iron, zinc, phytic acid, phosphorus, magnesium and manganese in common bean genotypes grown in Tanzania and that seed iron and zinc contents can be increased in yellow beans.

1.6 Significance of the study

Common bean is an important crop and source of essential micronutrient iron and zinc to humans. It is the mostly grown and consumed grain legume in Tanzania. This study is very important as it aimed at determination of seed iron and zinc contents of the common bean genotypes grown in Tanzania. Further importance of the current study is the aim the increase in seed iron and zinc contents of the widely consumed yellow bean varieties for the purpose of developing yellow bean varieties with high seed iron and zinc contents so as to improve the nutritional status of common bean consumers and contributing in alleviating iron and zinc deficiencies in Tanzania. Thus, information generated by this study will inform common bean breeders on the genotype to use when developing high seed iron and zinc-containing

varieties. Additionally, F2 lines will be further evaluated for developing breeding lines high in seed iron and zinc contents. In detail the study will:

- (i) Determine the influence of different agro-ecological zones of Tanzania on yield and yield components of common bean genotypes to identify stable and high seed yielding genotypes across sites as well as specifically adapted genotypes, which will be further evaluated and proposed for release as varieties or used in breeding programs for improving common bean seed yield.
- (ii) Determine the levels of common bean seed iron and zinc as influenced by different agro-ecological zones of Tanzania and identify high and stable seed iron and zinc-containing genotypes across agro-ecologies. The identified genotypes will be further evaluated and used in genetic iron and zinc biofortification by common bean breeders.
- (iii) Determine the levels of common bean seed phytic acid, ferritin, phosphorus, magnesium and manganese and identified those genotypes with low phytic acid, high ferritin, phosphorus, magnesium and manganese for further evaluation and use in common bean breeding programmes. In addition, phytic acid and mineral contents will be used to calculate phytic acid to mineral (iron, zinc, magnesium, and manganese) molar ratio, which is used to predict the minerals, such as iron and zinc bioavailability in human gut

1.7 Delineation of the study

This study is delimited to the followings:

- (i) The study aimed at determining levels of iron and zinc among common bean genotypes grown in Tanzania as influenced by three agro-ecological zones and increases the level of seed iron in yellow common bean varieties for improved iron status of common bean consumers in Tanzania
- (ii) Ninety-nine common bean genotypes were planted and harvested from three experimental sites, each located in a different agro-ecological zone and evaluated for seed minerals (iron and zinc) contents. Genotypes that were stable and had high seed iron content were selected and used as donor parents in developing crosses with high seed iron contents from widely consumed yellow beans in Tanzania.

- (iii) Sixty-one common bean genotypes from one experimental site (TARI-Selian) were used in determination of phytic acid, ferritin, phosphorus, magnesium and manganese levels.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is an annual herbaceous crop that belongs to Fabaceae family (Singh *et al.*, 2014). It originates from central and south America, whereby the crop was introduced in Africa by Portuguese (Bitocchi *et al.*, 2012). Common bean comprises of several cultivated genotypes that greatly differs in morphological and agronomical traits. The differences includes seed size and color, flower color and growth habit (Ng *et al.*, 2011). There are mainly two common bean growth habits, bush type that grows between 20 and 60 cm high and climbers, which grow between 2 and 3 m length. Most of the cultivated common bean genotypes have 4 to 6 seeds per pod, though some may contain up to 12 seeds per pod. Seed size varies from 15 to 90 g per 100 seeds (OECD, 2016). Common bean is mostly grown and consumed in Latin America and sub-Saharan Africa. It is a good source of vegetable protein, complex carbohydrates, vitamins and minerals (Celmeli *et al.*, 2018). The crop also contains ant nutritional factor such as phytic acid, which is believed to bind essential minerals like iron, zinc and magnesium (Hoppler *et al.*, 2014a).

Common bean performs well in environmental conditions with a temperature of 15 °C to 30°C, rainfall of 300 mm to 600 mm, and well-drained, loamy soils with pH ranging from 5.5 to 7.0 (Ossom *et al.*, 2006; Salcedo, 2008). In Tanzania, common bean is mainly grown in altitudes above 1000 m.a.s.l. for home consumption and income (Musimu, 2018). Worldwide Tanzania is ranked number seven and the largest producer of common bean in Africa followed by Uganda and Kenya. It is mostly grown in the Lake zone, Southern highlands, Northern and Western Tanzania (MoA, 2010). Despite the importance of common bean for food and incomes in Tanzania, the crop has been reported to be affected by extreme environmental conditions including: (a) very low or very high rainfall (below 300 mL or above 600 mL) as such conditions result in intermittent and or terminal drought, which negatively affects photosynthesis, causing a reduction in plant sugars, energy, quality and yield (Ntukamazina *et al.*, 2017; Diaz *et al.*, 2018). Too much rainfall, results in water logging, causing poor gas exchange between root and soil pore spaces. It also causes foliar diseases and root rot, thus reduces yield (Beebe *et al.*, 2013) (b) High temperature, such as day temperature of above 30 °C and night temperature above 20 °C as these conditions cause

flower bud, flower and pod abortion resulting in common bean seed yield reduction (De la Peña *et al.*, 2011; De Ron *et al.*, 2016) (c) Poor soil fertility, such as low nitrogen and available phosphorus causes a reduction in common bean yield through the reduction in nitrogen fixation activities and photosynthesis (Diaz *et al.*, 2018). Too acidic soils lead to aluminum toxicity which also reduces bean yield (Chekanai *et al.*, 2018). As a result of these adverse environmental factors, common bean production and productivity in Tanzania continue to be very low 1,035.4 kg/ha (FAOSTAT, 2016), compared to the potential yield of 1500 – 3000 kg/ha (the majority of non-climbing cultivars) (Namugwanya *et al.*, 2014) or up to 6000 kg/ha for some climbing bean cultivars (Williams, 2016). Nevertheless, the performance of bean and reaction to different environmental conditions vary between genotypes (Acquaah, 2013). Thus, there is a strong need to screen different bean genotypes (landraces, lines and improved varieties) performances in different environments and identify a few with superior stabilities and high seed mineral (e.g., iron and zinc) levels, yield, and yield components across different agro-ecologies for use in plant breeding programs targeting bean varietal development and release.

2.2 Iron deficiency anemia in sub-Saharan Africa

Micronutrient deficiency, commonly referred to as ‘hidden hunger’ among the Sub-Saharan African population is high, despite years of interventions taken by governments and international organizations to combat it. Among micronutrient deficiency (including iron, vitamin A and zinc), iron deficiency has been estimated to be a major health delinquent affecting approximately one third of the population (WHO, 2016; Abul-fadl *et al.*, 2018). Iron deficiency anemia affects pregnant women and children under the age of five more than other population groups, especially in developing countries such as those in Sub-Saharan Africa (Black *et al.*, 2013). In children, iron deficiency anemia is linked to poor cognitive and motor development while in adults it causes fatigue and low productivity (Balarajan *et al.*, 2011). Pregnant women have a high demand for iron to support fetal growth and development. Iron deficiency during pregnancy has been related to low birth weight, premature delivery and other perinatal complications, particularly hemorrhage (Mawani & Aziz, 2016).

About 62.3% (83.5 million) of children aged 6 – 59 months, 37% (69.9 million) of non-pregnant women and 46.3% (9.2 million) pregnant women aged 15 – 45 years in sub-

Saharan Africa are anemic (WHO, 2011) . Comparatively, the percentage of anemic children in Africa is higher than other regions globally (Fig. 1).

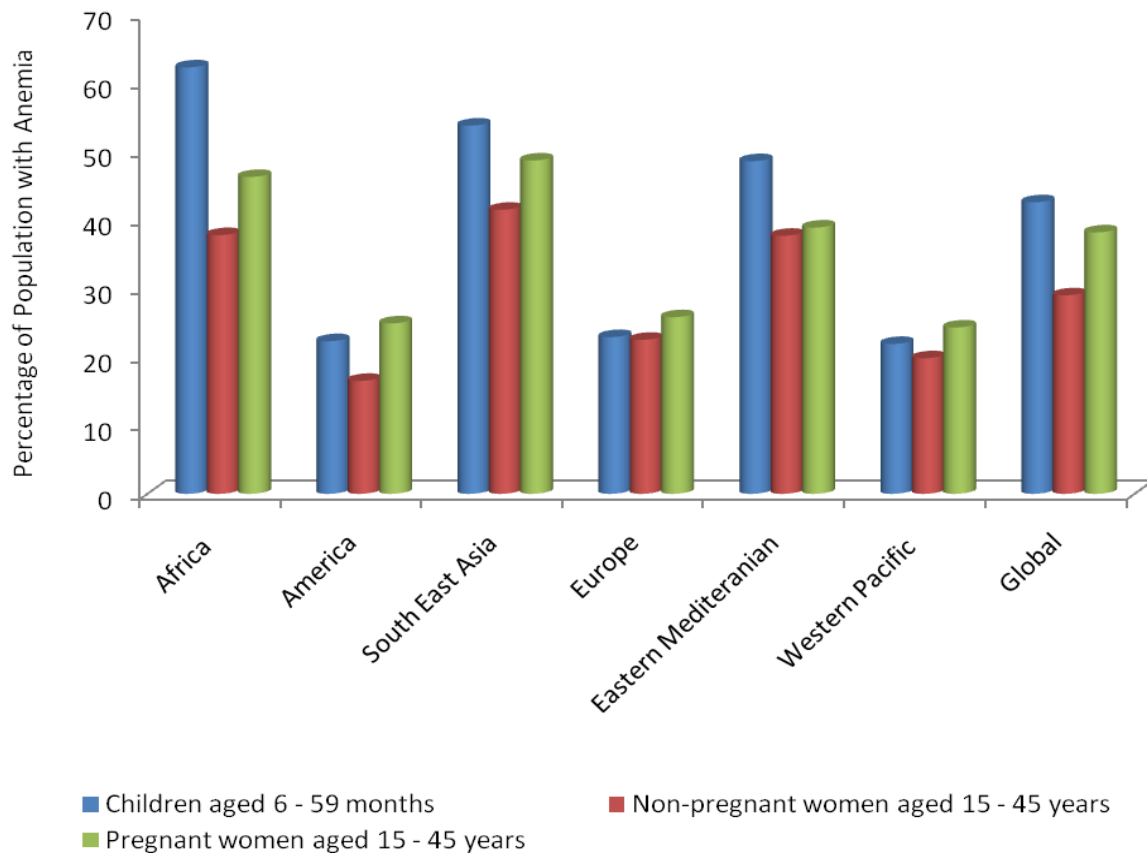


Figure 1: Anemia prevalence in preschool-age children, pregnant women, and non-pregnant women in each WHO region (WHO, 2011) report on anemia prevalence

Iron deficiency anemia was ranked number one by WHO (2016) report on Global Burden of Disease among the leading twenty causatives of the years lost due to disability (YLD) whereby the population of sub-Saharan Africa lived unhealthy life due to diseases (Fig. 2). Iron deficiency anemia causes 9.8% of all cases of YLDs which translates to 8.95 million YLDs in sub-Saharan Africa

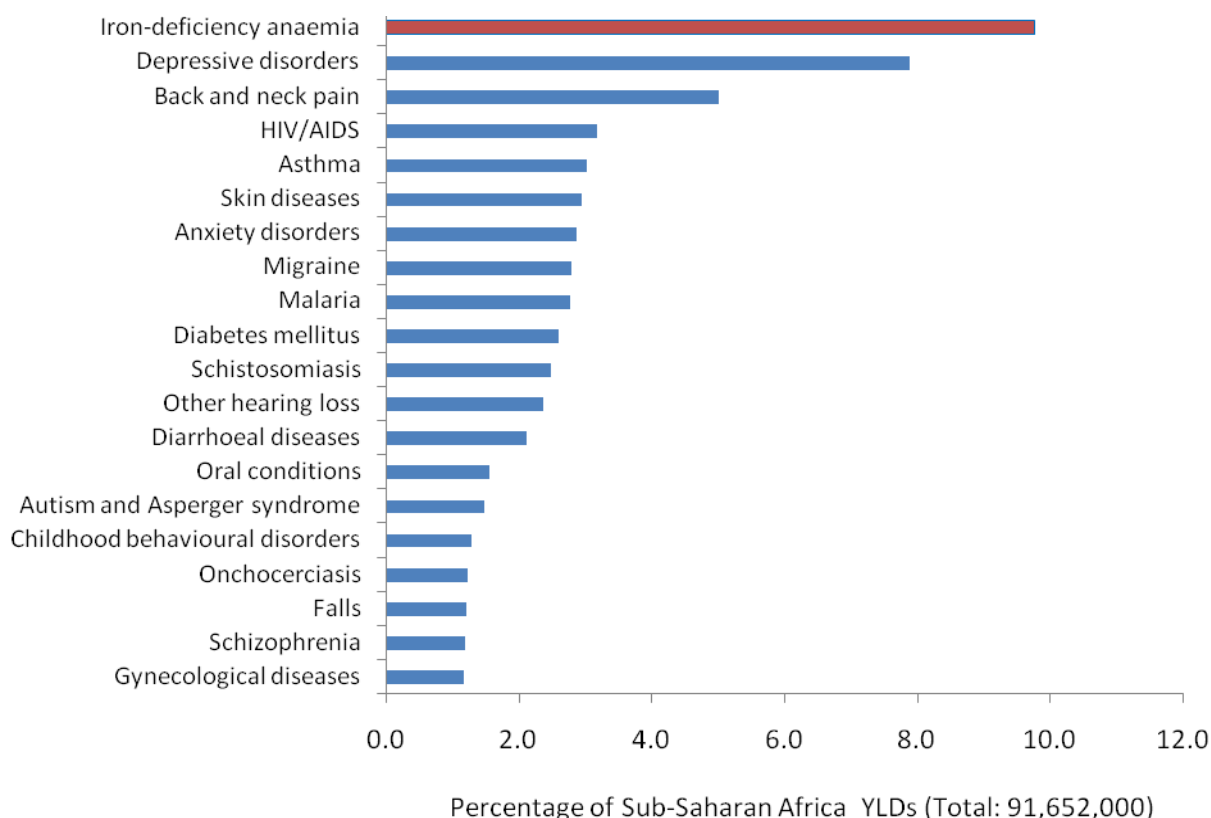


Figure 2: Percentage of YLDs (years lost due to disability) attributed to 20 leading causatives, in sub-Saharan Africa by 2015. Data sourced from WHO (2018) report on Global Health Estimates

The WHO (2016) report on Global Burden of Diseases ranked several diseases, injuries and risk factors by disability-adjusted life years lost (DALYs), a metric that combines years of life lost due to premature death, illness and disability. Iron deficiency anemia was ranked number 15 among the twenty main causes of DALY in sub-Saharan Africa (Fig. 3). This is translated to 11.6 million DALYs.

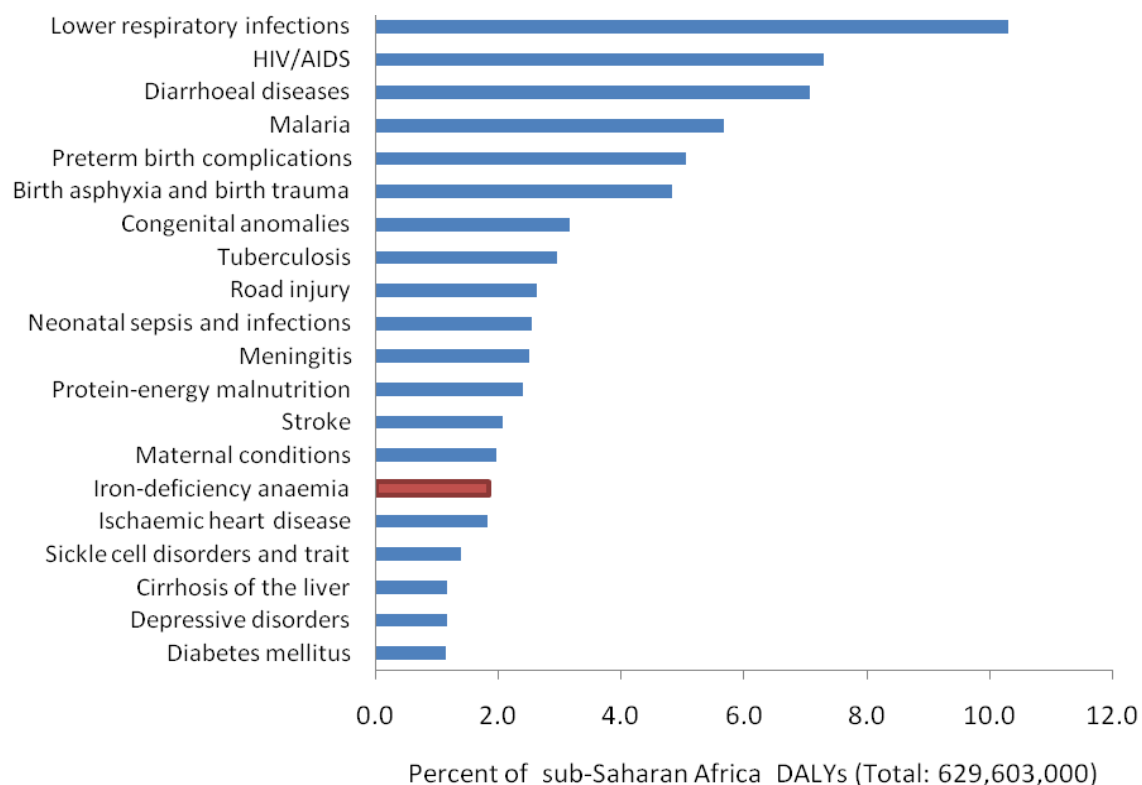


Figure 3: Percentage of DALYs (disability-adjusted life years lost) attributed to 20 leading risk factors, in sub-Saharan Africa region by 2015. Data sourced from World Health Organization (2018) report on Global Health Estimates

In Sub-Saharan Africa, the major cause of iron deficiency is consumption of diets which are poor in nutritional iron (FAO *et al.*, 2017). Limited availability and high cost of iron-rich food such as meat, fish, poultry and their products makes most of the populations residing in this region dependent on cereals and legumes (Osungbade & Oladunjoye, 2012). Furthermore, foods consumed in Sub-Saharan Africa are poor in vitamin C, which is said to increase absorption of iron in the human gut (Mwangi *et al.*, 2017). On the hand, cereals and legumes contain high levels of phytic acid and polyphenols which impair iron absorption from these foods making only 1 – 22% of the total iron contained in cereals and legumes available for absorption (Nielsen *et al.*, 2013).

2.3 Zinc deficiency status in sub-Saharan Africa

Malnutrition due to micronutrient deficiencies is a global public health problem despite several ongoing interventions to combat the problem (WHO, 2013). Compared to other global regions, sub-Saharan Africa as home for most resource poor population is more affected by micronutrient deficiencies (Fanzo, 2012). Zinc deficiency is among major risks to

human health, whose measured adverse outcome of exposure includes; diarrhea, pneumonia and malaria (WHO, 2009). Zinc deficiency ranks number three, after iron and vitamin A among the micronutrient deficiencies (WHO, 2013). Globally zinc deficiency effects has an estimate range of 4 – 73% across sub regions (WHO, 2002). In sub-Saharan Africa, zinc deficiency accounts for 18 – 22% attributable fractions for lower respiratory tract infections, 11 – 13% attributable fractions for diarrheal diseases and 10 – 22% attributable fractions for malaria (WHO, 2002; WHO, 2013). Lower respiratory infections, Diarrheal diseases and Malaria are among the leading cause of disability-adjusted life-year (DALY) in sub-Saharan (Fig. 4). In Tanzania about 37.5 % of the population is at risk of insufficient zinc intake, this puts the country in the group of high zinc deficiency countries globally (Ministry of Health, Community Development, Gender, Elderly and Children [MoHCDEC], 2014).

Zinc deficiency increases the risk of incidence for these infectious diseases as it impairs multiple aspects of immune function. These includes barrier and non-specific immunity, specific immune components, and mediators of immune function such as glucocorticoid and thymulin activity, and cytokine function (Bagherani & Smoller, 2016). Zinc deficiency negatively influence human body iron and vitamin status. It triggers synthesis of hepcidin molecule in the human gut, which decreases iron absorption (Kondaiah *et al.*, 2019). Vitamin A metabolism in humans depends on zinc-containing enzymes (Rahman *et al.*, 2002). In most cases zinc deficiency is associated with insufficient intake or absorption of zinc from the diet, however to some extent excess losses of zinc during diarrhea may also contribute (Plum *et al.*, 2010). People with gastrointestinal, chronic liver and renal, sickle cell and diabetes diseases are at high risk of suffering from zinc deficiency, due to reduction in zinc absorption and increased endogenous zinc losses (Bailey *et al.*, 2015; Kondaiah *et al.*, 2019). Pregnant and lactating women are also at risk of being zinc deficiency, due to high need of the mineral for growing and development of the fetus, on the other hand lactation reduces maternal zinc store (Rahman *et al.*, 2002; Ryz *et al.*, 2009). Children are at high risk of becoming zinc deficient, as they need more zinc for growth and development, zinc regulated cell growth and growth hormone metabolism (Nishi, 1996).

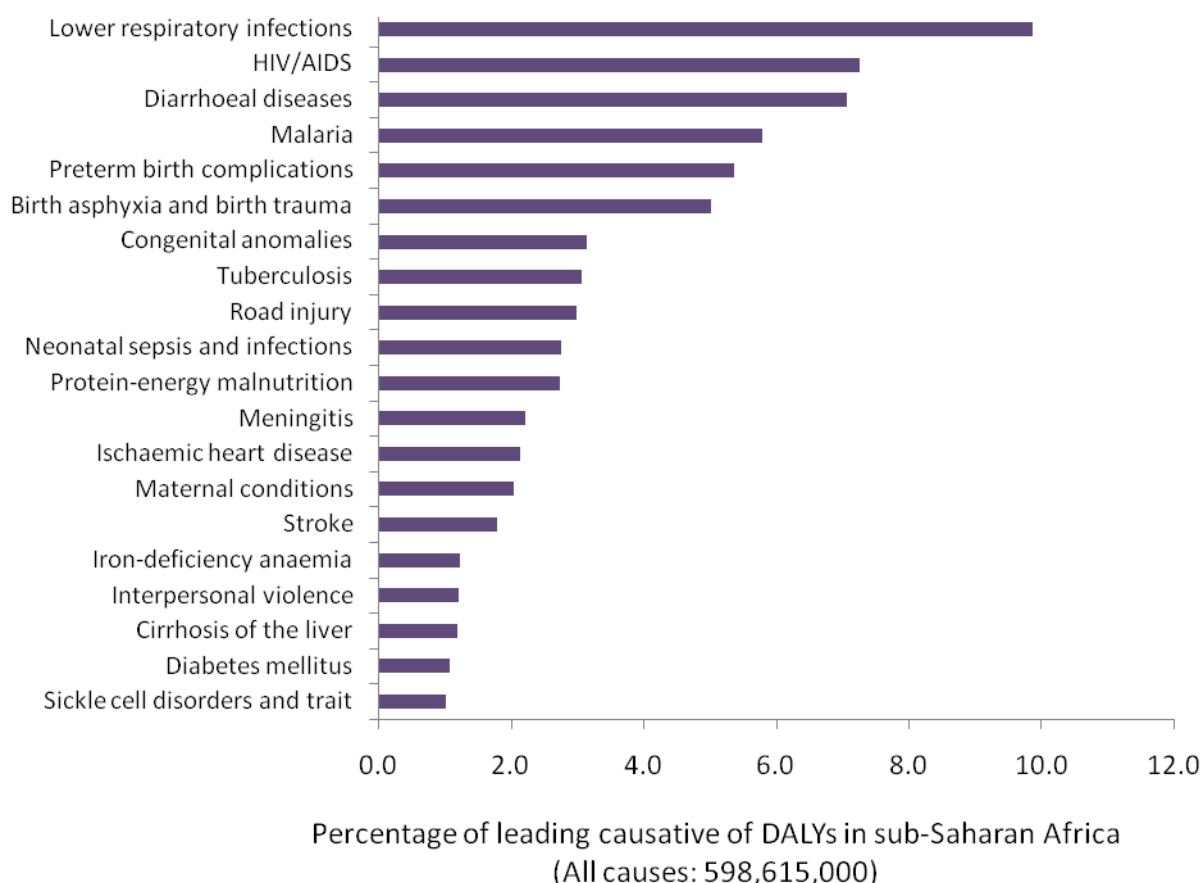


Figure 4: Percentage of DALYs (disability-adjusted life years lost) attributed to 20 leading causes in sub-Saharan Africa region by 2016. Data sourced from WHO (2018) report on Global Health Estimates

The global health risks on mortality and burden of disease attributable to selected major risks report for sub-Saharan Africa ranked zinc deficiency number 7 among the 20 leading risk factor causes of DALYs (Fig. 5). Disability-adjusted life years losts are calculated as the sum of the years of life lost due to premature mortality in the population and the years lost due to disability for incident cases of the disease or injury. Among the leading risk factor, zinc deficiency accounts for 2.4% of all DALYs cases, which translates to 8.96 million in sub-Saharan Africa (WHO, 2009).

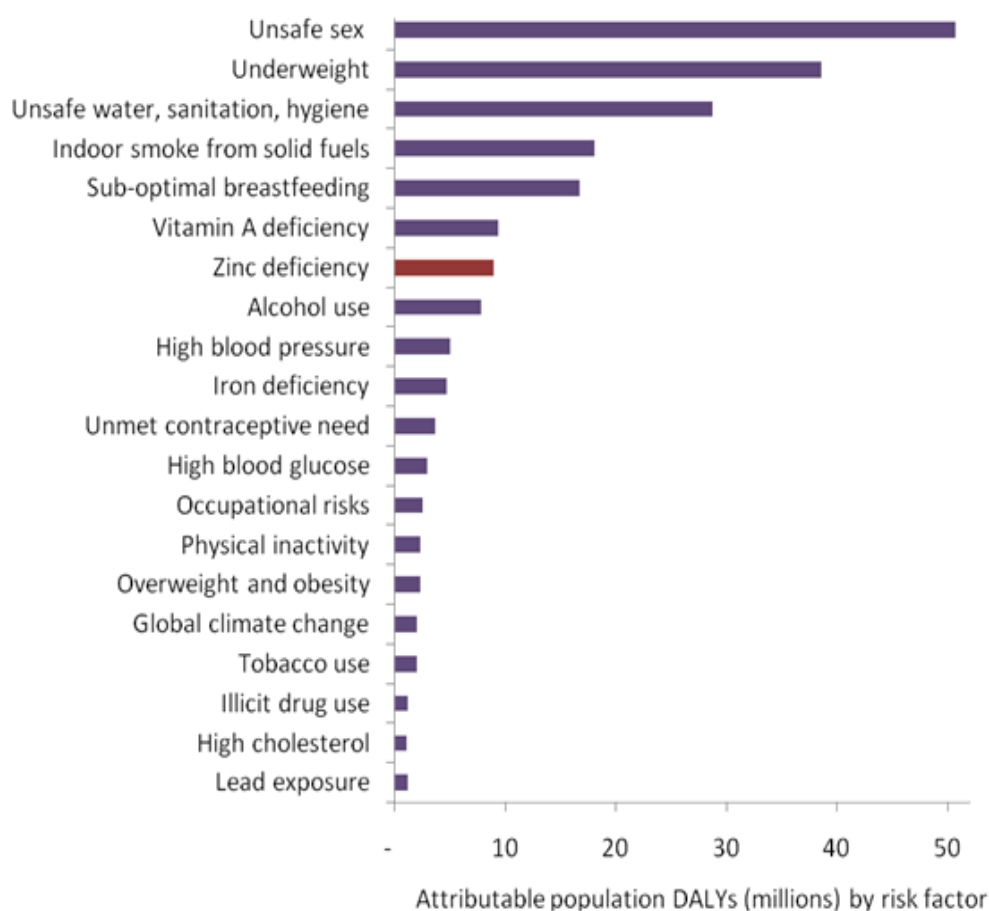


Figure 5: Estimates of DALYs attributable to 20 major health risks in sub-Saharan Africa region. Data sourced from global health risks report (WHO, 2009)

Zinc deficiency was ranked number 9 among the 20 leading risk factor causes of deaths in sub-Saharan Africa (Fig. 6). It was reported that 2.2% of all deaths which translates to 249 thousands deaths in Sub-Saharan Africa was caused by zinc deficiency (WHO, 2009).

In developing countries such as those in sub-Saharan Africa, zinc deficiency is mainly caused by utilization of food with low nutritional zinc (WHO, 2018; Kondaiah *et al.*, 2019). Poor availability of animal and fish-source foods and low per capita income, makes most of the populations in sub-Saharan region not afford meat-source foods which are rich in available zinc and thus depends much on cereals, legumes and roots and tubers (Rahman *et al.*, 2002; Ryz *et al.*, 2009). Additionally, there is low consumption of fruits and vegetables, foods rich in vitamin C, proven to increase absorption of zinc in the human gut (WHO, 2009). Even though bioavailability of zinc from plant foods in human gut is low, being negatively influenced by inhibitors such as phytic acid, tannins, dietary fiber and calcium (Hess & King,

2009; Liu *et al.*, 2017). Bioavailability of zinc from plant based foods ranges from 5.5 – 56.5% (Hemalatha *et al.*, 2007).

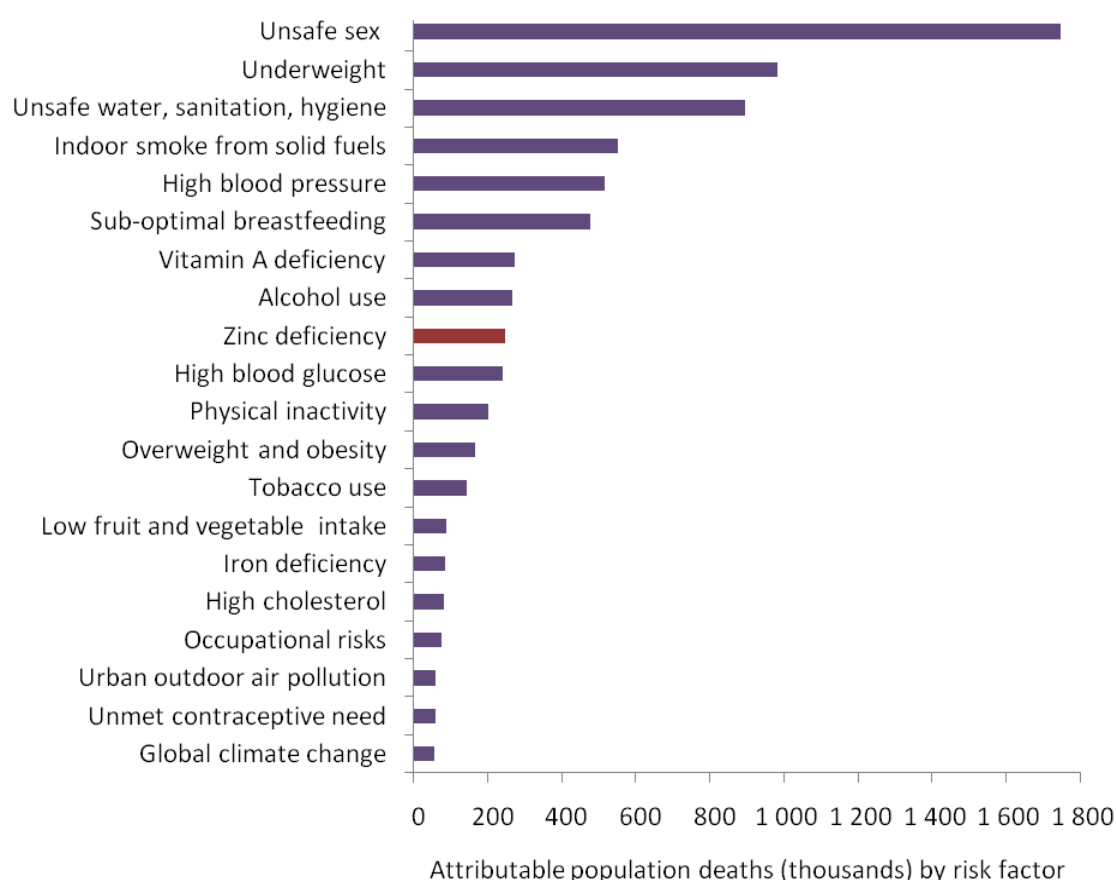


Figure 6: Estimates of mortality attributable to 20 major health risks in sub-Saharan Africa region. Data sourced from global health risks report (WHO, 2009)

Despite the measures being taken to alleviate zinc deficiency prevalence in sub-Saharan Africa, its effect among the populations showed no significant decrease. Thus there is a need of applying supplementation, food chemical fortification and currently biofortification so that, there is a complementation of the options as there is no single existing method that can alleviate micronutrient deficiency in sub-Saharan African countries including Tanzania (Bouis & Saltzman, 2017).

2.4 Strategies used in combating iron and zinc deficiencies

To date, there are several interventions which have been used by international organizations and governments to combat iron and zinc deficiencies in sub-Saharan Africa. The interventions include supplementation, fortification and recently biofortification (WHO, 2014).

2.4.1 Supplementation

Supplementation involves provision of minerals and vitamins in the form of low-cost tablets, capsules and syrups to the population groups exposed to micronutrients deficiencies. It is advised that, every pregnant woman in sub-Saharan Africa and other developing countries have to receive iron supplement during pregnancy time in the form of a tablets or syrup composed of 60 mg of iron and 400 µg of folic acid for body hemoglobin concentration and iron status improvement (Osungbade & Oladunjoye, 2012; WHO, 2014). In populations where anemia prevalence is $\geq 40\%$ among pregnant women, daily dose of iron supplement is recommended while in populations where anemia prevalence is less than $\leq 20\%$ a dose of iron supplement once per week is recommended (WHO, 2017). Sufficient iron intake is of much importance particularly to children and pregnant women, in most cases, the recommended dietary allowance (RDA) for iron (Table 1), should be achieved through food intake, when not met, iron supplementation is used as an alternative (National Institutes of Health, [NIH], 2019).

Table 1: The recommended dietary allowance (RDA) for iron

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.27 mg	0.27 mg		
7–12 months	11 mg	11 mg		
1–3 years	7 mg	7 mg		
4–8 years	10 mg	10 mg		
9–13 years	8 mg	8 mg		
14–18 years	11 mg	15 mg	27 mg	10 mg
19–50 years	8 mg	18 mg	27 mg	9 mg
51+ years	8 mg	8 mg		

Although supplementation of iron through providing universal iron-folate to pregnant women, iron deficiency anemia in sub-Saharan Africa among pregnant women is still a public health problem (Derso *et al.*, 2017). In Tanzania a daily oral dose of 60 mg of iron supplement (as ferrous sulfate) given to pregnant women only reduced the risk of maternal iron deficiency and iron deficiency anemia by 52 % and 66 % respectively (Etheredge *et al.*, 2015). Iron supplement provided to pregnant women in sub-Saharan Africa have not decreased iron deficiency problem among women due to poor distribution of the tablets and syrups, side effects (high hemoglobin concentration greater than 130 g/L), drop out and inappropriate use of iron supplement (Pena-Rosas *et al.*, 2012).

There a number of zinc supplements present and used to improve human health status, these include zinc acetate, zinc gluconate, zinc picolinate, and zinc sulfate (Mayo-Wilson *et al.*, 2014). Sufficient zinc intake is of much importance particularly to children and pregnant women, in most cases, the recommended dietary allowance (RDA) for zinc (Table 2), should be achieved through food intake, when not met, zinc supplementation is used as an alternative (Trumbo *et al.*, 2001).

Table 2: The recommended dietary allowance (RDA) for zinc

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	2 (AI)	2 (AI)
Infants	7-12 months	3	3
Children	1-3 years	3	3
Children	4-8 years	5	5
Children	9-13 years	8	8
Adolescents	14-18 years	11	9
Adults	19 years and older	11	8
Pregnancy	18 years and younger	-	12
Pregnancy	19 years and older	-	11
Breast-feeding	18 years and younger	-	13
Breast-feeding	19 years and older	-	12

In clinical management of diarrhea, particularly in developing countries like Tanzania and those found in sub-Saharan Africa, WHO recommends that children older than six months should be supplemented with zinc at a dose of 20 mg/day while infants under age of six months should be given zinc at a dose of 10 mg/day for 10 to 14 days (WHO, 2005). Zinc supplementation in a dose of 10 mg/day provided for 168 days have significance increase in growth of children under age of 5 years (Imad, 2011). According to American Society for Clinical Nutrition a zinc supplement at the dose of 400 µg/kg/day is recommended for the premature newborn (Bagherani & Smoller, 2016). Though zinc supplementation has been administered to children and other people in need for some decades, zinc deficiency is still a public health problem. It's coverage is limited by human and infrastructural capacity, of which in most cases these are poor in developing world, the intervention needs always trained personnel and training programs to the populations, thus making it not cost-effective and difficult to reach poor resource population residing in rural areas (Stein *et al.*, 2007; Mayo-Wilson *et al.*, 2014). There is a need of advocating other friendly alternative methods like biofortification which can easily reach resource poor populations such as those residing in Tanzania and other sub-Saharan African countries complementing iron and zinc supplementation in reducing iron and zinc deficiencies.

2.4.2 Food Fortification

Fortification involves adding vitamins and minerals to foods that are frequently eaten, for example flour (iron, thiamin, riboflavin, niacin, folic acid, vitamin A and Zinc), sugar (vitamin A), salt (iodine) cooking oil (vitamin A, D) and condiments (iron) (Allen *et al.*, 2006; World Health Organization, 2016). Iron is added in the form of iron salts (NaFeEDTA) and iron powder to cereal flour, as approved by FAO/WHO at the rate of 0.2 Fe/kg body weight per day, in case of high-phytate food, addition of Na₂EDTA plus ferrous sulfate is recommended (Allen *et al.*, 2006; WHO, 2017). Globally food fortification mandatory countries have been increasing from one in 1940 to 133 in 2017. In Africa, until 2017, forty countries were mandatory for food fortification, of the forty countries 36 are sub-Saharan African countries (Global Fortification Data Exchange, [GFDE], 2018). Up to 2017, twenty-five countries in sub-Saharan Africa were implementing iron fortification in wheat and or maize flour. The quantities of iron added to wheat and maize flour varied from one country to another, with range of 15 – 40 ppm (Fig. 7) for maize and 20 - 60 ppm for wheat flour (Fig. 8).

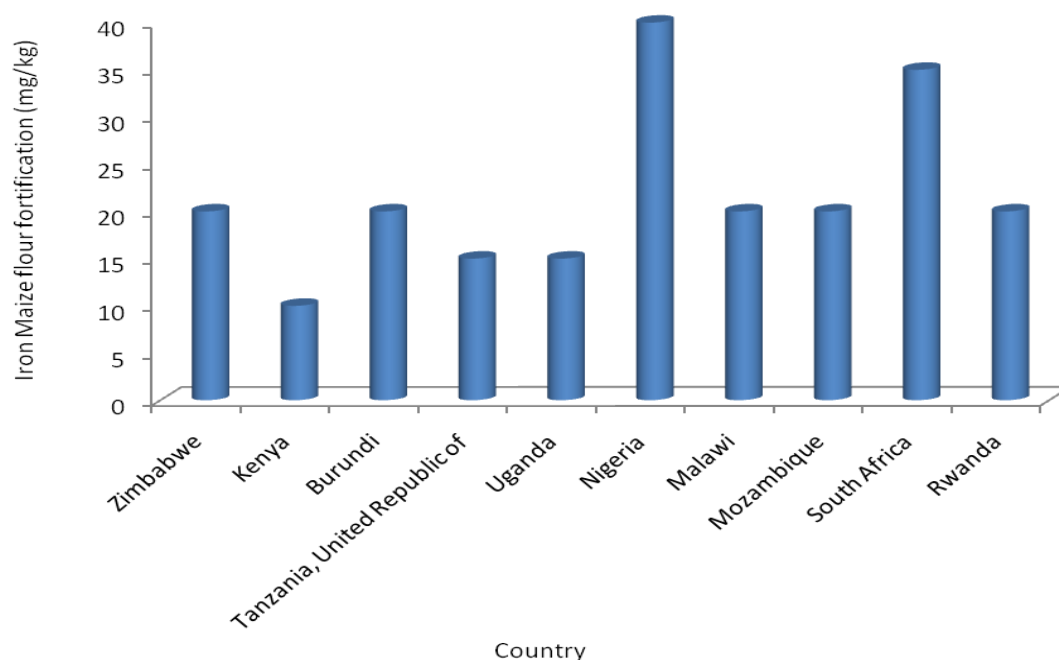


Figure 7: Recommended Iron quantity for fortification of maize flour in sub-Saharan Africa, data sourced from Global Fortification Data Exchange (2018)

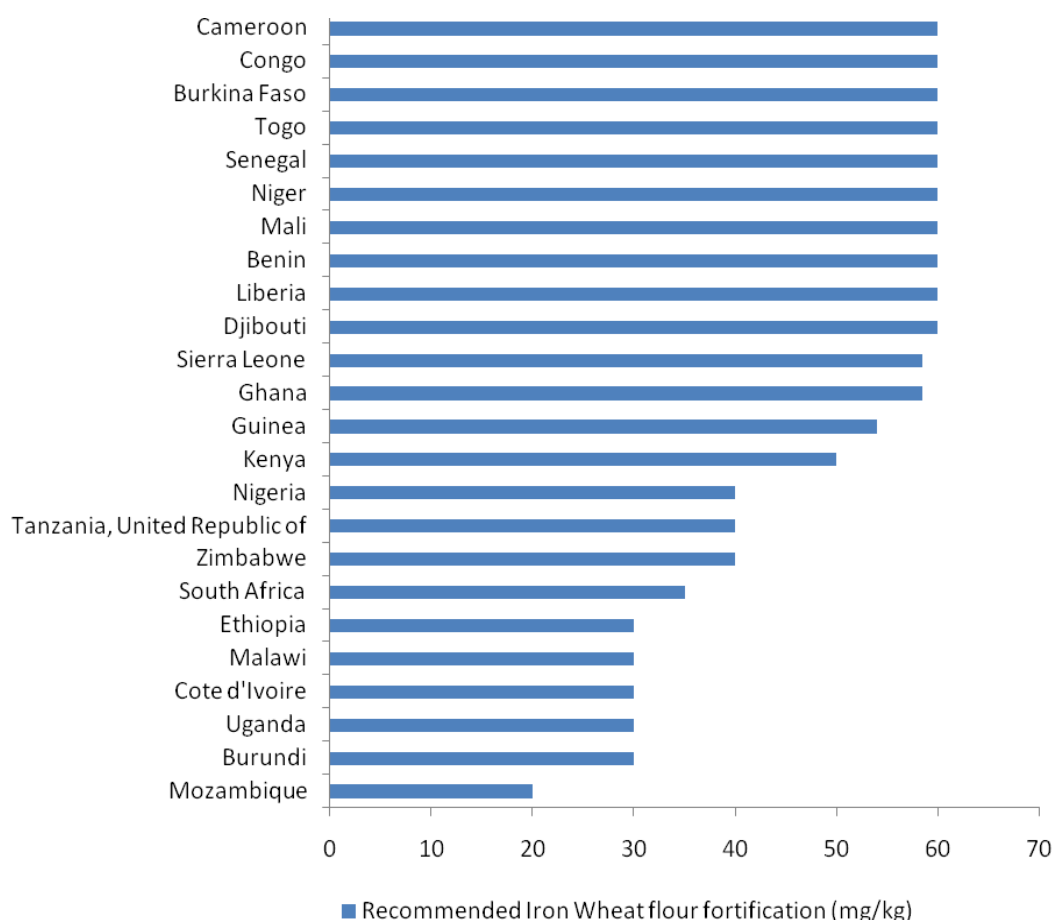


Figure 8: Recommended Iron quantity for fortification of wheat flour in sub-Saharan Africa, data sourced from GFDE (2018)

The most commonly used fortificants of zinc food fortification are zinc oxide and zinc sulfate with zinc oxide being more preferred, as it is the most cheapest fortificants compared to others (WHO, 2005; Jha & Warkentin, 2020). In sub-Saharan Africa, zinc fortification is mostly applied in maize and wheat flour, whereas until 2017 a total of 10 and 14 countries had mandatory fortification of maize and wheat flour, respectively (Global Fortification Data Exchange [GFDE], 2020a). The levels of zinc added to wheat and maize flour varied from one country to another, with range of 15 - 95 and 15 - 50 mg/kg, respectively (Fig. 9).

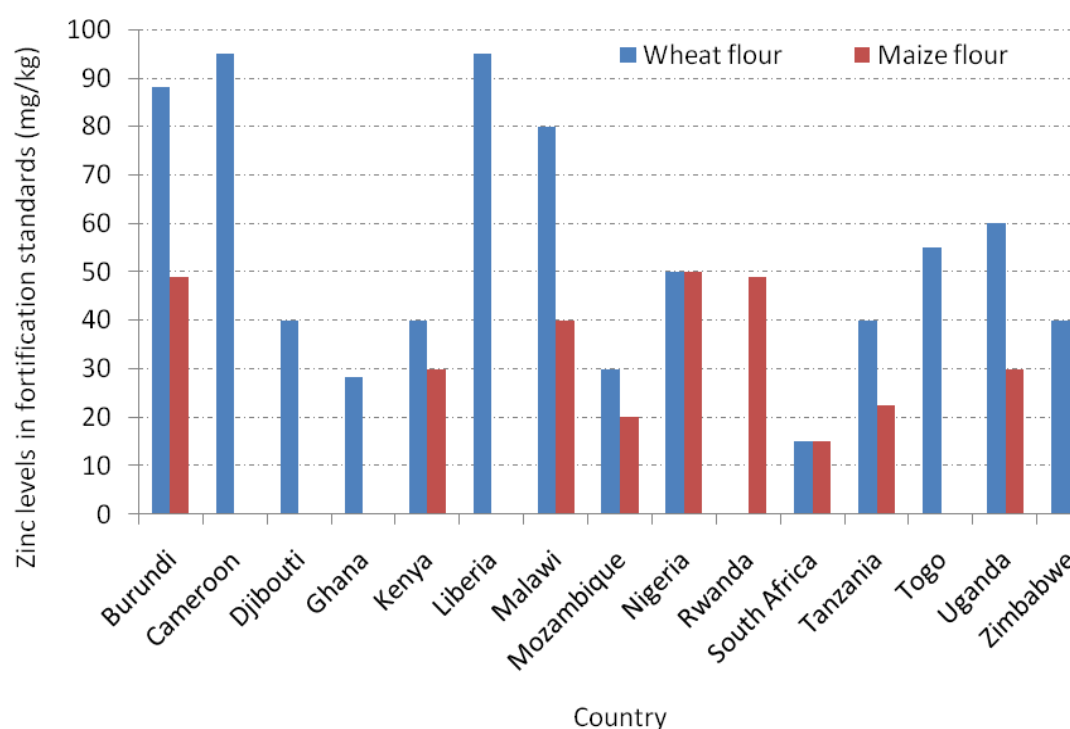


Figure 9: Recommended zinc levels for fortification of wheat and maize flour in sub-Saharan Africa, data sourced from GFDE (2020a)

Despite the facts that zinc fortification results into high and faster food zinc content increment to the satisfactory level, the intervention has not been successful in sub-Saharan Africa as it was expected. The technique requires infrastructure to develop fortificants, ability of consumers to buy or access to markets, and most grains are milled by small scale millers in both urban and the villages (Ferrão *et al.*, 2017). For instance, the proportion of industrially processed maize, which is a staple food in the region, is very low (Fig. 10). In Tanzania only 2.5 % and 33.1% of the population consume fortified maize and wheat flour respectively (GFDE, 2020b).

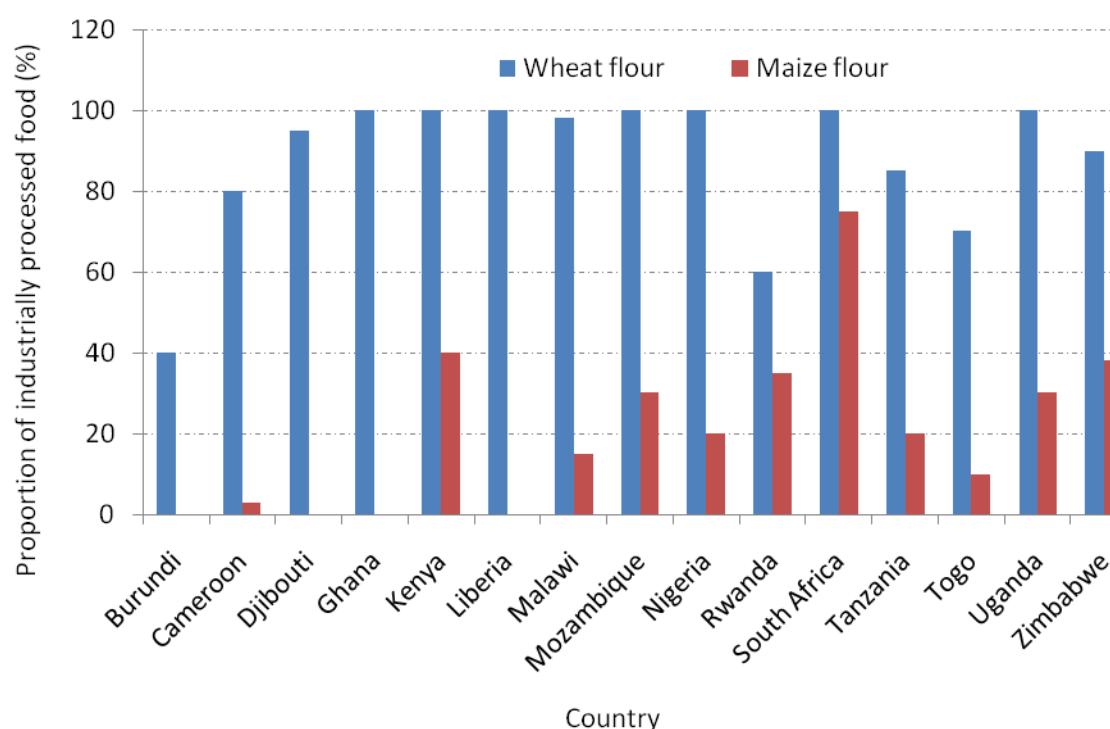


Figure 10: Proportion of industrially processed mandatory maize and wheat zinc fortification countries in sub-Saharan Africa, data sourced from GFDE (2020b)

Although food chemical fortification increases micronutrient content e.g., iron and zinc instantly to the required level, the intervention faces a lot of challenges in sub-Saharan Africa. The practice requires infrastructure to develop fortificants, ability of consumers to buy or access to markets, and most grains are milled by small scale millers in both urban and the villages (Ferrão *et al.*, 2017). Based on the fact that, iron and zinc deficiency prevalence is still high in sub-Saharan African countries e.g., Tanzania, there is a need of including other sustainable, friendly and affordable interventions such as biofortification for reduction of this public health problem.

2.4.3 Biofortification

Iron and zinc biofortification can be done through two main approaches namely agronomic practices and plant breeding (Jha & Warkentin, 2020)

(i) Agronomic biofortification

Agronomic biofortification is the application of soil or foliar fertilizers for the purpose of increasing the micronutrient content of edible parts of the plant (Mao *et al.*, 2014). This

works well for zinc (Mao *et al.*, 2014; Liu *et al.*, 2017) and for selenium (Mao *et al.*, 2014; Reis *et al.*, 2018). In contrast, iron fertilization has not successfully increased the iron content in edible parts of crops (Liu *et al.*, 2017). Some studies involved application of foliar iron fertilizer combined with chelates, reported significance increase in grain iron concentration (Márquez-Quiroz *et al.*, 2015; Ram *et al.*, 2016). However some studies which involved foliar application of iron and chelated iron fertilizer reported to have no significance increase in grain iron content (Sánchez *et al.*, 2012; Ciccolini *et al.*, 2017). Iron is the fourth most abundant element in the earth's crust, but it is poorly bioavailable because it binds to soil particles and forms insoluble complexes under aerobic conditions at neutral or alkaline pH, (Márquez-Quiroz *et al.*, 2015). Thus, iron fertilization can be possible for alkaline soils, in particular for growing fruit trees (peach, apricot). When amount of iron is more than required for physiological processes by plants, it becomes toxic, this causes accumulation of hydroxyl radicals through Fenton reaction, which in turn damage plant cells (Saaltink *et al.*, 2017; Connorton *et al.*, 2017). Plants have developed mechanisms which regulate the concentration of iron in the cells so that it does not accumulate to toxicity level. These mechanisms includes, uptake, transport and distribution of iron from the soil into different parts of the plants where it is metabolized and excess stored in iron storing protein called ferritin (Krohling *et al.*, 2016; Connorton *et al.*, 2017).

In most cases zinc mineral fertilizer is applied as zinc chelates (contain approximately 14% zinc), zinc sulphate (25-36% zinc) and zinc oxide (70-80% Zinc), where zinc sulphate is the widely used zinc mineral fertilizer (Chattha *et al.*, 2017; SMART, 2020). Zinc mineral fertilizers are applied into soils when there is poor phytoavailability of zinc mineral (Ramzan *et al.*, 2020). Application of zinc mineral fertilizer increases its availability, uptake by plants and contents in plant edible parts (Aciksoz *et al.*, 2011). A number of studies revealed increase in plants zinc content after zinc soil fertilization. An increase of up to 75.2 % in wheat grain zinc content was reported after zinc soil fertilization in China (Wang *et al.*, 2016). Rice grain zinc increase of up to 92.6 % was reported in India, after basal soil zinc sulphate application at maximum tillering and flowering stage (Saha *et al.*, 2017). In common bean 100 % increase in seed zinc content was reported in Brazil when zinc sulphate was applied as a soil fertilizer (Cambaia *et al.*, 2019). Application of zinc sulphate as foliar fertilizer increased wheat grain zinc content by 47.8 – 83.0 % whereas an increase of up 27 % in rice grain zinc content was reported as a result of zinc sulphate foliar fertilizer application (Chattha *et al.*, 2017; Saha *et al.*, 2017). A non-significant to significant increase of up to

14.7 % in grain zinc concentration was reported in common bean as a result of foliar zinc sulphate fertilizer application (Cambraia *et al.*, 2019). Zinc increment in grains among other factors, influenced by the variety, type of the crop used, and soil zinc status (Aciksoz *et al.*, 2011; Saha *et al.*, 2017).

Since agronomic biofortification require farmers to buy fertilizer every planting season, it is a challenge to resource poor farmers of Tanzania and other sub-Saharan African countries. Thus plant breeding to improve plants on iron and zinc uptake, transport, distributions and storage particularly in edible parts is more useful. As in most soils iron is not a limiting factor, but the ability of plants to absorb from the soils, transport up the plant, distribution into different plant parts and storage in edible parts differs from one plant to another and among varieties of the same crop.

(ii) Genetic biofortification

Genetic biofortification is the process of increasing the concentration of vitamins and minerals to the edible part of the crop through plant breeding so that when consumed regularly they can generate measurable improvement in nutritional status in humans and other organisms (La Frano *et al.*, 2014; Vasconcelos *et al.*, 2017). Iron and zinc biofortification started with rice in 1992 in Asia (Gregorio *et al.*, 2000) while for common bean it was reported in 1999 in South America (Welch & Graham, 2004). Genetic iron and zinc biofortification is cost-effective and can more easily reach rural and resource poor populations. Moreover, farmers can grow and re-grow iron biofortified varieties at zero cost and consume them for improved nutritional iron status (Blair, 2013; Garcia-Casal *et al.*, 2017). In sub-Saharan Africa, mineral biofortification mainly focuses on staple food crops which are widely cultivated and consumed, that include cassava, maize, sweet potato, legumes, sorghum, rice and wheat (Kodkany *et al.*, 2013; Rawat *et al.*, 2013). According to MoA (2019), common bean is ranked number one in terms of production quantity among leguminous crops and number five among staple food crops grown in Tanzania (Fig. 11).

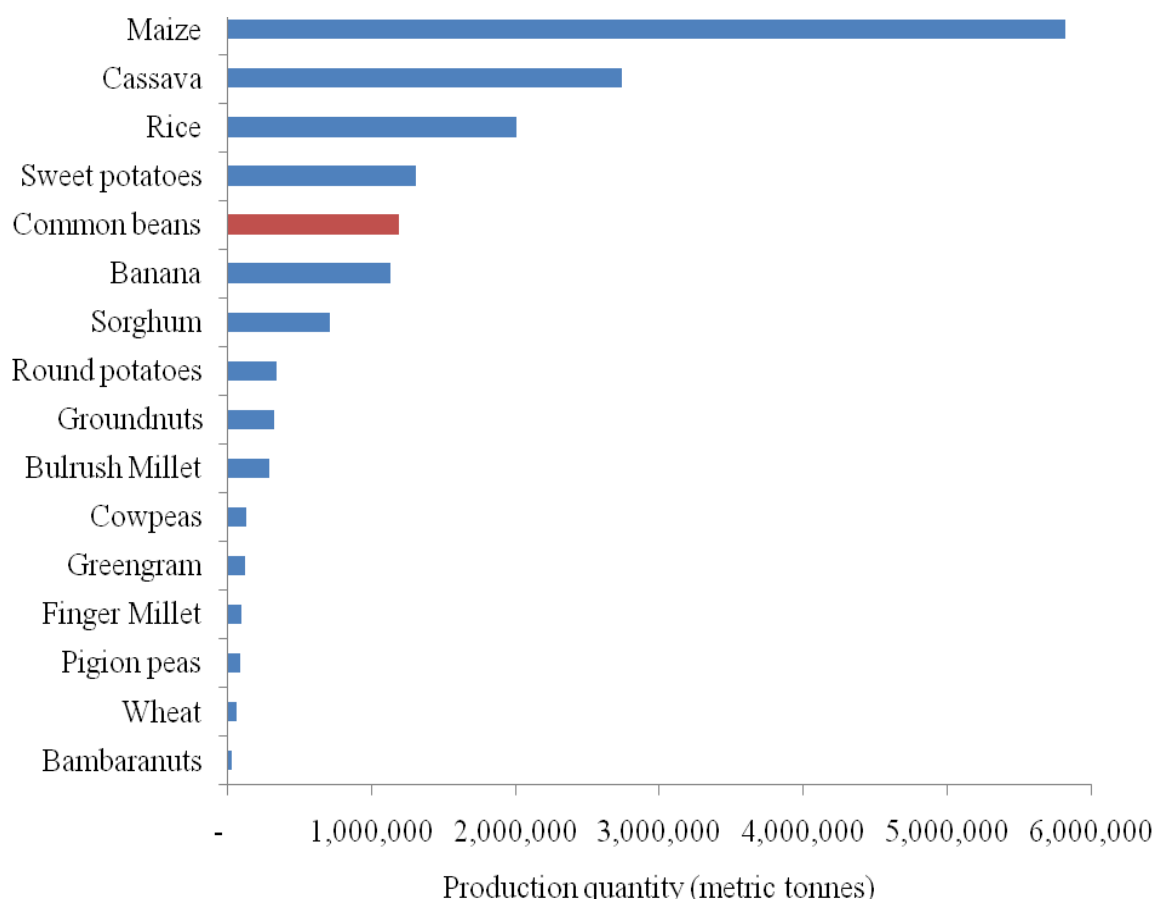


Figure 11: Production quantity of the leading 16 food crops in Tanzania, data sourced from MoA (2019)

In cereals including maize minerals like iron and zinc are more localized in embryo and aleurone layer, whereas in common beans the minerals are almost distributed in all seed parts (Bityutskii *et al.*, 2014) (Fig. 12 and 13). In most cases cereal grains are consumed after milling, a process which removes zinc highly concentrated parts (embryo and aleurone) leaving endosperm which has very low zinc concentration (Cakmak & Kutman, 2018). On the other hand, common bean grain are consumed as whole making the crop a good source of plant based zinc and thus good for genetic biofortification (Jha & Warkentin, 2020).

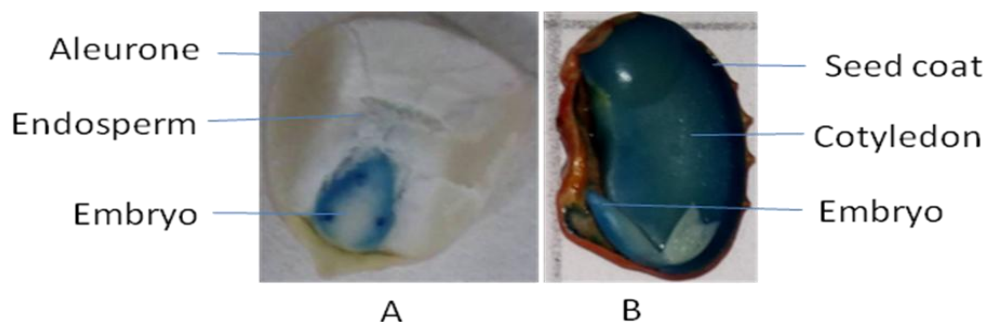


Figure 12: Distribution of Fe in maize (A) and common bean (B), visualized with Perl's staining (blue) as Fe-sensing dye that develops a blue complex with Fe. The intensity of the blue color is associated with the Fe content. Sourced from Plant Metabolism for Improved Nutrition and Health Summer School (2017)

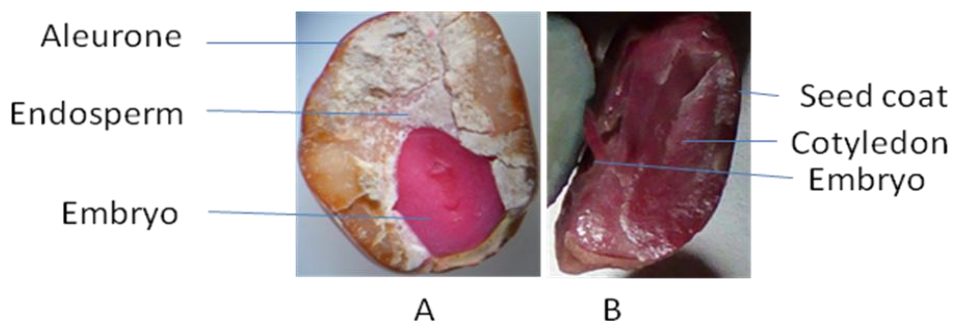


Figure 13: Distribution of Zn in maize (A) and common bean (B), visualized with dithizone as a Zn-sensing dye that develops a red complex with Zn. The intensity of the red color is associated with the Zn content. Sourced from Plant Metabolism for Improved Nutrition and Health Summer School (2017)

Most populations in Tanzania consume a diet based on cereals, roots and tubers, usually maize, cassava, rice with a legume crop mostly dominated by common beans compared to other legumes. Common beans and other grain legumes are used as supplements to cereal based diet as they are rich in protein and minerals compared to cereals, roots and tubers that are rich in carbohydrates. Being the most consumed grain legume with cereals, common beans become an important crop for genetic iron and zinc biofortification.

Common bean iron biofortification in Sub-Saharan Africa have been implemented in Rwanda, Uganda and Democratic Republic of Congo (DRC), that resulted into release of several iron biofortified farmer and consumer preferred varieties in these countries (Petry *et al.*, 2015; Bouis & Saltzman, 2017). In Rwanda, biofortified common beans had an increase in seed iron content of up to 90%, by having 94 ppm from a baseline start of 50 ppm

(Saltzman *et al.*, 2016; Bouis *et al.*, 2017). Subsequent nutritional studies in Rwanda showed the positive effect of the high-iron, improving the iron nutritional status of women aged 18 – 27 years significantly. In particular, increase in their hemoglobin, cognitive and performance upon consumption of the iron biofortified varieties with iron concentration of 86 ppm for 128 days at a rate of 336 g cooked common beans per day, which is equivalent to 14.5 ± 1.6 mg Fe per day, hemoglobin increased from 121 ± 13.9 to 124 ± 13.8 g/L (Haas *et al.*, 2016; Murray-Kolb *et al.*, 2017; Finkelstein *et al.*, 2017).

Globally the target for genetic zinc biofortification in common bean has been to develop cultivars with 40 % more seed zinc contents than the low zinc-containing genotype without compromising farmers and consumers preferred agronomic properties (Blair, 2013). In most cases common bean genetic zinc biofortification in sub-Saharan Africa has been treated as secondary mineral after iron regardless the potential of zinc to human health (Ritchie, 2017; Yu *et al.*, 2019). In this region, common bean zinc biofortification have been implemented in Democratic Republic of Congo (DRC), Ethiopia, Rwanda, Sudan and Uganda (Petry *et al.*, 2015a; FAOSTAT, 2018). The programme resulted into 50 % increase in seed zinc contents, though the primary focus was breeding for high seed iron content (Ugen *et al.*, 2009). Nine (6-bush and 3-climber type) high zinc-containing bean varieties have been released in the above named countries (Bouis *et al.*, 2013; Bouis & Saltzman, 2017). Zinc biofortified common bean varieties have been reported to retain zinc concentration up to 99.4 % after undergoing preparations for home recipes (Hummel *et al.*, 2020). Several studies have revealed that there is no significance correlation between iron and zinc mineral in grains (Ugen *et al.*, 2009; Liu *et al.*, 2017; Philipo, *et al.*, 2020). Even though there are some zinc biofortification programmes going on in sub-Saharan Africa, there is limited information on the effect of zinc biofortified varieties on nutritional zinc status of the target populations.

Iron and zinc genetic biofortification of the widely consumed yellow common bean varieties have not been implemented in Tanzania which is the largest producer of common beans in Africa. Thus, there is a need to adopt iron and zinc biofortification strategy so as to reduce and control iron and zinc deficiencies in women, children and all other resource poor population categories. Genetic iron and zinc biofortification in crops including common beans can be done through several methods which involves, conventional breeding, marker assisted breeding and genetic engineering (Welch, 2002; Muluaalem, 2015)

(a) Conventional Breeding

Conventional plant breeding is the development or improvement of varieties for traits of interest by crossing closely related individual plants (Caligari & Forster, 2015). For iron and zinc biofortification, varieties with contrasting seed iron and or zinc contents are crossed, in order to transfer genes from a high iron or zinc containing variety (cultivated or wild related species) into a popular variety with low iron or zinc (Welch & Graham, 2004; Saltzman *et al.*, 2013; Caligari & Forster, 2015). In common beans natural variation exist in both cultivated and wild species in terms of seed iron and zinc contents (Tryphone & Nchimbi-Msolla, 2010; Acquah *et al.*, 2013)

Most of iron and zinc biofortified varieties of common beans that had been developed and some released for public use in sub-Saharan Africa, were developed through conventional breeding (Bouis *et al.*, 2013). Although biofortification by Conventional breeding is easy and cheaper, selection of plants is mostly influenced by environmental conditions (soil characteristics and climate) and requires many years to release a variety (Hirschi, 2009; FAO, 2010). In crops like common beans the complications is even more as selection should involve some other market type traits like seed color, size and shape apart from selecting for high seed iron content, therefore adoption of molecular breeding techniques in sub-Saharan Africa is of much importance.

(b) Marker assisted Breeding

Molecular marker-assisted breeding is the technique of improving crops through the use of genetic marker(s) linked to the trait of interest (Jiang, 2013). Segments of DNA that are linked to the trait(s) of interest are used for indirect selection of those trait(s) from a segregating or non segregating population (Collard & Mackill, 2008). Selection of individual plants involves two main stages. First, identification and validation of DNA markers associated with the trait(s) of interest in parents and second, use of the validated DNA markers to select individual plants from a target breeding population at early seedling stage, based on the presence of markers associated with trait(s) of interest (Lim *et al.*, 2014; Diapari *et al.*, 2015). Compared to conventional breeding, marker assisted breeding has advantages in improving traits including seed iron content in common bean. First is reduction in time of selection, whereby individual plants can be selected for any trait(s) of interest at younger stage, secondly selection is not affected by environmental conditions, and thirdly, recessive

alleles in heterozygous individual plants can be easily selected using co-dominant markers (e.g. SSR and SNP), thus avoiding selfing and or test crossing like in conventional breeding, thus shortened time for variety development (Semagn *et al.*, 2006).

The quantitatively inheritance of seed iron and zinc contents in common beans, was clearly revealed after the identification of genetic markers which control these traits (Blair *et al.*, 2009; Cichy *et al.*, 2009). The genetic markers were later used in construction of genetic linkage map, identification of chromosomal regions which control high seed iron and improvement of the traits in common bean (Blair *et al.*, 2009; Cichy *et al.*, 2009; Blair *et al.*, 2016). To date, knowledge of the genetic bases for many seed iron and zinc concentration in common bean relies on linkage and quantitative trait loci (QTL) analysis using biparental populations, which has low resolution due to limited number of recombination incidents and thus results into genetic markers which are cross specific and show only fractions of genetic variability underlying the common bean high seed iron and zinc contents (Blair, 2013; Kamfwa *et al.*, 2015). There is a need of using techniques like Genome Wide Association Study (GWAS) in studying genetic variability underlying iron and zinc contents in common bean, since it involves use of diverse germplasm of a crop collected from different environments, which when genotyped gives a clear picture of the candidate genes responsible for expression of the trait of interest. In sub-Saharan Africa, there is limited information on application of marker assisted breeding in improving seed iron content in common bean, thus adoption of the technique is of much importance as many iron biofortified common bean will be developed within short time period compared to conventional breeding.

(c) Genetic engineering

Plant genetic engineering is the practice of manipulating genetic makeup of the plant through genome editing and or transfer of gene(s) from a closely related or distant organism aimed at developing superior plant varieties with traits of interest (Carvalho & Vasconcelos, 2013; Goudia & Hash, 2015). The advancement in DNA knowledge and biotechnology has enabled studies on plant genome, identification and validation of several genes controlling plant agronomic and biochemical traits including grain micronutrient contents (Das *et al.*, 2014). In the process of transferring genes coding for traits of interest, the identified and validated gene(s) are isolated from the source organism and transferred into tissues of the target plant via DNA micro particle bombardment or *Agrobacterium tumefaciens* mediated transfer (Kwapata *et al.*, 2012; Dutta *et al.*, 2014). Transferring genes into unrelated species (e.g.

from bacteria to plants) is called transgenesis, while transferring from similar species or sexually compatible species (e.g. from wild to cultivated varieties) is called cisgenesis (Shrestha *et al.*, 2018). The plant developed from transgenesis is known as transgenic while that from cisgenesis is called a cisgenic (Acquaah, 2013). The benefits of employing plant genetic engineering over conventional breeding in improving plant traits, including grain iron and zinc contents are, first it is the fastest method of developing varieties, though it needs high initial financial investment (Keshavareddy, 2018). Second, genes from a distant species can be isolated and used to improve another plant species, thus can be applied even when there is no genetic variation in the trait of interest (Connorton *et al.*, 2017). Third, only the genes of interest are transferred to the plant to be modified whereas in conventional breeding there is transfer of even the unwanted genes during artificial hybridization (Borrill *et al.*, 2014).

Gene transfer technique has been used in developing several plant varieties with increased grain iron and zinc contents (Dias & Ortiz, 2012). Over expression of nicotianamine synthase (NAS) encoding genes, resulted into increase in grain iron and zinc contents of transgenic rice by 2 – 3 folds (Borrill *et al.*, 2014), whereas over expression of a metal transporter (HvMTP1) encoding genes in barley led to 25 % increase barley cis-genic grain (Menguer *et al.*, 2018). To date, there is limited information on seed iron and zinc content increase by gene transfer techniques in common bean, though the already identified and validated transporters and genes involved in grain zinc accumulations in other crops can be used to develop transgenic or bean plants with increased seed zinc contents (Oblessuc *et al.*, 2012; Sperotto & Ricachenevsky, 2017). The limited information is reported to be due to very long common bean genetic transformation protocol, poor reproducibility and in vitro regeneration (Sperotto & Ricachenevsky, 2017).

Recently genome editing has been advocated as a precision breeding technique and a compliment to conventional genetic engineering gene transfer as it does not necessarily involve transformation (Mao *et al.*, 2019). Genome editing involves several molecular biological methods, which includes zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) (Vinoth & Ravindhran, 2017), and recently clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems (Krohling *et al.*, 2016; Hummel *et al.*, 2018). These methods use sequence-specific engineered nucleases, which when induced results into identification of specific DNA sequences and give rise to double-

stranded breaks (DSBs) (Zhang *et al.*, 2018). The endogenous repair systems of plants correct the DSBs either by no homologous end joining (NHEJ), which can lead to the insertion or deletion of nucleotides causing gene knockouts, or by homologous recombination (HR), which can result into gene replacements and insertions (Mao *et al.*, 2019). The DSBs repair outcomes are predictable and thus selection of mutations with benefits to plant breeding can be done (Veillet *et al.*, 2020). In most cases, genome editing techniques have been used in improving plants abiotic stress tolerance and biotic stress resistance traits with very few studies focusing on food nutritional quality (Kwapata *et al.*, 2012; La Frano *et al.*, 2014). For example, target genome editing of *OsERF922* gene in rice using CRISPR/Cas9 technique resulted into development of rice with enhanced blast resistance (Zhang *et al.*, 2018), whereas drought tolerance wheat was developed by editing *TaDREB2* and *TaERF3* genes (Ansari *et al.*, 2020). Likewise, CRISPR/Cas9 genome editing was used in editing of soybean *E1* gene and developed early flowering mutants (Han *et al.*, 2019). There is limited information in common bean genome editing particularly for grain iron and zinc improvement, though there are several genes identified to control different traits (agronomic, biotic and abiotic stress response and grain quality) (Sperotto & Ricachenevsky, 2017; Veillet *et al.*, 2020; Ansari *et al.*, 2020). Genetic engineering is a quick and precision method for developing iron and zinc biofortified varieties, however the technique faces lots of challenges in most sub-Saharan African countries including Tanzania on the acceptability of the varieties by the communities and government regulatory organs due to fear of the unknown outcomes to human health and environment that could be caused by genetically modified crops (GMCs).

2.5 Mineral bioavailability

Mineral bioavailability is the percentage absorption of the total amount of mineral found in food (Petry *et al.*, 2013). Bioavailability of divalent mineral cations from cereal and legumes grains is negatively influenced by phytic acid (PA) (Nielsen *et al.*, 2013). Phytic acid, a Myo-inositol-1, 2, 3, 4, 5, 6-hexakisphosphate is a major phosphorus and mineral storage compound, which stores 70 – 85% of iron and located in cotyledons of legume seeds, it inhibits absorption of nutritional divalent cations such as Fe^{2+} , Zn^{2+} , Ca^{2+} , and Mg^{2+} and decreases bioavailability of such important divalent minerals in the monogastric intestinal (Fig. 14). Its inhibition capacity increase as its concentration increases (Petry *et al.*, 2013; Sparvoli & Cominelli, 2015). In common bean, phytic acid concentrations have been reported to range from 400 to 2600 mg/100 g (Petry *et al.*, 2015). For better absorption of divalent

minerals in human gut, PA concentration of <700 mg/100 g is advised to be attained during genetic biofortification (Petry *et al.*, 2013). The compound is reported to be highly dependent on variety and soil type where the variety is grown and it is positively correlated with iron (Greiner *et al.*, 2006; Sparvoli & Cominelli, 2015; Petry *et al.*, 2015). The inhibitory effect of PA on iron, zinc and other divalent ions absorption in the human gut increases with increase in its concentration (Chattha *et al.*, 2017). Sparvoli and Cominelli (2015) reported that PA: mineral molar ratio of the grain is more important in mineral bioavailability in human gut than concentration of PA. The lower the PA: mineral molar ratio, the higher the bioavailability of the mineral in human gut (Nielsen *et al.*, 2013). Thus, genetic biofortification increases grain iron and or zinc concentration and lowers PA: Mineral molar ratio.

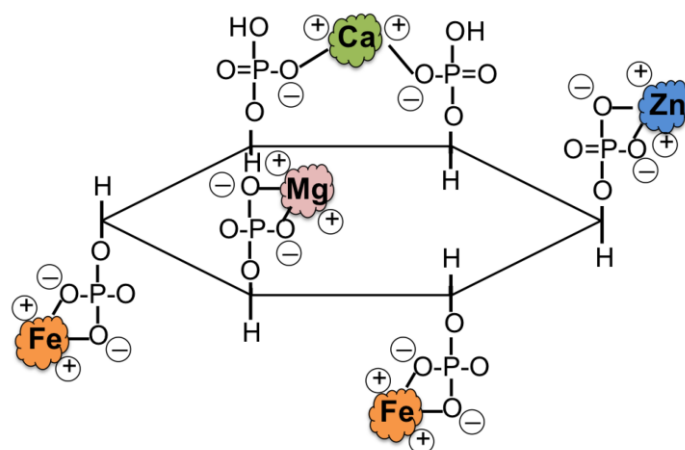


Figure 14: The hexavalent phytic acid binding divalent cations

About 13 - 42% of total iron is stored in ferritin, a protein which can store up to 4500 Fe^{3+} atoms (Hoppler *et al.*, 2014). Ferritins in common beans were found to range from 50 to 70 mg/kg, which correlates to iron content of 10 mg/kg, iron stored in ferritin is readily available for absorption in the gastrointestinal (Hoppler *et al.*, 2014; Zielińska, 2015). Therefore, for increased iron bioavailability from biofortified varieties, it is uttermost important to understand the levels of ferritins and phytic acid concentrations in common bean seeds particularly in Tanzania, where there is limited information on their levels among the cultivars of common beans grown in the country.

The current literature review has clearly revealed that, genetic iron, and zinc biofortification particularly of staple food is the most current cost-effective intervention in controlling iron and zinc deficiencies in sub-Saharan African countries such as Tanzania. Development of high iron and or zinc-containing common bean varieties, which is the mostly cultivated and

consumed grain legume in Tanzania, is the best approach. Unlike cereals which need to be milled before consumption and thus ending up losing high iron and zinc-containing parts (embryo and aleurone layer), common bean grain is consumed whole, providing sufficient zinc to consumers. Among the strategies of genetic iron and zinc biofortification, conventional and or marker assisted breeding are the best compared to genetic engineering, as high iron and or zinc-containing varieties can be developed at low cost particularly in developing countries like those in sub-Saharan Africa. In many sub-Saharan African countries including Tanzania, nutritional profiling of the local varieties of staple food crops has to be done, as these would offer high diversity in protein, carbohydrates, vitamins and minerals particularly zinc and iron. This may lead to identification of varieties for use in breeding programs. Development of high iron and zinc-containing plant varieties by genetic engineering is the precise method, this method lacks acceptability in most of the sub-Saharan African countries due to fear of the unknown and thus no zinc biofortified variety developed by this technique have been released in the region. High zinc-containing bean varieties can easily reach resource poor farmers and consumers particularly in remote areas of sub-Saharan Africa compared to supplementation and fortification which need advanced infrastructures to operate.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Common bean genotypes

This study used ninety-nine common bean genotypes, that included fifty-nine local varieties collected from randomly selected farmers of the largest bean producing regions in the country; namely Morogoro, Mbeya, Arusha, and Kagera. Whereas thirty-two improved cultivars that are recommended for cultivation in a wide range or specific environment and eight breeding lines were obtained from research institutions, which include; Sokoine University of Agriculture (SUA), located in Morogoro, Tanzania Agricultural Research Institute (TARI) Uyole, Selian and Maruku stations found in Mbeya, Arusha, and Kagera respectively. Geographical and weather description of Morogoro region (United Republic of Tanzania [URT], 2006; Kahimba *et al.*, 2015), Mbeya region, Arusha region (Chuwaa, 2012), and Kagera region (URT, 2006) are presented in Table 3. The characteristics of the common bean genotypes used in this study are presented in Table 4.

Table 3: Geographical information and weather conditions of regions where seeds were obtained

Region	Geographical position		Mean annual rainfall (mm)	Mean annual temperature (°C)
	Latitudes	Longitudes		
Morogoro	05°58' - 09°32'S	35°25' - 38°30'E	500 -2200	18-30
Mbeya	07°00' - 09°35'S	32°00' - 35°00'E	650-2600	16-25
Arusha	02°00' - 06°00'S	35°00' - 38°00'E	250-1200	21-26
Kagera	01°00' - 02°45'S	30°25' - 32°40'E	500-2000	20-28

Table 4: Characteristics of the 99 common bean genotypes used in this study

GN	Genotype name	Source	Growth habit	Seed Color	Seed size	Status
G1	ACC 714	Mbeya	Semi climber	Black	Small	Line
G2	Bagara Ompigize	Kagera	Bush	Cream	Medium	Local
G3	Bangaya Akatebe	Kagera	Bush	White	Small	Local
G4	Bilfa 4	Mbeya	Climber	Brown	Small	Released
G5	Bilfa Uyole	Mbeya	Bush	Red mottled	Large	Released
G6	Buji	Kagera	Bush	Grey	Large	Local
G7	Burushu	Kagera	Semi climber	Tan mottled	Medium	Local
G8	CAL 96	Mbeya	Semi climber	Red mottled	Large	Line
G9	Calima Uyole	Mbeya	Semi climber	Red mottled	Large	Released
G10	Cheupe	Arusha	Climber	White	Medium	Released
G11	Chumba Neroza	Kagera	Climber	Red	Medium	Local
G12	CODMLB 033	Mbeya	Climber	Red mottled	Large	Line
G13	DOR 500	Mbeya	Climber	Black	Small	Line
G14	Fibea	Mbeya	Bush	Pale Yellow	Large	Local
G15	Jabeyila	Kagera	Climber	Brown	Small	Local
G16	Jesca	Arusha	Semi climber	Brown mottled	Large	Released
G17	KAB o6F2-8-35	Mbeya	Bush	Red mottled	Large	Line
G18	KAB o6F2-8-36	Mbeya	Climber	Red mottled	Large	Line
G19	Kabanima	Mbeya	Semi climber	Red mottled	Medium	Local
G20	Kabumburi	Kagera	Climber	Yellow	Large	Local
G21	Kachele	Kagera	Bush	White	Small	Local
G22	Kaempu	Kagera	Climber	Green key lime	Small	Local
G23	Kainja	Kagera	Bush	Red	Medium	Local
G24	Kaisho kamugole	Kagera	Climber	Brown	Small	Local
G25	Kakaritusi	Kagera	Climber	Dark bay red	Small	Local
G26	Kamoshi	Kagera	Bush	Ivory	Small	Local
G27	Kamosi	Kagera	Climber	Ivory	Small	Local
G28	Kanade	Kagera	Bush	Red	Medium	Local
G29	Kashule	Kagera	Bush	Dark Red mottled	Large	Local
G30	Kasukari	Kagera	Bush	Ivory	Small	Local
G31	Katuku	Kagera	Bush	Red	Small	Local
G32	Katuku2	Kagera	Bush	Red	Medium	Local
G33	Kibugu	Kagera	Bush	Orange	Large	Local
G34	Kigoma	Mbeya	Bush	Yellow	Large	Local
G35	Kikobe	Kagera	Climber	Olive	Medium	Local
G36	Kilindi	Mbeya	Climber	Grey	Medium	Local
G37	Kinyobya	Kagera	Bush	Tan red mottled	Large	Local
G38	Kipapi	Mbeya	Climber	Grey	Large	Local
G39	Kisapuri	Kagera	Climber	Red	Medium	Local
G40	Kitebe	Kagera	Climber	White	Medium	Local
G41	Kituntunu	Kagera	Bush	Ivory	Medium	Local
G42	Kyababikira	Kagera	Climber	Ivory Mottled	Large	Local
G43	Kyakaragwe	Arusha	Bush	Ivory	Small	Local
G44	Lyamungo 85	Arusha	Bush	Red mottled	Large	Released
G45	Lyamungo 90	Arusha	Bush	Red mottled	Large	Released
G46	Maharage Kamba	Kagera	Climber	Purple	Medium	Local
G47	Maharage Mbeya	Kagera	Climber	Pale Yellow	Medium	Local
G48	Malirahinda	Kagera	Climber	Brown	Small	Local
G49	Masusu	Mbeya	Semi climber	Brown	Large	Local
G50	Meupe Uyole	Mbeya	Semi climber	White	Large	Released
G51	Mshindi	Morogoro	Bush	Grey	Medium	Released
G52	Msolini	Mbeya	Climber	Brown Cocoa	Large	Local
G53	Mwami Kola	Kagera	Semi climber	Purple	Small	Local
G54	Ngoma za bahaya	Kagera	Climber	Cream	Small	Local
G55	Ngwakungwaku	Mbeya	Climber	Orange	Large	Local
G56	Njano fupi	Kagera	Bush	Yellow	Large	Local
G57	Njano Uyole	Mbeya	Bush	Yellow	Medium	Released
G58	Nyeupe Kubwa	Kagera	Climber	White	Medium	Local

GN	Genotype name	Source	Growth habit	Seed Color	Seed size	Status
G59	Nyeupe ndogo	Mbeya	Climber	White	Medium	Local
G60	Pasi	Mbeya	Semi climber	Ivory	Large	Released
G61	Pesa	Morogoro	Semi climber	Dark bay red	Medium	Released
G62	Raja	Kagera	Bush	Red mottled	Large	Local
G63	Rojo	Morogoro	Semi climber	Dark bay red	Medium	Released
G64	Rosenda	Mbeya	Bush	Red mottled	Large	Released
G65	Rozikoko fupi	Kagera	Bush	Red mottled	Medium	Local
G66	Ruondera	Kagera	Bush	Grey	Large	Local
G67	RWR 2154	Mbeya	Semi climber	Red mottled	Large	Line
G68	Selian 05	Arusha	Climber	Tan	Small	Released
G69	Selian 06	Arusha	Bush	Purple	Medium	Released
G70	Selian 10	Arusha	Bush	White	Small	Released
G71	Selian 11	Arusha	Bush	White	Small	Released
G72	Selian 12	Arusha	Bush	Red	Large	Released
G73	Selian 13	Arusha	Bush	Yellow	Large	Released
G74	Selian 14	Arusha	Climber	Red mottled	Large	Released
G75	Selian 15	Arusha	Climber	Almond	Large	Released
G76	Selian 9	Arusha	Climber	White	Small	Released
G77	Selian 94	Arusha	Climber	Tan mottled	Medium	Released
G78	Selian 97	Arusha	Bush	Red	Large	Released
G79	Selundo	Mbeya	Semi climber	Olive	Large	Released
G80	Sinon	Mbeya	Bush	Almond	Large	Local
G81	SMC 17	Mbeya	Semi climber	White	Medium	Line
G82	SMC 18	Mbeya	Climber	White	Medium	Line
G83	Soya	Kagera	Climber	Greyish brown	Medium	Local
G84	Soya Mbeya	Kagera	Bush	Grey	Large	Local
G85	SUA 90	Morogoro	Bush	Brown	Medium	Released
G86	Tema	Kagera	Bush	White	Small	Local
G87	Tikumba Nyama	Kagera	Climber	Dark red Mottled	Large	Local
G88	Urafiki	Mbeya	Climber	Red	Medium	Released
G89	Uyole 03	Mbeya	Bush	Cream mottled	Large	Released
G90	Uyole 04	Mbeya	Bush	Pale Yellow	Medium	Released
G91	Uyole 16	Mbeya	Bush	Orange	Large	Released
G92	Uyole 18	Mbeya	Climber	Grey	Large	Released
G93	Uyole 84	Mbeya	Semi climber	Beige	Medium	Released
G94	Uyole 94	Mbeya	Bush	Cream mottled	Large	Released
G95	Uyole 96	Mbeya	Bush	Red	Large	Released
G96	Uyole 98	Mbeya	Semi climber	Orange	Medium	Released
G97	Wanja	Mbeya	Climber	Olive	Large	Released
G98	Wifi Nyegela	Kagera	Climber	Ivory	Small	Local
G99	Zawadi	Morogoro	Bush	Brown	Medium	Released

GN = Genotype number

3.2 Description of test locations

The three field experiments of this study were planted at TARI-Selian in Arusha, TARI-Uyole in Mbeya and Sokoine University of Agriculture (SUA) in Morogoro (Fig. 15). Geographical positions and altitude, where the field trials were planted at each test location are presented in Table 5. Laboratory determination of seed iron and zinc contents was done at SUA, while the determination of phytic acid, ferritin, phosphorus, magnesium, and manganese contents was done at the John Innes Centre (JIC) in the United Kingdom.

Table 5: Geographical information of the test locations

Test Location	Latitude	Longitude	Altitude (m.a.s.l)
TARI-Selian station, Arusha	3° 22' S	36° 37' E	1430.0
SUA, Morogoro	6° 50' S	37° 39' E	541.7
TARI-Uyole station, Mbeya	8° 55' S	33° 30' E	1772.0

m.a.s.l = meters above sea level.

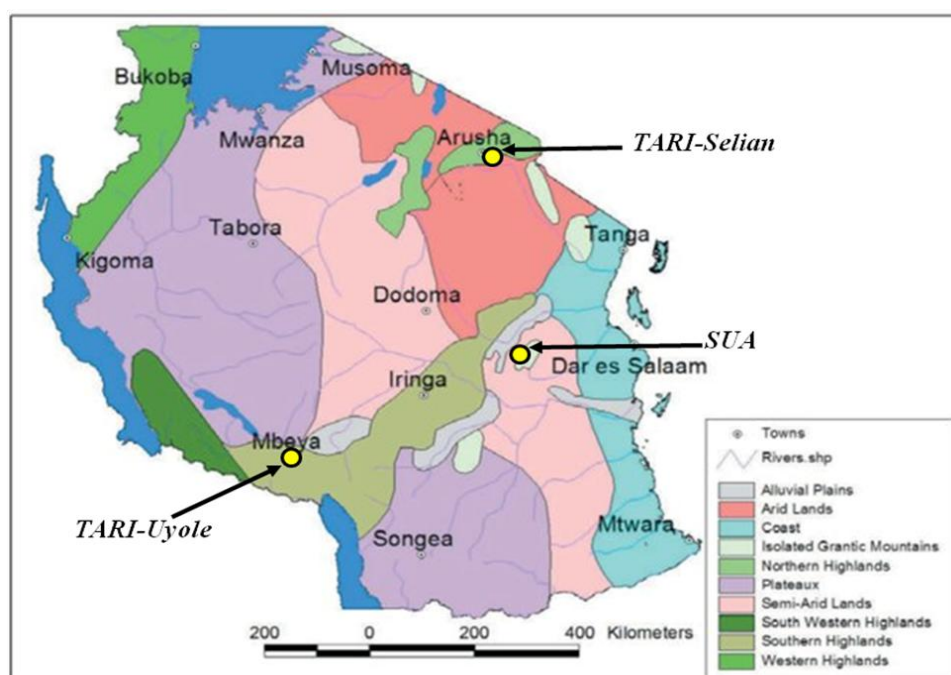


Figure 15: Map of Tanzania showing Agro-ecological zones (Ministry of Agriculture Food Security and Cooperatives, 2014), and the experimental sites (TARI-Selian, SUA, and TARI-Uyole)

3.3 Soil collection from experimental fields and analysis

Soil samples were collected at each experimental field at a depth of 20 cm before planting. The soil samples were air-dried, ground, sieved using a 2.0 mm mesh and used in laboratory for the determination of soil physical and chemical characteristics. Texture of the soils was

obtained using the hydrometer method whereas soil pH was determined on 2.5:1 water to soil suspension (Okalebo *et al.*, 2002). Available phosphorus (AP) for TARI-Selian experimental field soil (basic) was determined using the Olsen method while that of SUA and TARI-Uyole soils (acidic), were determined using Bray 1 method (Estefan *et al.*, 2013). Exchangeable bases (Ca, Mg, Na, and K) were extracted using ammonium acetate and determined by atomic absorption spectrophotometry. Walkley-Black wet combustion method was used to determine organic carbon (OC), whereas total nitrogen (TN) was measured using the Kjeldahl method (van Reeuwijk, 2002).

3.4 Field trial details

The field experiments at all three test locations (TARI-Selian, SUA, and TARI-Uyole) were laid out in alpha lattice design with three replications, each replication containing five blocks of 20 plots (Fig. 16). Every experimental plot was planted with one common bean genotype in two rows of 1.5 m length spaced at 50 cm apart. Within rows plants were spaced at 10 cm from one plant to another. Planting at TARI-Uyole and Selian station, was done on March 2018 and harvested on July 2018, whereas common bean genotypes planting at SUA was done on May 2018 and harvested on August 2018.

3.5 Data Collection

3.5.1 Yield and yield components of common bean genotypes in three bean-growing agro-ecological zones of Tanzania

At each of the three test locations, days to 75 % flowering in each genotype were observed and recorded during flowering time. At harvesting, all plants in a plot were harvested and heaped at the center of a plot. Ten plants were randomly selected and the number of pods in each of the selected plants was counted and recorded to determine the number of pods per plant. The number of seeds per pod was counted and recorded from twenty randomly selected pods. Pods were shelled and air-dried for three days, the weight of 100 seeds, and all seeds per plot (g/plot) were measured using weighing balance and recorded. The weight (g) of seeds per plot (m^2) was later converted into kg/ha as follows; $1 \text{ g/m}^2 \times 1 \text{ kg}/1000 \text{ g} \times 10\,000 \text{ m}^2/\text{ha} = 10 \text{ kg/ha}$, thus the weight (g) per plot (m^2) was multiplied by 10 to get kg/ha.

Rep I																				
Block 1	G85	G75	G2	G40	G44	G74	G81	G47	G20	G5	G76	G91	G71	G61	G19	G14	G98	G95	G78	G63
Block 2	G30	G57	G17	G18	G37	G53	G92	G73	G13	G59	G34	G9	G10	G54	G28	G41	G84	G64	G43	G69
Block 3	G72	G1	G88	G49	G52	G3	G51	G31	G42	G79	G94	G68	G22	G83	G11	G36	G15	G45	G46	G70
Block 4	G65	G55	G90	G25	G26	G62	G21	G6	G99	G8	G38	G16	G97	G86	G32	G7	G66	G48	G12	G4
Block 5	G62	G35	G77	G87	G33	G58	G23	G93	G96	G39	G67	G60	G82	G80	G50	G89	G56	G29	G27	G24
Rep II																				
Block 1	G87	G64	G11	G23	G83	G49	G13	G39	G15	G29	G48	G42	G4	G40	G59	G31	G99	G12	G44	G38
Block 2	G74	G28	G10	G55	G89	G94	G66	G32	G50	G14	G37	G46	G52	G69	G33	G8	G6	G62	G82	G51
Block 3	G78	G5	G95	G71	G62	G22	G65	G41	G73	G75	G27	G34	G54	G98	G24	G96	G97	G25	G60	G53
Block 4	G92	G35	G86	G7	G63	G91	G72	G70	G93	G16	G80	G9	G45	G79	G20	G30	G47	G56	G84	G67
Block 5	G21	G76	G88	G77	G61	G17	G85	G2	G18	G81	G43	G90	G57	G3	G19	G68	G58	G1	G36	G26
Rep III																				
Block 1	G76	G44	G16	G68	G88	G38	G18	G32	G34	G58	G85	G79	G75	G30	G61	G12	G62	G33	G20	G26
Block 2	G13	G71	G35	G6	G80	G60	G37	G24	G43	G55	G31	G17	G7	G15	G77	G56	G66	G72	G81	G97
Block 3	G99	G2	G98	G10	G65	G21	G57	G82	G5	G27	G14	G42	G78	G89	G36	G95	G49	G47	G63	G48
Block 4	G40	G84	G4	G91	G86	G73	G87	G59	G28	G69	G53	G51	G83	G3	G50	G29	G52	G8	G94	G22
Block 5	G64	G92	G93	G19	G67	G39	G46	G25	G23	G11	G70	G62	G54	G45	G96	G9	G41	G90	G74	G1

Figure 16: Alpha lattice design field layout of the experiment

3.5.2 Levels of iron and zinc among common bean genotypes in three bean-growing agro-ecological zones of Tanzania

At each of the three test locations, pods were harvested, shelled, seeds were air-dried and put into separate paper bags for each of the 99 genotypes. The air-dried seeds were taken into the laboratory, whereby 5.0 g of each genotype was ground by Cyclotec 1093 sample mill. The atomic absorption spectrophotometer (AAS) method was used to determine seed iron and zinc contents (Estefan *et al.*, 2013). A sample of 0.5 g dry and ground common bean seeds from each genotype was weighed and put into porcelain crucibles. The porcelain crucibles with samples were placed into the furnace. The samples were heated into ashes at the temperature of 550 °C for 5 hours. After 5 hours the furnace was turned off allowing sample ashes to cool. The cooled ashes were dissolved into 6 N HCl and thoroughly mixed. After 10 minutes the mixtures were made up to 50 mL by addition of distilled water. The solutions were filtered using whatman No. 42 filter paper. The filtrates were used to determine absorbances of iron and zinc using AAS at the wavelengths of 248.3 and 213.9 nm respectively, the mineral absorbances were later used to calculate sample iron and zinc concentrations using standard curve drawn from standard solutions of known iron and zinc concentrations.

3.5.3 Levels of phytic acid, P, Mg and, Mn among common bean varieties grown in Tanzania

(i) Phytic acid

About 5 g of air-dried seeds of each of the 61 common bean genotypes selected based on seed iron and zinc contents among the 99 genotypes from TARI-Selian station was obtained. Based on seed iron content, the selected genotypes were grouped into subgroups as follows; 19.7 to 39.4 mg/kg low that were 16, 47.3 to 69.4 mg/kg (23) (Moderate) and 71.8 to 150.8 mg/kg (22) (High). Based on seed zinc content the selected genotypes were grouped into subgroups as follows; 14.6 to 24.1 mg/kg low that were 37, 25.1 to 30 mg/kg (20) (Moderate) and 32.2 to 33.3 mg/kg (4) (High) (Tryphone & Nchimbi-Msolla, 2010; Kimani & Warsame, 2019) The seeds of the selected genotypes were ground in a coffee grinder into pieces and then the pieces were ground into fine flour using a pestle and mortar. The Megazyme method (Megazyme, 2017) was used to determine phytic acid contents. 0.2 g flour of each common bean genotype was weighed and put into a falcon tube, followed by the addition of 4 mL of 0.66 M hydrochloric acid. The mixture was incubated overnight at room temperature. The

next day, an extract of 1 mL was transferred into a 1.5 mL microfuge tube and centrifuged at full speed for 10 minutes. After centrifugation, 0.5 mL of the supernatant was transferred in to a new 1.5 mL tube and neutralized with an equal volume (0.5 mL) of 0.75 M sodium hydroxide solution. The neutralized sample extract was used as follows:

The following were added into 1.5 mL microfuge tubes	Free phosphorus (no phytase)	Total Phosphorus (Phytase added)
Water	620 μ L	600 μ L
Solution 1	200 μ L	200 μ L
Sample extract	50 μ L	50 μ L
Suspension 2 (phytase)	-	20 μ L

The added phytase enzyme hydrolyses phytic acid and releases phosphate from it. The mixture was mixed by vortex and incubated in a water bath at 40 °C for 10 minutes. After incubation, the following solutions were added

The following were added into 1.5 mL microfuge tubes	Free phosphorus (no ALP enzyme)	Total Phosphorus (ALP enzyme added)
Water	20 μ L	-
Solution 3	200 μ L	200 μ L
Suspension 4 (ALP)	-	20 μ L

The added alkaline phosphatase (ALP) enzyme further hydrolyses phytic acid and releases the remaining phosphates. The mixture was mixed by vortex and incubated in a water bath at 40 °C for 15 minutes. After incubation, 300 μ L of 50% trichloroacetic acid (TCA) was added to stop the reaction, followed by centrifugation at 13 000 runs per minute (rpm) for ten minutes. After centrifugation, 1 mL of the supernatant was transferred into new 1.5 mL microfuge tube. A standard curve was prepared by using solution 5 (Phosphorus solution), whereas 1 mL was made as follows

The following were added into 1.5 mL microfuge tubes	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Water	5 mL	4.95 mL	4.75 mL	4.5 mL	4.25 mL
Solution 5	0	50 μ L	250 μ L	500 μ L	750 μ L

To each 1.5 mL containing 1 mL of the sample supernatant and those with 1 mL of the phosphorus solution (for standard curve), 500 μ L color reagent was added. The mixture was incubated in a water bath at 40 °C for 1 hour and then mixed by vortex after 1 hr of incubation. After incubation and mixing, 1 mL of each sample supernatant mixed with color

reagent and 1 mL of each phosphorus solution mixed with color reagent was transferred into a semi-micro cuvette and total phosphorus absorbance was read using a spectrophotometer at wavelength 655 nm. The phytic acid concentration was obtained using the Mega Calc: Phytic Acid / Total Phosphorus on the Megazyme website after entering the absorbances of the samples and standard solutions absorbances and concentrations <https://secure.megazyme.com/Phytic-Acid-Total-Phosphorus-Assay-Kit>.

(ii) Minerals

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) method was used to determine seed phosphorus (P), magnesium (Mg), and manganese (Mn) contents (Hou *et al.*, 2016). For each of the 61 common bean genotypes, a sample of 0.05 g overnight oven-dried flour at 55 °C was added into a digestion tube of known weight. While in the fume cabinet, 2 mL of Nitric acid (67-69 % low metal), was added followed by 0.5 mL hydrogen peroxide (30-32 % low metal) (Fig. 17 A). The samples mixed with nitric acid and hydrogen peroxide in digestion tubes were overnight heated using a heating block at 95 °C in the fume cabinet (Fig. 17 B and C). After digestion, 25.1 mL of Milli-Q water was added, and the full weight of the diluted sample was obtained as follows, full tube weight (g) - empty tube weight (g) = full volume measurement (g), and dilution ratio was calculated as follows, dilution ratio = full volume weight (g) / flour weight (g). The ICP-OES at different wavelength P (213.6 nm), Mg (285.2 nm) and Mn (259.4 nm) was used to determine sample digestion solutions absorbance. The mineral concentration of the sample was calculated using a standard curve drawn from standard solutions with known mineral concentration and absorbance.

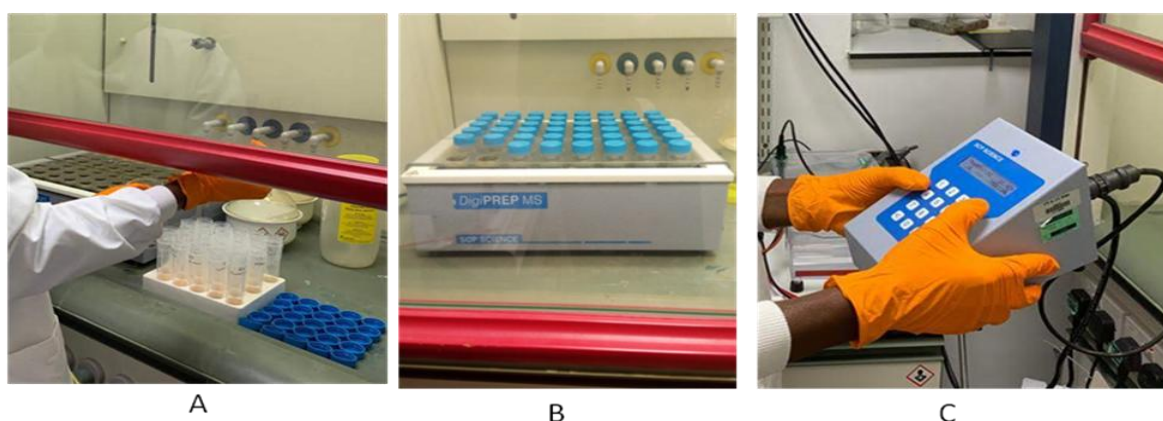


Figure 17: (A) adding nitric acid and hydrogen peroxide into samples; (B and C) heating block being set at temperature of for 990 minutes

3.5.4 Levels of ferritin protein using Western blot analysis

(i) Protein extraction

The western blot method was used to determine the levels of ferritin protein (Cvitanich *et al.*, 2010; GE Healthcare, 2011). About 0.02 g of sample flour of each of the 61 common bean genotypes harvested from TARI-Selian station site were weighed into 1.5 mL Eppendorf, followed by addition of 200 μ L of 100 mM Tris-HCl pH 8, 0.1% Sodium dodecyl sulfate (SDS), while putting on ice and vortex thoroughly to mix. Centrifugation of the mixture at 4 °C for 10 minutes was done, followed by pipetting of 1 mL supernatant into another clean 1.5 mL Eppendorf tube, on ice. Standard solutions were prepared by diluting Bovine Serum Albumin (BSA), which is the universally accepted reference protein 2mg/mL in total volume of 100 μ L at 0(x2), 1.25, 2.5, 3.75, 5, 7.5 μ L. About 2 μ L of sample protein extract was diluted in 100 μ L Milli-Q water. 1 mL of diluted Bradford reagent 1:5 with H₂O was added to standards and samples and left to stay for 5 minutes. The absorbances of the standards and samples were determined using spectroscopy, whereas protein content of the samples was determined using the absorbance and the standard curve equation.

(ii) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) preparation

Two types of gels were prepared into falcon tubes as follows:

Materials	Separating 2 gels at 12.5%	Stacking 2 gels at 5%
Acrylamide (30%) (mL)	4.4	0.9
Water (mL)	3.4	3.0
Solution 2 (mL)	2.5	1.3
APS (μ L)	100.0	50.0
TEMED (μ L)	10.0	5.0

(iii) Electrophoresis

After preparation of the gels, the racks were assembled for gel solidification (Fig. 18 A). Separating gel was added first followed by isopropanol to remove bubbles left for some minutes to solidify, the isopropanol was washed with distilled water and then addition of stacking gel followed by comb was done. Once the stacking gel was solidified, the comb was removed and the gel placed in the electrophorator. Gel solidification was confirmed by the solidification of gel solution left in falcon tubes. The running buffer was poured into the

electrophorator until it covered the gel. The marker (protein ladder) at 5 μ L was loaded in one lane of each gel followed by common bean protein extract of 15 μ L to the rest gel lanes. The electrophorator was connected to power supply and the gel was run with voltage of 140 V for 45 min – 1hr, or until the dye front runs off the bottom of the gel (Fig. 18 B).

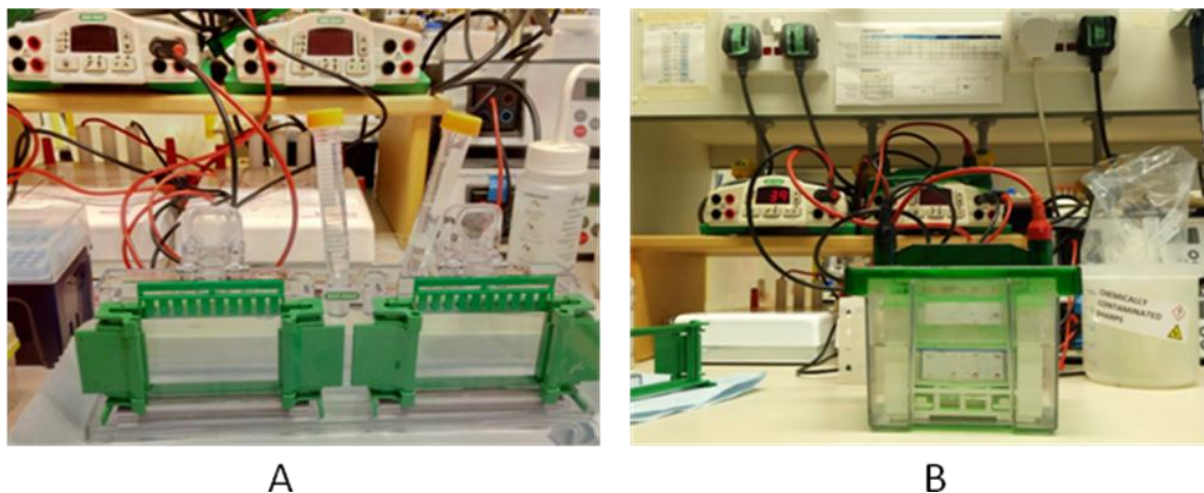


Figure 18: Instruments used in gel electrophoresis; (A) Assembled rack for gel solidification, and (B) running electrophorator for separating proteins based on molecular weight

(iv) Proteins Transfer from the Gel onto a Blotting Membrane

Eight filter sheets of 7.5 x 9.0 cm to fit the gel and one pure nitrocellulose transfer and immobilization membrane with the same dimensions were prepared. Four filter papers wetted in transfer buffer were placed on block surface of the biometrica semi-dry blotting machine. The glass plates holding the gel were separated, and the gel was retrieved and put onto the filter papers followed by nitrocellulose transfer and immobilization membrane. No air bubbles were allowed between the gel and membrane and the extra liquid between them was squeezed. The other four filter papers were placed on top, air bubbles and extra liquid were removed by a roller. The biometrica semi-dry blotting machine was closed and run at 200 mA for 45 minutes for the two gels.

(v) Blocking and antibody incubation

After transferring of the proteins to blotting membrane, the membrane was blocked with 5% skim milk in Tris Buffered Saline (TBS) + Tween 0.1% and incubated overnight in a cold room at 4 °C on a shaker. On the next day, the 5% skim milk in TBS + Tween 0.1% were poured and primary antibody α -ferritin \neq 34 final bleed dilution 1: 5000 (1.6 μ L of antibody α -

ferritin \neq 34 in 8 mL of 5% skim milk in TBS + Tween 0.1%) was added and incubate for 1 hour on a shaker. The membrane was washed with TBS + Tween 0.1% for 5 minutes three times. (Note: All washing and antibody incubation steps were done on a shaker at room temperature to ensure even agitation). The secondary antibody α -rabbit dilution 1: 5000 (1.6 μ l of antibody α -rabbit in 8 mL of 5% skim milk in TBS + Tween 0.1%) was added and incubated for 1 hour on a shaker. The membrane was washed three times with TBS + Tween 0.1%, for 5 minutes. The electrogenerated chemiluminescence (ECL) mix was prepared (following the proportion of ECL solution 1 + (ECL solution 2 + 60 μ L 30% H_2O_2) provided by the manufacturer), then, 2 mL of ECL solution 1 and 2 mL of ECL solution 2 were added and mixed in a new tube. Using the 1000 μ L pipette, 2 mL of the ECL mixture was pipetted into each membrane to cover the top and bottom of the membrane and incubated for 5 minutes. The membrane ferritin band was visualized using Image Quant LAS 500 and Image J software was used to calculate the pixel values of sample ferritin bands.

3.5.5 Development of F2 population of common bean crosses by crossing widely consumed yellow bean varieties with a high and stable seed iron and zinc content cultivar

Two widely adapted and consumed yellow common bean varieties (Kigoma and Njano Uyole) were crossed with high seed iron and zinc-containing common bean variety (CODMLB 033) (Fig. 19). The three common bean genotypes were planted in screen house at TARI-Selian in the year 2019. Each parental genotype was planted in 30 soil filled pots making a total of 90 pots, where two seeds were planted per pot, the plants in pot were thinned to one after two weeks of germination.

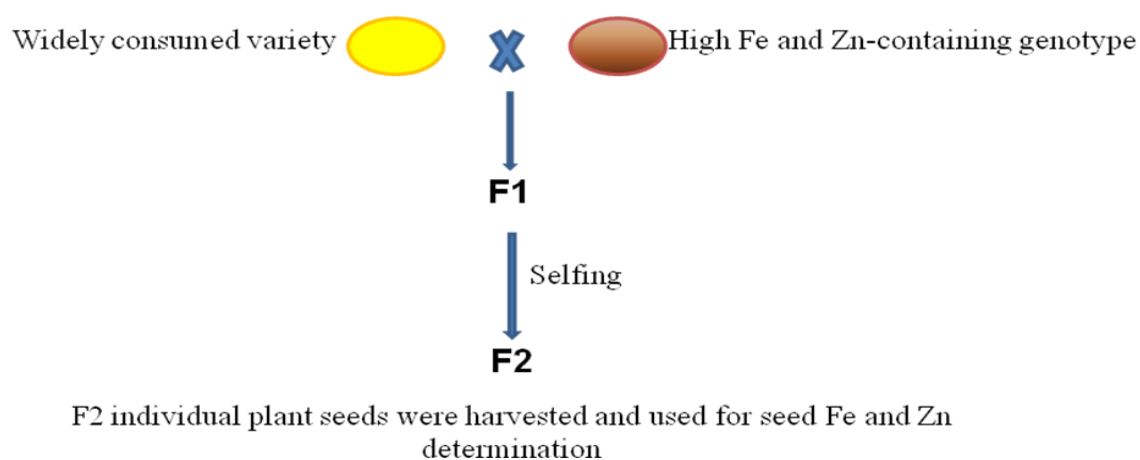


Figure 19: Steps in high iron and zinc common bean second generation development

(i) Artificial hybridization

During flowering time, artificial hybridization was performed in the morning at 08:00 to 10:30 a.m. Before flower opening, optimum size unopened flower buds were selected from female parents (Kigoma and Njano Uyole) and were opened artificially. After opening, using forceps, the anthers were cautiously removed leaving the stigma uncovered. Flowers which are about to open from the male parents (CODMLB 033) were opened and their anthers were collected using forceps and rubbed gently onto the stigma of female parent emasculated flowers for pollen transfer (Bliss, 1980). After artificial pollen transfer, with the help of forceps, the female flowers were closed with their petals and tagged for easy identification (Fig. 20).



Figure 20: Artificial pollination of common bean flowers

The plants were watered until maturity where, the artificially hybridized pods were harvested and seeds stored in paper bags. The seeds of each successful cross plant were planted as first-generation (F1) plants and watered until maturity. Hand weeding was done whenever, weeds occurred. After maturity, seeds of each F1 plant were harvested and later planted as F2 plants for segregation. The seeds of each maturing F2 plants were harvested and some of their seeds from each harvested plant were air-dried, ground into fine powder using pestle and mortar and used for laboratory determination of iron and zinc using AAS as in objective (ii).

3.6 Statistical analysis

3.6.1 Analysis of variance

Analysis of variance (ANOVA) on days to 75 % flowering, yield, and yield components, seed iron, zinc, magnesium, manganese, phosphorus and phytic acid contents from individual test location, was performed using GenStat 15th edition statistical package, so as to determine their significant variability among the tested common bean genotypes. Common bean genotypes seed yield and yield components, phytic acid and minerals means were separated using Duncan's new multiple range test (DNMRT) methods at a 5% level of probability, while Pearson's correlations were used to determine the relationship between the variables at a 5% level of probability. Minitab 14 statistical software was used to draw box plot graphs. Bar and column charts were drawn using Microsoft excel sheet.

3.6.2 The AMMI and GGE-biplot analysis

Plant Breeding Tools (PBTools) version 1.4 was used for drawing Additive main effects and multiplicative interaction (AMMI) and Genotype and Genotype by Environment interaction (GGE) biplot graphics. The AMMI-biplot analysis combines both analysis of variance (ANOVA) and principal component analysis (PCA) into one analysis. The ANOVA is used to partition the variation into genotype (G), environment (E) and genotype by environment interaction (GE) main effects, whereas PCA is used to estimate the residual multiplicative genotypes by environments interaction (Priyadarshan, 2019). The AMMI1 biplot x-axis shows the main effects of genotype and environment while y-axis shows principal component one (PC1) scores and hence used to visualize genotypes and environments mean performance, and their stability in relation to PC1 scores (Neisse *et al.*, 2018). In this study, AMMI biplot graphics were used to determine stability and adaptability of common bean genotypes across experimental sites on number of pods per plant, number of seeds per pod,

weight (g) of 100 seeds, seed iron and zinc. The AMMI model Balakrishnan *et al.* (2016) using GenStat 15th edition statistical package (Equation 1), was used to assess the main effects of genotypes, environment and genotype by environment interaction on seed yield and its components and seed minerals.

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} \dots\dots\dots (1)$$

Where Y_{ge} is the yield or mineral for genotype g in environment e , μ is the grand mean, μ_g the mean for genotype g (over environments), and μ_e the mean for environment e (over genotypes), $\alpha_g = \mu_g - \mu$ be the genotype deviation and $\beta_e = \mu_e - \mu$ is the environment deviation, λ_n the singular value for n component, γ_{gn} be the eigenvector value for genotype g and let δ_{en} be the eigenvector value for environment e , ρ_{ge} is the residual term. The AMMI Stability Value (ASV) as explained by Purchase *et al.* (2000) was used to quantify and rank the common bean genotypes based on their yield and mineral (iron and zinc) stability (Equation 2).

$$ASV = \sqrt{\left[\frac{SSIPC1}{SSIPC2} (IPC1) \right]^2 + (IPC2)^2} \dots\dots\dots (2)$$

Where $SSIPC1$ is the interaction principal component one sum of the square, $SSIPC2$ is the interaction principal component two sum of the square, $IPC1$ and $IPC2$ are interaction principal component 1 and 2 respectively

Genotype stability index (GSI) or Yield Stability Index (YSI_i) of each common bean genotype in terms of seed yield and minerals was calculated based on the rank of the i th genotype across environments based on AMMI Stability Value ($RASV_j$) and rank of the i th genotype based on mean yield or mineral across environments (RY_i) (Bose *et al.*, 2014) as:

$$YSI_i = RASV_i + RY_i \dots\dots\dots (3)$$

The genotype main effect and genotype by environment interaction effect (GGE) Biplot is mostly used in graphic analysis of two-way data particularly in visualization of genotype main effects (G) plus genotype by environment interaction effects (GE) (Yan *et al.*, 2007). When the first principal component (PC1) is largely associated with G, it represents the fraction explained by genotype while second principal component represents the fraction explained by GE. These two first principal components are used to produce the GGE-Biplot

graphic (Yan & Tinker, 2006). The GGE-biplot graphics were used for visualization of the ideal genotypes across sites in terms of seed mineral, experimental sites relationship, discriminating ability and representativeness for common bean genotypes on number of pods per plant, number of seeds per pod, weight (g) of 100 seeds weight, seed yield, iron, and zinc.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Test locations weather and soil physico-chemical characteristics

All the test locations received enough rainfall above 300 mm in planting season of the year 2018, though at different rates. The crop requires rainfall above 300 mm for it to perform well. The other monthly weather parameters during the growing season are as presented in Table 6. Tanzania Agricultural research Institute (TARI) Selian recorded the highest amount of rainfall, 645.5 mm, followed by SUA 565.7 mm, while the lowest rainfall was recorded at TARI-Uyole, 336.1 mm. The highest temperature of 28.6 °C was recorded at SUA, Morogoro followed by TARI-Selian which recorded mean temperature of 24.0 °C whereas TARI-Uyole recorded the lowest mean temperature of 23.3 °C. The highest relative humidity at TARI-Selian (88.0 %) and SUA (87.0 %) was recorded in April, while TARI-Uyole recorded highest relative humidity of 92.6 % in March. All the test locations recorded the lowest relative humidity in August.

Table 6: Test locations weather information during experimental period

Month	TARI-Selian				SUA				TARI-Uyole			
	Max Temp (°C)	Min Temp (°C)	Rain (mm)	Rh (%)	Max Temp (°C)	Min Temp (°C)	Rain (mm)	Rh (%)	Max Temp (°C)	Min Temp (°C)	Rain (mm)	Rh (%)
March	28.9	19.3	302.7	83.0	30.4	21.2	186.7	82.0	23.4	14.6	156.7	92.6
April	24.3	17.5	195.3	88.0	28.9	21.1	228.6	87.0	23.7	14.8	149.9	84.8
May	22.9	17.4	137.5	87.0	28.4	19.9	111.0	86.0	23.6	11.2	29.5	74.2
June	22.3	14.5	7.4	83.0	28.3	17.1	6.4	80.0	22.5	7.9	0.0	71.0
July	22.1	14.3	0.8	78.0	27.1	16.8	33.0	76.0	21.9	8.9	0.0	72.0
August	23.4	13.4	1.8	76.0	28.5	16.5	0.0	71.0	24.7	7.8	0.0	70.0
Total	143.9	96.4	645.5	495.0	171.6	112.6	565.7	482.0	139.8	65.2	336.1	464.6
Mean	24.0	16.1	107.6	82.5	28.6	18.8	94.3	80.3	23.3	10.9	56.0	77.4

Max = maximum, Min = minimum, Rh = relative humidity, Temp = temperature

Test locations soil characteristics are presented in Table 7. Analysis of variance revealed no significant difference ($P \leq 0.05$) in % sandy soil particles, whereas there was a significant difference ($P \leq 0.05$) in % clay and silt soil particles among the test location soils. Soils at TARI-Uyole and TARI-Selian were classified as sandy clay loam while soils at SUA were classified as clay. Significant variations ($P \leq 0.05$) among the test location soils were observed in total nitrogen (TN), organic carbon (OC), and available phosphorus (P), a highly significant variation ($P \leq 0.01$) was observed in exchangeable calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) among the test location soils. Furthermore, soils from the test locations had no significant difference ($P \leq 0.05$) in soil pH.

Table 7: Soil characteristics of the test locations

Soil Properties	Location			Mean	Optimal levels	CV%	LSD (0.05)	P-value
	Selian	SUA	Uyole					
Soil pH	7.15a	6.00a	5.84a	6.33	5.5 – 7.5	6.2	1.69	0.132
% Clay	28.12b	52.12a	30.12b	36.79		5.9	9.29	0.013
% Silt	22.92a	9.92b	22.92a	18.59		11.6	9.30	0.04
% Sand	48.96a	37.96a	46.96a	44.63		4.8	9.30	0.064
Soil textural class	SCL	SCL	C					
TN %	0.16b	0.25a	0.15b	0.19	0.25 – 0.5	4.4	0.04	0.011
OC %	2.21b	4.52a	1.92b	2.88	>2	8.2	1.01	0.013
P (mg/kg)	23.93a	2.24c	13.26b	13.14	20 - 100	15.9	9.01	0.018
Ca ²⁺ (CmolKg ⁻¹)	22.14a	8.14b	8.05b	12.78	> 10	5.5	3.02	0.004
Mg ²⁺ (CmolKg ⁻¹)	5.15a	4.98a	2.61b	4.25	> 1.5	2.8	0.52	0.004
Na ⁺ (CmolKg ⁻¹)	1.03a	0.48c	0.58b	0.70	< 1.5	1.2	0.04	0.001
K ⁺ (CmolKg ⁻¹)	5.58a	0.96c	1.54b	2.69	0.6 – 2.0	0.8	0.09	0.001

TN = Total nitrogen, OC = Organic carbon, C = Clay, SCL = Sand clay loam, Different letters among samples = significant differences by Duncan's new multiple range test ($p \leq 0.05$), CV = coefficient of variation, LSD = Least significance difference, and P-value = F probability.

4.1.2 Yield and yield components of common bean genotypes in three bean growing agro-ecological zones of Tanzania

(i) Genotypes seed yield and yield components variation

The highest common bean seed yield was recorded at TARI-Selian, followed by TARI-Uyole and lastly SUA. Seed yield at TARI-Selian, ranged from 1252.1 to 5121.9 kg/ha with a mean of 2336.0 kg/ha, while at SUA, seed yield ranged from 668.5 to 2499.4 kg/ha with a mean yield of 1347.7 kg/ha, and at TARI-Uyole it has a range of 903.4 – 2773.1 kg/ha with mean yields of 1579.4 kg/ha. The large variation in seed yield among the common bean genotypes was observed at TARI-Selian due to the larger interquartile range of the box plot compared to the rest experimental sites (Fig. 21D). The highest 100 seed weight was recorded at TARI-Selian compared to other experimental sites. Weight of 100 seeds per genotype at TARI-Selian had a range of 20.3 – 66.0 g with a mean of 42.9 g. At SUA, the weight of 100 seeds ranged from 15.6 to 44.9 g with a mean of 30.1 g, whereas at TARI-Uyole, the weight of 100 seeds had a range of 17.0 – 50.1 g with a mean of 32.7 g. There was greater variability in the weight of 100 seeds among genotypes at TARI-Selian compared to other sites. Most of the tested bean genotypes weighted 100 seeds below the mean in all sites (Fig. 21C). The highest number of pods per plant and the largest variability among bean genotypes were recorded at TARI-Selian compared to other sites. Most of the bean genotypes at TARI-Selian and SUA had the number of pods per plant greater than their site means (Fig. 21A). The largest variation and highest number of seeds per pod among the experimental sites was recorded at TARI-Selian (Fig. 21B).

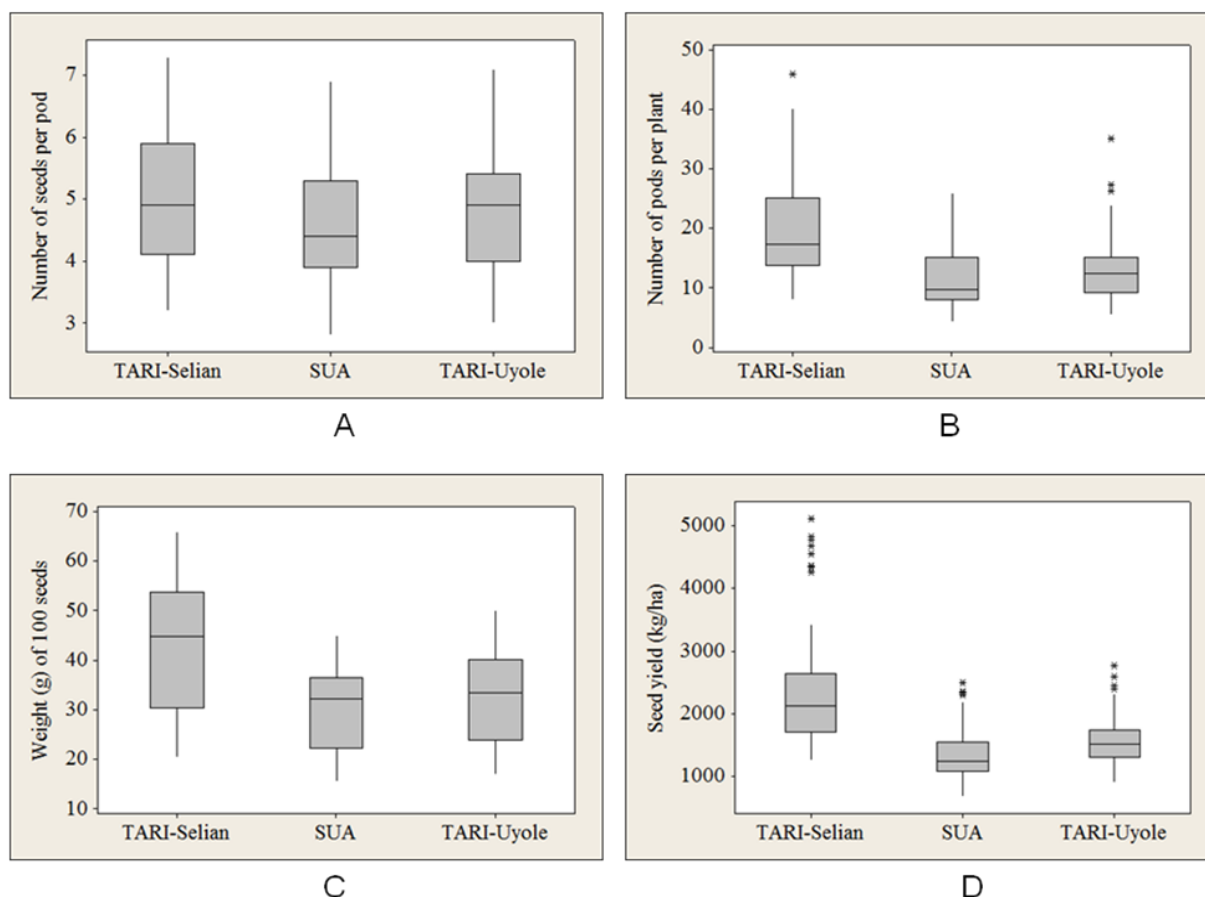


Figure 21: Distribution of 99 common bean genotypes for seed yield and yield components across sites (TARI-Selian, SUA and TARI-Uyole); (A) Number of pods per plants; (B) Number of seeds per pod; (C) 100 seed weight; (D) Seed yield

The highest seed yielding genotype at TARI-Selian was Cheupe (5122 kg/ha), closely followed by Uyole 84 (5116 kg/ha) and Selian 05 (4831 kg/ha). Among the common bean genotypes harvested at SUA, Jabeyila recorded the highest seed yield of 2499 kg/ha, followed by Cheupe (2353 kg/ha) and Mwamikola (2329 kg/ha). At Uyole-Mbeya, the highest seed yielding genotypes was Selian 14 (2773 kg/ha), followed by DOOR 500 (2772 kg/ha) and Selian 15, which had 2588 kg/ha (Table 8).

At TARI-Selian, the highest number of pods per plant was recorded from Cheupe (45.9) followed by Ruondera (40.1) and Kaisho kamugole (37.2). Cheupe also recorded the highest number of pods per plant (25.9) at SUA, closely followed by Jabeyila (25.1) and Mwamikola (21.8), whereas Wifi nyegela had the highest number of pods per plant (35.0) at TARI-Uyole, followed by Kikobe (27.3) and DOOR 500 (26.3). In terms of the number of seeds per pod, Malirahinda (7.3), Cheupe (7.3), and Ngoma za bahaya (7.0) were the best three genotypes at TARI-Selian. At SUA the best three genotypes in the number of seeds per pod were Kaempu

(6.9), Kikobe (6.6), and Kyakaragwe (6.5), whereas Cheupe (7.1), kamosi (7.0), and kaempu (7.0) had the highest number of seeds per pod at TARI-Uyole (Table 9).

The highest 100 seed weight-containing common bean genotypes at TARI-Selian were Lyamungo 90 (66.0 g), CAL96 (65.0 g), and Msolini (64.0 g), Whereas Lyamungo 90 (44.9 g), Msolini (44.5 g), and Selian 15 (44.2 g) recorded the highest 100 seed weight at SUA. At TARI-Uyole Selian 15 (50.1 g), Msolini (47.0 g) and Uyole 94 (46.7 g) were the highest 100 seed weight-containing common bean genotypes. The earliest flowering 3 common bean genotypes at TARI-Selian were Jesca (34.3 days), Kigoma (35.0 days), and Selian 12 (35.0 days), whereas Pesa (33.0 days), Rojo (33.0 days), and Zawadi (33.3 days) flowered early at SUA. At Uyole Calma Uyole (36.0 days), Kigoma (36.0 days), and Kintuntunu (36.0 days) were observed as the earliest flowering common bean genotypes (Table 10).

Table 8: Test locations seed yield mean and ranking of 99 common bean genotypes based on seed yield, AMMI stability value (ASV), and yield stability index (YSI)

GN	Genotype	Common bean seed yield (kg/ha)					Common bean genotypes ranking					
		Selian	Uyole	SUA	Mean	IPCA1	IPCA2	ASV	RASV _i	RY _i	YSI _i	RYSI _i
G1	ACC 714	2629no	1888g-l	1639h-m	2052j-n	0.06	0.24	0.4	1	22	23	1
G2	Bagara Ompigize	2041xyz	1681j-t	1278o-A	1667w-E	3.09	2.76	15.6	43	43	86	35
G3	Bangaya Akatebe	1863A-D	1029C-F	893H-M	1261R-W	-0.22	-1.33	1.7	2	92	94	45
G4	Bilfa 4	1708E-J	1507m-z	811KLM	1342M-T	2.97	6.71	16.2	45	86	131	77
G5	Bilfa Uyole	2400qrs	1347s-C	919F-M	1555D-K	-4.26	2.06	21.2	51	57	108	58
G6	Buji	1694F-K	1414m-B	1358m-y	1489F-M	6.01	-1.53	29.9	69	64	133	78
G7	Burushu	2637n	1096A-F	1542j-p	1758t-A	-4.04	-9.75	22.3	55	39	94	44
G8	CAL 96	1884ABC	903F	1456k-t	1414K-S	2.42	-10.27	15.8	44	71	115	66
G9	Calima Uyole	1403O-R	1141y-F	1239p-E	1261R-W	7.13	-3.45	35.5	76	93	169	96
G10	Cheupe	5122a	1957f-l	2353ab	3144a	-21.19	-11.50	105.7	96	1	97	49
G11	Chumba Neroza	3175hi	1602l-x	1260p-B	2012k-o	-9.14	0.19	45.3	86	23	109	60
G12	CODMLB 033	2518n-q	1645k-v	1732f-k	1965n-q	0.72	-4.21	5.5	9	27	36	6
G13	DOR 500	3012jk	2772a	1137u-J	2307fgh	-3.12	18.54	24.1	60	16	76	27
G14	Fibea	2490pqr	1728h-q	1245p-C	1821q-w	-1.57	3.18	8.4	19	35	54	15
G15	Jabeyila	2536n-q	2393bcd	2499a	2476de	8.41	-3.38	41.9	84	10	94	43
G16	Jesca	1467M-P	1328t-D	1243p-D	1346M-S	7.30	-0.96	36.2	78	84	162	92
G17	KAB o6F2-8-35	1758C-H	1446m-A	854J-M	1353M-S	2.43	5.21	13.1	32	81	113	62
G18	KAB o6F2-8-36	2312stu	1428m-B	1093x-L	1611z-H	-1.94	1.12	9.7	25	52	77	28
G19	Kabanima	1768C-G	1257x-F	936C-M	1321N-U	2.01	1.50	10.1	26	87	113	64
G20	Kabumburi	1836B-E	1171y-F	1477j-t	1495F-M	4.21	-6.68	21.9	53	61	114	65
G21	Kachele	2498opq	1607l-x	1225q-F	1777s-y	-2.29	1.70	11.5	27	37	64	20
G22	Kaempu	2413qrs	1762h-o	1421l-v	1865o-u	0.43	1.54	2.6	3	33	36	9
G23	Kainja	1628H-L	1357q-C	1106w-L	1364L-S	4.93	0.95	24.5	62	79	141	85
G24	Kaisho kamugole	2824lm	1609l-x	858I-M	1764t-z	-7.90	5.90	39.6	82	38	120	67
G25	Kakaritusi	1969yzA	1604l-x	1301o-z	1624y-G	3.63	1.48	18.1	47	49	96	48
G26	Kamoshi	2093wxy	1455m-A	1116v-K	1555D-K	0.59	1.53	3.3	7	56	63	19
G27	Kamosi	2212uvw	1671k-t	1531j-q	1805r-x	2.80	-0.85	13.9	35	36	71	25
G28	Kanade	3260gh	2160c-g	1759f-j	2393ef	-4.58	1.64	22.8	56	11	67	21
G29	Kashule	1559K-N	940EF	942C-M	1147VW	2.84	-2.77	14.4	36	98	134	80
G30	Kasukari	2145vwx	1660k-u	1097x-L	1634y-G	0.82	4.60	6.1	10	46	56	16
G31	Katuku	2833lm	1954f-l	1692g-l	2160ijk	-1.44	0.19	7.2	15	19	34	4
G32	Katuku2	4270e	2063d-i	820KLM	2384ef	-21.16	10.68	105.5	95	12	107	56
G33	Kibugu	1734D-I	1281v-E	1114v-K	1376L-S	3.55	-0.37	17.6	46	75	121	71
G34	Kigoma	1598I-M	1400n-B	1333m-y	1444I-Q	6.80	-1.27	33.8	75	68	143	86

GN	Genotype	Common bean seed yield (kg/ha)					Common bean genotypes ranking					
		Selian	Uyole	SUA	Mean	IPCA1	IPCA2	ASV	RASV _i	RY _i	YSI _i	RYSI _i
G35	Kikobe	3316fg	2311b-e	2011def	2546d	-2.98	0.49	14.8	38	9	47	11
G36	Kilindi	1658G-K	1246x-F	1221q-F	1375L-S	4.83	-2.12	24.0	59	76	135	81
G37	Kinyobya	1562K-N	1141y-F	1066y-L	1256S-W	4.44	-1.49	22.1	54	94	148	87
G38	Kipapi	1775C-G	1511m-y	1487j-s	1591B-J	6.37	-1.92	31.6	71	54	125	73
G39	Kisapuri	2364rst	1395o-C	1110v-L	1623y-G	-2.52	0.36	12.5	31	50	81	30
G40	Kitebe	2520n-q	1998e-k	1242p-D	1920n-s	-0.72	6.98	7.8	17	31	48	12
G41	Kituntunu	2900kl	1188y-F	1073x-L	1720u-C	-9.20	-2.89	45.7	87	41	128	74
G42	Kyababikira	1782C-G	1398n-B	1297o-z	1493F-M	4.66	-1.11	23.1	58	62	120	68
G43	Kyakaragwe	2424qrs	1184y-F	1178r-H	1595A-I	-3.65	-3.58	18.4	49	53	102	52
G44	Lyamungo 85	1682F-K	1720i-r	1467j-t	1623y-G	8.12	1.44	40.3	83	51	134	79
G45	Lyamungo 90	1356P-S	1265w-E	892H-M	1171UVW	6.06	2.77	30.2	70	96	166	95
G46	Maharage Kamba	2764m	1882g-l	1167t-I	1938n-r	-4.21	5.93	21.7	52	29	81	29
G47	Maharage Mbeya	2209uvw	1891g-l	1887e-h	1996l-o	5.93	-2.23	29.5	68	25	93	41
G48	Malirahinda	2038xyz	1427m-B	1222q-F	1562C-K	1.67	-0.12	8.3	18	55	73	26
G49	Masusu	3110ij	1719i-s	2186bcd	2338e-h	-2.36	-9.80	15.3	40	14	54	14
G50	Meupe Uyole	1706E-J	1427m-B	1225q-F	1453H-P	5.14	0.32	25.5	64	67	131	76
G51	Mshindi	1415OPQ	1063B-F	1370m-y	1283Q-V	7.46	-6.23	37.5	80	90	170	97
G52	Msolini	2812lm	2035e-j	2098b-e	2315fgh	1.58	-3.77	8.7	21	15	36	5
G53	Mwami Kola	2214uvw	1886g-l	2329abc	2143i-l	8.53	-7.92	43.0	85	20	105	55
G54	Ngoma za bahaya	2150vwx	1630l-w	1208r-G	1663x-E	1.32	2.76	7.1	14	44	58	17
G55	Ngwakungwaku	2892klm	1511m-y	2295abc	2232ghi	-0.33	-13.80	13.9	34	17	51	13
G56	Njano fupi	1945zAB	1150y-F	1181r-H	1426K-R	1.20	-3.40	6.8	13	70	83	33
G57	Njano Uyole	1456NOP	1477m-z	1103x-L	1345M-S	7.21	2.95	35.9	77	85	162	93
G58	Nyeupe Kubwa	4356e	2440bc	1886e-h	2894b	-13.98	2.37	69.4	91	4	95	46
G59	Nyeupe ndogo	2469pqr	1769h-n	1538j-p	1925n-s	0.60	0.06	3.0	6	30	36	7
G60	Pasi	2501opq	1712i-s	1439k-u	1884o-t	-0.58	0.48	2.9	4	32	36	8
G61	Pesa	1805C-F	1504m-z	1571i-o	1627y-G	6.54	-3.12	32.6	73	48	121	70
G62	Raja	1960y-B	1604l-x	854J-M	1473G-N	1.03	7.17	8.8	22	65	87	36
G63	Rojo	1280RS	1409n-B	1416l-w	1368L-S	10.63	-1.73	52.7	88	77	165	94
G64	Rosenda	1791C-G	1429m-B	668M	1296O-V	0.91	7.28	8.6	20	88	108	59
G65	Rozikoko fupi	1615I-L	1209y-F	981A-L	1268R-W	3.66	0.47	18.2	48	91	139	84
G66	Ruondera	4548d	2237c-f	1933d-g	2906b	-16.56	-1.38	82.1	93	3	96	47
G67	RWR 2154	2642n	2053e-i	1119v-K	1938n-r	-2.50	9.14	15.4	42	28	70	23
G68	Selian 05	4831b	1313t-D	1988def	2711c	-23.17	-15.57	115.9	97	7	104	54
G69	Selian 06	4785bc	1385p-C	958B-M	2376efg	-28.61	-1.43	141.9	99	13	112	61
G70	Selian 10	1763C-H	1639k-v	1070y-L	1491F-M	4.53	5.20	23.1	57	63	120	69
G71	Selian 11	2893klm	2144c-g	1543j-p	2193hij	-2.15	4.69	11.6	28	18	46	10

GN	Genotype	Common bean seed yield (kg/ha)					Common bean genotypes ranking					
		Selian	Uyole	SUA	Mean	IPCA1	IPCA2	ASV	RASV _i	RY _i	YSI _i	RYSI _i
G72	Selian 12	1293QRS	1253x-F	901G-M	1149VW	6.71	2.59	33.4	74	97	171	98
G73	Selian 13	1669F-K	1249x-F	941C-M	1286P-V	3.03	1.46	15.1	39	89	128	75
G74	Selian 14	3429f	2773a	2132b-e	2778bc	-1.43	5.33	8.9	23	5	28	2
G75	Selian 15	4678c	2588ab	1993def	3086a	-16.02	2.63	79.5	92	2	94	42
G76	Selian 9	2775lm	1782h-m	1844e-i	2134i-m	-0.67	-4.07	5.3	8	21	29	3
G77	Selian 94	1520L-O	1258x-F	1430k-u	1403K-S	7.58	-4.40	37.8	81	72	153	88
G78	Selian 97	1766C-H	1460m-A	818KLM	1348M-S	2.20	5.87	12.4	30	83	113	63
G79	Selundo	2259tuv	1672k-t	2066cde	1999l-o	5.55	-7.68	28.6	67	24	91	39
G80	Sinon	1760C-H	1637k-v	1265o-B	1554D-K	5.73	2.71	28.5	66	58	124	72
G81	SMC 17	1514L-O	1366p-C	1176s-H	1352M-S	6.57	0.36	32.6	72	82	154	89
G82	SMC 18	1982yzA	1155y-F	946C-M	1361L-S	-0.59	-0.39	2.9	5	80	85	34
G83	Soya	1857A-D	1309t-D	932D-M	1366L-S	1.28	2.15	6.7	12	78	90	38
G84	Soya Mbeya	4343e	2081d-h	1614h-n	2680c	-17.04	0.75	84.5	94	8	102	51
G85	SUA 90	1278RS	1333t-D	945C-M	1185T-W	7.48	3.17	37.2	79	95	174	99
G86	Tema	2245tuv	1512m-y	802LM	1520E-L	-2.64	6.09	14.4	37	60	97	50
G87	Tikumba Nyama	2060xyz	1671k-t	1489j-r	1740t-B	4.12	-0.09	20.4	50	40	90	37
G88	Urafiki	1393O-R	975DEF	969A-M	1112W	4.89	-2.37	24.3	61	99	160	91
G89	Uyole 03	2575nop	1500m-z	1432k-u	1836p-v	-2.31	-2.56	11.7	29	34	63	18
G90	Uyole 04	2201uvw	1135z-F	1322n-y	1553D-K	-0.67	-5.76	6.7	11	59	70	24
G91	Uyole 16	2087wxy	1500m-z	1480j-s	1689v-D	3.04	-2.44	15.3	41	42	83	32
G92	Uyole 18	1581J-N	1220y-F	2090b-e	1630y-G	10.77	-13.38	55.1	89	47	136	82
G93	Uyole 84	5116a	1736h-p	1313n-y	2722c	-28.37	-1.44	140.7	98	6	104	53
G94	Uyole 94	1787C-G	1291u-E	1071y-L	1383L-S	2.78	0.23	13.8	33	74	107	57
G95	Uyole 96	2296stu	1634l-w	1001z-L	1644y-F	-1.44	5.23	8.9	24	45	69	22
G96	Uyole 98	2124vwx	1323t-D	929E-M	1458H-O	-1.44	1.99	7.4	16	66	82	31
G97	Wanja	1663G-K	1351r-C	1185r-H	1400K-S	5.01	-0.18	24.9	63	73	136	83
G98	Wifi Nyegela	2211uvw	2391bcd	1363m-y	1988m-p	4.91	11.47	26.9	65	26	91	40
G99	Zawadi	1252S	1650k-v	1385m-x	1429J-R	11.78	2.12	58.5	90	69	159	90

Different letters among genotype values = significant differences by Duncan's new multiple range test (DNMRT) ($p \leq 0.05$), GN = Genotype number, IPC1 and IPC2 are interaction principal component 1 and 2 respectively, ASV = AMMI Stability Value, RASV = rank of the genotype across environments based on AMMI Stability Value, YSI = Yield Stability Index, RY = rank of the genotype across environments based on mean yield across environments, RYSI = rank of the genotype based on Yield Stability Index

Table 9: Variation in number of pods per plant and seeds per pod among bean genotypes at the three test locations

GN	Genotype	Number of pods/plant				Number of seeds/pod			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G1	ACC 714	17.9AB	10.5p-z	13.5p-z	14.0r-u	5.9g-m	5.2l-u	5.9b-f	5.7i-m
G2	Bagara Ompigize	23.6op	18.1c-g	20.1e-i	20.6e-h	4.1F-J	4.1z-I	4.3j-o	4.2y-F
G3	Bangaya Akatebe	20.2tuv	8.7u-G	9.0 D-M	12.6t-A	6.1e-j	5.7f-m	5.0ghi	5.6i-m
G4	Bilfa 4	15.5GHI	6.9D-I	13.0q-C	11.8x-G	5.0s-y	5.5g-n	4.0op	4.8p-s
G5	Bilfa Uyole	15.5GHI	4.3I	7.4J-M	9.1L-Q	3.3MN	3.8D-M	5.0ghi	4.0D-H
G6	Buji	8.0Y	8.3v-H	7.6 I-M	8.0QR	4.9t-z	4.5v-D	5.5c-g	5.0pqr
G7	Burushu	20.0u-x	14.2j-o	9.1C-M	14.4p-s	5.0s-y	5.3k-s	4.3nop	4.8p-s
G8	CAL 96	10.9STU	10.1r-C	7.8H-M	9.6K-P	3.6LMN	4.6s-B	5.0ghi	4.4v-C
G9	Calima Uyole	9.9VW	7.0C-I	5.5M	7.5R	4.0H-L	4.2y-G	4.0nop	4.1C-H
G10	Cheupe	45.9a	25.9a	14.0o-x	28.6a	7.3a	6.0b-i	7.1a	6.8ab
G11	Chumba Neroza	15.4GHI	7.9x-H	14.1o-v	12.4u-B	5.9g-m	5.9c-ij	5.9b-f	5.9f-i
G12	CODMLB 033	21.7rs	16.1e-k	13.8p-y	17.2lmn	3.4MN	2.9OP	3.9op	3.4MN
G13	DOR 500	27.5j	12.3n-t	26.3bc	22.0cde	6.3c-g	4.9n-w	6.0bcd	5.8h-l
G14	Fibea	16.2D-H	6.4E-I	10.3u-L	11.0A-K	4.2E-J	3.1NOP	4.0nop	3.8H-L
G15	Jabeyila	25.7 lm	25.1a	23.0de	24.6b	5.5m-r	5.0n-v	6.0bcd	5.5j-n
G16	Jesca	12.2O-R	7.9x-H	9.0 D-M	9.7J-P	4.1F-J	4.6s-B	5.0ghi	4.6s-x
G17	KAB o6F2-8-35	15.5GHI	8.3 v-H	12.0r-F	11.9w-E	4.0H-L	3.4I-P	4.0nop	3.8G-L
G18	KAB o6F2-8-36	17.3ABC	8.7u-G	11.3r-J	12.5u-B	3.9I-L	3.7FG-N	3.0q	3.5LM
G19	Kabanima	16.4C-G	6.9D-I	12.3r-E	11.9w-F	4.0H-L	3.5H-O	4.0nop	3.8G-L
G20	Kabumburi	11.6RST	12.3n-t	8.8 D-M	10.9B-K	5.3p-u	4.1yz-H	4.0nop	4.5u-z
G21	Kachele	30.7g	18.1c-h	20.8d-h	23.2c	6.7bc	6.5a-d	4.0nop	5.7h-l
G22	Kaempu	24.1o	16.7e-j	22.4def	21.1def	6.7bc	6.9a	7.0a	6.9a
G23	Kainja	17.9AB	8.0x-H	10.5t-K	12.1v-C	4.3D-J	3.7F-N	4.3j-o	4.1B-H
G24	Kaisho kamugole	37.2c	10.3p-B	19.0f-j	22.2cde	5.1s-x	6.4a-e	6.1bc	5.8g-j
G25	Kakaritusi	16.7C-F	11.3p-v	13.3q-A	13.8r-v	6.4c-f	6.0b-i	5.1ghi	5.8g-k
G26	Kamoshi	27.4j	11.0p-x	14.1o-v	17.5j-m	6.3c-g	6.4a-e	6.0bcd	6.2cde
G27	Kamosi	28.7i	17.7c-i	19.0f-j	21.8cde	6.7bcd	5.9d-k	7.0a	6.5bc
G28	Kanade	24.3no	16.5e-j	18.0g-m	19.6fgh	5.4n-s	4.4v-E	6.3b	5.4mn
G29	Kashule	15.8F-I	7.5y-H	8.0F-M	10.4D-M	4.9u-A	4.1y-H	4.0nop	4.3w-E
G30	Kasukari	23.0pq	9.7r-D	14.1o-v	15.6opq	6.3d-h	6.3a-f	6.0b-e	6.2def
G31	Katuku	22.3qr	15.9f-k	18.7f-k	19.0h-k	4.8v-B	4.0A-J	5.2ghi	4.7r-w
G32	Katuku2	35.5d	8.1 v-H	14.4m-t	19.3ghi	5.2q-v	4.8p-y	4.0nop	4.7r-w
G33	Kibugu	11.0STU	5.8GHI	8.5D-M	8.4O-R	4.7w-C	4.1z-I	4.3k-p	4.4w-C
G34	Kigoma	11.9P-S	7.5y-H	9.0 D-M	9.4K-Q	5.1r-w	4.3w-F	4.0nop	4.5u-z
G35	Kikobe	35.3d	19.7bcd	27.3b	27.4a	6.1e-j	6.6ab	4.7h-m	5.8g-l

GN	Genotype	Number of pods/plant				Number of seeds/pod			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G36	Kilindi	14.1JKL	6.9D-I	7.7 I-M	9.6K-Q	4.3C-I	4.0A-J	4.0nop	4.1A-G
G37	Kinyobya	16.8C-F	9.3t-E	9.0 D-M	11.7x-G	5.9g-m	4.2y-G	4.0nop	4.7q-v
G38	Kipapi	16.9B-E	15.7g-k	11.0s-K	14.5pqr	4.5z-F	3.7F-N	4.0nop	4.1C-H
G39	Kisapuri	17.9AB	8.7v-G	12.0r-F	12.9r-z	4.7x-D	4.1y-H	4.0nop	4.2x-F
G40	Kitebe	16.2D-H	7.5y-H	13.0q-C	12.2u-C	6.1e-j	5.2l-u	6.0b-e	5.8h-l
G41	Kituntunu	25.1mn	8.7u-G	13.1q-C	15.6opq	5.8i-n	5.7f-l	4.9ghi	5.5lmn
G42	Kyababikira	10.5UV	8.4v-H	7.5I-M	8.8M-R	3.7KLM	3.7F-N	3.9op	3.8H-L
G43	Kyakaragwe	23.1pq	9.2u-E	9.0 D-M	13.8r-v	4.8v-B	6.5abc	5.2gh	5.5 j-n
G44	Lyamungo 85	9.3WX	10.5p-z	11.0s-K	10.3E-N	3.2N	3.8D-M	4.0nop	3.7J-M
G45	Lyamungo 90	11.5RST	6.0F-I	7.1KLM	8.2PQR	3.6LMN	3.8D-M	5.0ghi	4.1z-G
G46	Maharage Kamba	32.3f	10.3p-A	17.7g-o	20.1fgh	6.9ab	5.1m-v	6.3b	6.1d-g
G47	Maharage Mbeya	11.9P-S	11.8o-u	13.3q-A	12.3u-B	6.0f-l	4.8p-y	5.0ghi	5.3no
G48	Malirahinda	22.5qr	11.3p-w	15.1k-r	16.3mno	7.3a	6.2b-f	6.1b	6.5bc
G49	Masusu	27.8j	16.7e-j	12.5r-D	19.0hij	4.8v-B	4.8o-y	3.9op	4.5t-y
G50	Meupe Uyole	19.7u-y	5.9F-I	8.6 D-M	11.4y-I	4.5A-G	3.2K-P	4.9g-j	4.2y-F
G51	Mshindi	16.0E-H	13.3k-p	10.3u-L	13.2r-x	5.2q-v	4.0A-J	5.0ghi	4.7p-v
G52	Msolini	20.2t-w	16.1e-k	14.8l-s	17.0l-o	3.3MN	3.3J-P	3.0q	3.2N
G53	Mwami Kola	17.2A-D	21.8b	18.5g-l	19.2hi	5.7j-o	6.1b-g	5.9b-f	5.9f-i
G54	Ngoma za bahaya	22.6qr	12.6m-s	17.3h-p	17.5jlm	7.0ab	6.1b-h	6.0b-e	6.4cde
G55	Ngwakungwaku	18.1zA	15.0i-n	6.4LM	13.2r-x	4.3D-J	3.9C-K	3.9op	4.0E-I
G56	Njano fupi	13.8KLM	9.1u-F	7.1KLM	10.0H-O	4.2E-J	4.4v-E	4.0nop	4.2y-F
G57	Njano Uyole	13.7K-N	9.7r-D	13.0q-C	12.2v-C	3.9I-L	3.9B-J	4.0nop	4.0F-J
G58	Nyeupe Kubwa	27.3jk	15.5g-l	20.2e-i	21.0def	6.1e-k	5.5h-o	5.0ghi	5.5lmn
G59	Nyeupe ndogo	25.5lm	17.1d-j	16.8i-q	19.8fgh	5.8i-n	3.9C-K	4.9g-k	4.8p-s
G60	Pasi	26.1lm	15.0i-n	20.5d-i	20.5e-h	3.9JKL	2.8P	3.0q	3.2N
G61	Pesa	11.7Q-T	8.9u-G	10.4u-L	10.3E-M	5.3o-t	4.6s-B	4.6i-n	4.8p-s
G62	Raja	19.9u-x	7.0C-I	14.5m-t	13.8r-v	4.6y-E	4.3w-F	5.0ghi	4.6r-w
G63	Rojo	10.2UVW	14.5j-o	13.3q-A	12.7t-A	4.5z-F	5.0n-v	5.0ghi	4.8p-s
G64	Rosenda	18.9yz	5.4HI	14.9l-s	13.0r-y	4.1G-K	3.5G-O	4.2m-p	3.9F-K
G65	Rozikoko fupi	12.1O-R	9.6s-D	8.8 D-M	10.2G-N	4.8v-B	4.7r-A	4.9g-k	4.8p-u
G66	Ruondera	40.1b	18.9cde	23.9cd	27.6a	5.1r-w	3.3J-P	6.0b-e	4.8p-t
G67	RWR 2154	19.2vxy	8.0x-H	14.2m-u	13.8r-v	5.1r-w	3.9B-J	4.0nop	4.4w-D
G68	Selian 05	34.3e	17.5d-i	14.0o-x	22.0cde	5.3o-t	5.8e-l	4.0nop	5.1op
G69	Selian 06	25.8lm	8.7u-G	13.0q-C	15.8nop	6.1e-j	5.0n-v	5.0ghi	5.4mn
G70	Selian 10	15.9E-H	9.9r-D	15.0k-s	13.6r-w	6.5cde	6.1b-h	6.0b-e	6.2def
G71	Selian 11	32.6f	15.5g-m	19.3f-i	22.5cd	7.0ab	4.3w-F	5.9b-f	5.7i-m
G72	Selian 12	13.3K-N	6.9D-I	13.2q-B	11.1z-K	4.1G-K	3.0OP	4.0nop	3.7I-M

GN	Genotype	Number of pods/plant				Number of seeds/pod			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G73	Selian 13	12.2O-R	8.3v-H	10.0y-L	10.2G-N	3.9I-L	4.1z-I	4.0nop	4.0E-I
G74	Selian 14	34.2e	13.3k-q	17.7g-o	21.7cde	5.7k-p	4.9n-x	5.9b-f	5.5j-n
G75	Selian 15	25.7lm	16.0e-k	18.0g-n	19.9fgh	5.1s-x	4.1z-I	4.9g-l	4.7q-w
G76	Selian 9	23.9op	20.5bc	18.3g-l	20.9defg	6.4c-f	5.4i-p	6.3b	6.0e-h
G77	Selian 94	14.1JKL	10.6p-y	9.4A-M	11.4y-J	4.8v-B	4.7p-z	4.9ghi	4.8p-t
G78	Selian 97	12.7N-Q	6.9D-I	10.0y-L	9.9I-O	5.0s-y	5.0n-v	5.0ghi	5.0opq
G79	Selundo	26.4kl	8.3 v-H	13.5p-z	16.1mno	4.1G-K	3.9C-K	4.0nop	4.0F-J
G80	Sinon	17.3ABC	9.6s-D	12.0r-F	13.0r-y	4.0H-L	3.7E-M	3.7p	3.8G-L
G81	SMC 17	11.5RST	9.0u-F	11.7r-H	10.8B-L	4.2E-J	4.2y-G	3.9op	4.1A-G
G82	SMC 18	21.1st	6.4E-I	11.0s-K	12.8s-z	4.8v-B	3.9C-K	4.0nop	4.2y-F
G83	Soya	13.1L-O	7.3A-H	10.1v-L	10.2G-N	4.9u-A	4.1y-H	4.9g-j	4.6r-w
G84	Soya Mbeya	31.7f	15.1h-n	21.3d-g	22.7c	4.9t-z	4.1z-I	4.0nop	4.3w-E
G85	SUA 90	14.8IJ	8.6v-G	8.4E-M	10.6C-L	5.6l-q	5.4i-q	5.4d-g	5.5lmn
G86	Tema	29.7h	8.7u-G	15.3j-r	17.9i-l	5.9g-m	5.3j-r	5.4dfg	5.6 j-n
G87	Tikiumba Nyama	15.3HI	9.4t-E	11.4r-I	12.0w-D	4.7x-D	4.5u-C	4.0nop	4.4v-B
G88	Urafiki	15.3GHI	15.7g-k	11.8r-G	14.3q-t	4.0H-L	4.7p-z	5.0ghi	4.6s-x
G89	Uyole 03	14.3JK	7.7y-H	9.3B-M	10.4D-M	4.3D-J	4.1y-H	4.9ghi	4.4u-A
G90	Uyole 04	12.3O-R	12.7l-r	12.3r-E	12.4u-B	3.2N	4.1z-I	3.0q	3.4MN
G91	Uyole 16	13.7K-N	8.2 v-H	8.8 D-M	10.2F-N	4.4B-H	4.0A-J	5.0ghi	4.5u-z
G92	Uyole 18	12.9M-P	15.9f-k	7.9G-M	12.2v-C	5.0s-y	4.5u-C	4.0nop	4.5s-y
G93	Uyole 84	32.1f	7.1B-I	11.8r-H	17.0l-o	5.9h-m	5.9c-j	5.2ghi	5.7i-m
G94	Uyole 94	15.9E-H	9.7r-D	9.2B-M	11.6x-H	3.3MN	4.6s-B	4.1nop	4.0F-J
G95	Uyole 96	21.1st	7.3A-I	9.7z-L	12.7t-z	3.9I-L	4.1y-H	4.3k-p	4.1A-G
G96	Uyole 98	17.1A-D	7.0C-I	12.4r-E	12.2v-C	3.7KLM	3.5G-O	3.7p	3.6KLM
G97	Wanja	10.8TUV	7.4z-H	7.6 I-M	8.6N-R	4.3D-J	3.9B-J	5.0ghi	4.4v-C
G98	Wifi Nyegela	20.4tu	18.7c-f	35.0a	24.7b	6.2e-i	5.9c-j	7.0a	6.4cd
G99	Zawadi	8.4XY	10.3p-A	8.7 D-M	9.1L-Q	5.9h-m	5.3k-t	5.0ghi	5.4mn
Mean		19.8	11.3	13.3	14.8	5.0	4.6	4.8	4.8
LSD (0.05)		0.893	2.453	3.132	2.391	0.365	0.541	0.496	0.476
CV %		2.8	13.5	14.6	10.1	4.5	7.3	6.4	6.2
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Different letters among genotype values = significant differences by Duncan's new multiple range test (DNMRT) ($p \leq 0.05$); GN = Genotype number; CV % = Coefficient of variation; LSD = Least significance difference; P-value. = F probability

Table 10: Variation in weight of 100 seeds and days to 75% flowering among bean genotypes at the three test locations

GN	Genotype	Weight of 100 seeds (g)				Days to 75% flowering			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G1	ACC 714	21.7QR	19.6MNO	21.7OP	21.0ST	37.7m-u	43.0z	43.0xyz	41.2A-G
G2	Bagara Ompigize	35.0D	24.9BC	27.0IJ	29.0J	37.3m-p	40.0tu	38.7i-l	38.7k-p
G3	Bangaya Akatebe	23.3NO	16.0RS	19.4RS	19.6UV	37.0lm	42.0w-z	40.0m-q	39.7p-v
G4	Bilfa 4	26.0K	23.1EFG	24.8K	24.6NO	40.0L	40.0tu	40.0m-q	40.0r-y
G5	Bilfa Uyole	62.0d	41.2d	40.0no	47.7f	41.0M	43.0z	41.0qst	41.7D-J
G6	Buji	56.3h	41.8d	40.8mn	46.3hi	36.3cde	34.0a-e	42.0t-x	37.4e-j
G7	Burushu	35.0D	21.9HIJ	26.2J	27.7L	38.7v-E	38.0nr	39.0i-m	38.6k-o
G8	CAL 96	65.0b	37.7fgh	44.0f	48.9d	35.3b	37.0k-n	42.0t-x	38.1i-m
G9	Calima Uyole	61.3d	35.6k-n	40.8mn	45.9ij	39.0AEF	38.0nor	36.0a	37.7f-k
G10	Cheupe	33.0F	18.7OPQ	23.3LMN	25.0NO	43.7P	43.0z	43.0xyz	43.2LMN
G11	Chumba Neroza	30.3G	23.7DE	26.2J	26.7M	37.3l-q	43.0z	39.0i-m	39.8q-w
G12	CODMLB 033	51.0mn	38.4fg	41.8i-l	43.7m	39.7F-L	42.0w-z	42.0t-x	41.2A-G
G13	DOR 500	24.0MN	18.3PQ	20.0R	20.8T	40.0L	43.0z	42.0t-x	41.7D-J
G14	Fibea	62.0d	41.2d	45.0e	49.4c	38.0uv	43.0z	44.0z-D	41.7D-J
G15	Jabeyila	28.3I	21.1IJK	25.3K	24.9NO	37.7m-u	43.0z	39.0i-m	39.9q-x
G16	Jesca	52.0l	34.7m-p	39.6op	42.1n	34.3a	36.0hk	38.0ei	36.1a-d
G17	KAB o6F2-8-35	52.0l	32.3stu	36.8vw	40.4uvw	38.0uv	38.0n-r	38.0efi	38.0h-l
G18	KAB o6F2-8-36	52.0l	31.8u	37.8stu	40.5tuv	38.7v-E	43.0z	39.0i-m	40.2t-A
G19	Kabanima	45.0t	33.0rst	32.6BC	36.9zA	38.0puv	38.0n-r	41.0q-t	39.0l-r
G20	Kabumburi	51.3lm	36.9hij	35.3yz	41.2qrs	36.3c-f	38.0n-r	37.0ae	37.1d-i
G21	Kachele	24.0MN	16.9R	18.3TU	19.7UV	44.3Q	43.0z	44.0z-D	43.8N
G22	Kaempu	22.3PQ	19.2M-P	17.0V	19.5UV	43.7P	43.0z	40.7qrs	42.4I-L
G23	Kainja	41.0w	30.5vw	33.3AB	34.9D	38.3u-A	42.0w-z	41.0q-t	40.4u-C
G24	Kaisho kamugole	26.0K	19.7MNO	19.9R	21.9R	44.0PQ	45.0A	38.0e-i	42.3H-L
G25	Kakaritusi	27.7IJ	20.0K-N	22.6N	23.4Q	40.0L	42.0w-z	40.0m-q	40.7v-D
G26	Kamoshi	20.3S	19.0N-Q	19.0ST	19.4 V	41.0M	43.0z	42.0t-x	42.0G-K
G27	Kamosi	21.3R	18.6OPQ	18.3TU	19.4V	46.0S	43.0z	42.0t-x	43.7MN
G28	Kanade	42.0v	29.6wxy	39.7op	37.1z	38.3u-B	40.0tu	44.0z-E	40.8w-E
G29	Kashule	52.0l	34.9l-p	38.4qrs	41.8nop	39.0A-F	37.0k-n	38.0e-i	38.0h-l
G30	Kasukari	27.7IJ	21.7HIJ	22.5NO	23.9P	40.3L	42.3xyz	40.0m-q	40.9x-F
G31	Katuku	28.0I	23.0EFG	23.2LMN	24.8NO	38.7v-E	42.0w-z	42.0t-x	40.9x-F
G32	Katuku2	40.0x	25.6AB	30.0F	31.9G	39.0A-F	43.0z	45.0D	42.3H-L
G33	Kibugu	51.3lm	34.0pqr	34.9z	40.1vwx	37.3l-r	35.0egh	39.0i-m	37.1d-i
G34	Kigoma	50.3no	36.0jkl	36.8vwx	41.0q-t	35.0b	35.0e-h	36.0a	35.3a
G35	Kikobe	27.0J	23.1EFG	25.2K	25.1N	39.0A-F	38.0n-r	42.0t-x	39.7p-v

GN	Genotype	Weight of 100 seeds (g)				Days to 75% flowering			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G36	Kilindi	33.0F	27.0z	29.0G	29.8I	36.0c	34.3c-g	39.0i-m	36.4b-e
G37	Kinyobya	49.0p	37.3ghi	39.2pq	41.8nop	36.7c-l	40.0tu	40.0m-q	38.9l-q
G38	Kipapi	53.7jk	40.7d	41.0jlm	45.1k	38.0p-v	35.0e-h	38.0e-i	37.0c-h
G39	Kisapuri	44.7t	31.4uv	33.5A	36.5AB	36.0c	37.0k-n	40.0m-q	37.7f-k
G40	Kitebe	35.0D	25.7AB	31.1DE	30.6H	37.0e-m	36.0h-l	41.0q-t	38.0h-l
G41	Kituntunu	35.7BCD	27.0z	30.0F	30.9H	38.0p-v	42.0w-z	36.0ab	38.7k-p
G42	Kyababikira	54.0j	36.2ijk	40.7mn	43.6m	38.3u-C	40.0tu	42.0t-x	40.1s-z
G43	Kyakaragwe	27.7IJ	19.9LMN	21.6P	23.1Q	40.0L	40.0tu	40.7qrs	40.2t-A
G44	Lyamungo 85	62.0d	43.6bc	41.8ij	49.1cd	38.0p-v	42.7yz	39.0i-m	39.9q-x
G45	Lyamungo 90	66.0a	44.9a	45.9cd	52.3ab	38.0p-v	37.0k-n	40.0m-q	38.3j-n
G46	Maharage Kamba	36.3AB	30.4vw	31.6DE	32.8F	39.3E-I	43.0z	43.3y-C	41.9F-J
G47	Maharage Mbeya	44.7t	34.4opq	33.0AB	37.4yz	38.3u-D	43.0z	37.0a-f	39.4o-u
G48	Malirahinda	24.0MN	17.9Q	22.8MN	21.6R	39.3E-J	43.0z	41.0q-t	41.1z-G
G49	Masusu	56.0h	40.7d	46.0cd	47.6fg	36.0c	41.3wx	42.0t-x	39.8q-w
G50	Meupe Uyole	57.7g	38.3fg	43.4fg	46.4h	37.3l-s	37.0k-n	39.0i-m	37.8g-k
G51	Mshindi	37.0A	24.4CD	28.1H	29.9I	36.0c	36.3klm	39.0i-m	37.1d-i
G52	Msolini	64.0c	44.5ab	47.0b	51.8b	38.0p-v	43.0z	37.0a-g	39.3n-t
G53	Mwami Kola	29.3H	20.3KLM	23.8L	24.5O	40.0IL	43.0z	38.0e-i	40.3t-B
G54	Ngoma za bahaya	24.0MN	18.1PQ	17.9U	20.0U	40.0IJL	43.0z	40.0m-q	41.0y-G
G55	Ngwakungwaku	56.0h	39.5e	43.2g	46.2hi	36.3c-g	38.0n-r	39.0i-m	37.8g-k
G56	Njano fupi	49.0p	38.7ef	36.0wxy	41.2qrs	36.3c-h	35.0e-i	40.0m-q	37.1d-i
G57	Njano Uyole	42.0v	34.4opq	31.7DE	36.0BC	37.0e-m	37.0k-n	39.0i-m	37.7f-k
G58	Nyeupe Kubwa	35.3CD	22.5FGH	27.2I	28.3K	39.0A-F	40.0tu	42.3xy	40.4u-C
G59	Nyeupe ndogo	40.0x	34.5n-q	38.7qr	37.7y	39.0A-F	45.0A	38.0e-i	40.7v-D
G60	Pasi	50.0o	36.2ijk	37.0uv	41.1qrs	39.0A-G	40.0tuv	39.0i-m	39.3n-t
G61	Pesa	47.0r	34.0pqr	38.5qrs	39.9wx	36.0c	33.0a	39.0i-m	36.0abc
G62	Raja	52.0l	33.0rst	39.0pq	41.3pqr	39.3E-K	37.0k-n	41.0q-t	39.1m-s
G63	Rojo	43.0u	26.5zA	31.6DE	33.7E	37.0e-m	33.0ab	42.0t-x	37.3e-j
G64	Rosenda	55.0i	43.1c	46.6bc	48.3e	38.7v-E	40.0 tuv	41.0q-t	39.9q-x
G65	Rozikoko fupi	45.0t	29.2xy	31.1DE	35.1D	38.0p-v	38.0n-r	42.0t-x	39.3n-t
G66	Ruondera	53.7jk	35.3k-o	41.8ijk	43.6m	40.0I-L	43.0z	42.3xy	41.8E-J
G67	RWR 2154	52.0l	32.1tu	38.0rst	40.7stu	38.0p-v	43.0z	39.0i-m	40.0r-y
G68	Selian 05	25.0L	18.0Q	19.8RS	20.9T	40.0I-L	43.0z	36.0abc	39.7p-v
G69	Selian 06	39.0y	32.3stu	36.0wy	35.8C	39.0A-H	42.0w-z	42.3xy	41.1z-G
G70	Selian 10	23.0OP	16.0RS	19.0ST	19.3V	46.0S	43.0z	43.0xyz	44.0N
G71	Selian 11	27.0J	16.6RS	18.0U	20.5T	43.0O	41.0uw	44.0z-E	42.7J-M
G72	Selian 12	48.0q	36.5ijk	36.0wxy	40.2vwxy	35.0b	34.7d-g	39.0i-m	36.2a-d

GN	Genotype	Weight of 100 seeds (g)				Days to 75% flowering			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G73	Selian 13	54.0j	35.3k-o	35.4yz	41.6opq	38.0p-v	34.0a-e	38.0e-i	36.7b-f
G74	Selian 14	59.0ef	41.2d	43.0gh	47.7f	43.0O	43.0z	43.0x-A	43.0K-N
G75	Selian 15	63.7c	44.2ab	50.1a	52.7a	42.0N	41.3wx	48.0F	43.8N
G76	Selian 9	26.0K	15.6S	17.9U	19.8UV	45.0R	36.3klm	44.0z-E	41.8E-J
G77	Selian 94	41.7vw	35.8j-m	31.0E	36.2BC	39.7F-L	42.0w-z	41.0q-t	40.9x-F
G78	Selian 97	53.0k	33.4qrs	37.3tuv	41.2qrs	38.0p-v	37.0k-o	38.0e-i	37.7f-k
G79	Selundo	52.0l	33.1rst	41.0j-m	42.0no	39.0A-H	43.0z	40.0m-q	40.7v-D
G80	Sinon	56.0h	38.7ef	35.3yz	43.3m	37.0e-n	38.0n-r	41.0q-u	38.7k-p
G81	SMC 17	34.0E	23.6DEF	23.0MN	26.7M	40.3L	42.0w-z	42.0 t-x	41.4C-I
G82	SMC 18	36.0BC	20.9JKL	22.9MN	26.6M	40.3L	42.3xyz	42.0t-x	41.6D-I
G83	Soya	48.3pq	35.0l-p	36.0wxy	39.8x	35.3b	37.0k-p	38.0e-i	36.8b-g
G84	Soya Mbeya	51.7lm	32.3stu	38.7qr	40.9r-u	38.0p-w	43.0z	42.3xy	41.1z-G
G85	SUA 90	36.0BC	22.2GHI	23.9L	27.4L	36.0cd	33.7a-d	38.0e-i	35.9ab
G86	Tema	24.7LM	18.8N-Q	20.9Q	21.5RS	38.0p-x	43.0z	43.0x-B	41.3B-H
G87	Tikiumba Nyama	54.0j	33.3q-t	43.0gh	43.4m	38.0p-y	35.0e-j	39.0i-m	37.3e-j
G88	Urafiki	38.0z	30.2wx	28.1H	32.1G	36.3c-i	38.0n-s	39.0i-m	37.8g-k
G89	Uyole 03	62.0d	35.9j-m	38.9pq	45.6j	38.0p-z	39.0rt	41.0q-v	39.3n-t
G90	Uyole 04	45.0t	29.2xy	33.1AB	35.8C	38.7v-E	42.3xyz	40.0m-q	40.3t-B
G91	Uyole 16	55.0i	37.7fgh	40.8mn	44.5l	39.0A-H	34.0a-f	38.0e-i	37.0c-h
G92	Uyole 18	48.0q	36.4ijk	41.3j-m	41.9no	37.0e-o	40.0tuv	37.0a-h	38.0h-l
G93	Uyole 84	39.0y	26.0zAB	28.2H	31.1H	41.0M	43.0z	42.0t-x	42.0G-K
G94	Uyole 94	58.3fg	37.3ghi	46.7bc	47.4fg	37.3l-t	41.7wxy	39.0i-m	39.3n-t
G95	Uyole 96	58.0g	41.2d	42.3hi	47.1g	39.0A-H	38.0n-s	38.0e-j	38.3j-n
G96	Uyole 98	46.0s	30.0wx	32.0CD	36.0C	37.0e-o	38.0n-s	40.0m-r	38.3 j-n
G97	Wanja	59.3e	38.6ef	45.3de	47.7f	36.3c-j	35.0e-j	41.0q-w	37.4e-j
G98	Wifi Nyegela	27.0J	18.7OPQ	23.5LM	23.1Q	40.0I-L	37.0k-q	36.0a-d	37.7f-k
G99	Zawadi	40.0x	28.6y	30.1F	32.9F	36.3c-k	33.3abc	38.0e-k	35.9ab
Mean		42.9	30.1	32.7	35.221	38.7	39.7	40.239	39.539
LSD (0.05)		0.667	1.015	0.76	0.826	0.567	0.901	0.912	0.808
CV %		1.0	2.1	1.4	1.5	0.9	1.4	1.4	1.3
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Different letters among genotype values = significant differences by Duncan's new multiple range test (DNMRT) ($p \leq 0.05$); GN = Genotype number; CV % = Coefficient of variation; LSD = Least significance difference; P-value. = F probability

(ii) The AMMI analysis and yield stability index

Across locations, there was highly significant ($P < 0.001$) effects of genotypes, environments, and genotype by environment interaction on the days to 75 % flowering, number of pods per plant, number of seeds per pod, the weight of 100 seeds and seed yield (kg/ha). Mean seed yield across sites ranged from 1085.2 to 3068.7 kg/ha with a grand mean of 1736.9 kg/ha. AMMI analysis showed that the main effects of genotypes and environment accounted for 39.3 % and 31.4 % of seed yield treatment sum of the squares respectively, whereas genotype x environment interaction effect represented 26.8 % of seed yield treatment sum of the squares. The two interaction principal component axes (IPCA 1 and IPCA 2) were both highly significant ($P \leq 0.001$) for seed yield and accounted for 83.2 and 16.8 % respectively of the genotype by environment interaction for seed yield sum of the squares (Table 11).

Table 11: AMMI analysis of variance for seed yield of common bean genotypes across sites

Source of Variation	DF	SS	MS	F	P-value	%TSS	%GEISS
Total	890	506262438	568834				
Treatments	296	493659622	1667769	82.8	<0.001	97.5	
Genotypes	98	199047377	2031096	100.84	<0.001	39.3	
Environments	2	158873571	79436785	627.26	<0.001	31.4	
Block	6	759843	126640	6.29	<0.001	0.2	
Interactions	196	135738674	692544	34.38	<0.001	26.8	
IPCA	99	112960007	1141010	56.65	<0.001		83.2
IPCA	97	22778667	234832	11.66	<0.001		16.8
Error	588	11842974	20141				

DF = degree of freedom, SS = sum of square, MS = mean sum square, F = F value, P-value. = F probability, %TSS = percentage of total sum square and %GEISS = percentage of genotype by environment interaction sum square

The main effects of genotypes, environment, and genotype x environment interaction accounted for 55.5 %, 5.5 %, and 36.7 % of the days to 75 % flowering treatment sum of the squares respectively. The two interaction principal component axes (IPCA 1 and IPCA 2) were both highly significant ($P \leq 0.001$) for days to 75 flowerings and accounted for 67.8 and 32.1 % respectively of the genotype by environment interaction for days to 75 % flowering sum of the squares. Genotype main effect accounted for 49.2 %, while environmental main effect and genotype by environment interaction accounted for 26.0 % and 21.9 % of the number of pods/plant total sum of the squares respectively. Of the interaction, IPCA1 accounted for 74.6 % of the interaction sum of squares while IPCA2 accounted for 25.4 % of the interaction sum of the squares (Table 12).

Table 12: AMMI analyses of variance for days to 75% flowering and number of pods/plant of common bean genotypes across sites

Source of Variation	DF	Days to 75% flowering						Number of pods per plant					
		SS	MS	F	P-value	%TSS	%GEISS	SS	MS	F	P-value	%TSS	%GEISS
Total	890	6851	7.7					44930	50.5				
Treatments	296	6696	22.6	89.5	<0.001	97.7		43574	147.2	68.9	<0.001	97.0	
Genotypes	98	3804	38.8	153.6	<0.001	55.5		22094	225.5	105.5	<0.001	49.2	
Environments	2	376	188.1	167.2	<0.001	5.5		11660	5829.8	350.4	<0.001	26.0	
Block	6	7	1.1	4.5	<0.001	0.1		100	16.6	7.8	<0.001	0.2	
Interactions	196	2516	12.8	50.8	<0.001	36.7		9820	50.1	23.4	<0.001	21.9	
IPCA	99	1707	17.3	68.3	<0.001		67.8	7324	74	34.6	<0.001		74.6
IPCA	97	808	8.3	33.0	<0.001		32.1	2495	25.7	12.0	<0.001		25.4
Error	588	149	0.3					1257	2.1				

DF = degree of freedom, SS = sum of square, MS = mean sum square, F = F value, P-value. = F probability, %TSS = percentage of total sum square and %GEISS = percentage of genotype by environment interaction sum square

Table 13: AMMI analyses of variance for number of seed/pod and 100 seed weight of common bean genotypes across sites

Source of Variation	DF	Number of seeds per pod						100 seed weight (g)					
		SS	MS	F	P-value	%TSS	%GEISS	SS	MS	F	P-value	%TSS	%GEISS
Total	890	933.4	1.1					119863	135				
Treatments	296	881.1	3.0	34.5	<0.001	94.4		119705	404	1532.7	<0.001	99.9	
Genotypes	98	684.5	7.0	80.9	<0.001	73.3		85390	871	3302.4	<0.001	71.2	
Environments	2	22.3	11.2	45.7	<0.001	2.4		27393	13696	34089.6	<0.001	22.9	
Block	6	1.5	0.2	2.8	0.01	0.2		2	0	1.5	0.168	0.0	
Interactions	196	174.3	0.9	10.3	<0.001	18.7		6922	35	133.9	<0.001	5.8	
IPCA	99	100.2	1.0	11.7	<0.001		57.5	5819	59	222.8	<0.001		84.1
IPCA	97	74.1	0.8	8.8	<0.001		42.5	1103	11	43.1	<0.001		15.9
Error	588	50.8	0.1					155	0				

DF=degree of freedom, SS = sum of square, MS = mean sum square, F = F value, P-value = F probability, %TSS = percentage of total sum square and %GEISS = percentage of genotype by environment interaction sum square

The contribution of genotype main effect on the number of seeds/pod and 100 seed weight total sum of the squares was larger 73.3 % and 71.2 % respectively, compared to environmental main effect which contributed 2.4 % of the number of seeds per pod total sum of the squares and 22.9 % of 100 weight total sum of the square. Genotype by environment effect accounted for 18.7 % of the number of seeds per pod total sum of the squares and 5.8 % of 100 seed weight total sum of the squares. The IPCA1 and IPCA2 for both 100 seed weight and the number of seeds/pod were highly significant difference ($P \leq 0.001$) (Table 13).

The AMMI-1 biplot (Fig. 22) elaborates genotypic and environmental additive main effects against their corresponding first interaction principal component axis (PC1). Common bean genotypes placed on the right-hand side of the midline have higher seed yield compared to those on the left-hand side of Fig. 21. Genotype G74 (Selian 14) and G35 (Kikobe) had low PC1 scores close to zero and high seed yield. This indicates that the genotypes were less involved in genotype by environment interaction, therefore these were the most stable and high yielding genotypes. On the other hand, genotype G93 (Uyole 84), G69 (Selian 06), and G68 (Selian 05) exhibited the highest positive genotype by environment interaction while G99 (Zawadi) and G62 (Raja) expressed the highest negative genotype by environment interaction. Among the three environments, Uyole-Mbeya (E3) had a low contribution to genotype by environment interaction, as it is placed closer to the origin of the biplot, whereas Selian-Arusha (E1) and SUA-Morogoro (E2) showed larger environmental main effects with high contributions to genotype by environment interaction as their placed far from the interaction principal axis (PC1).

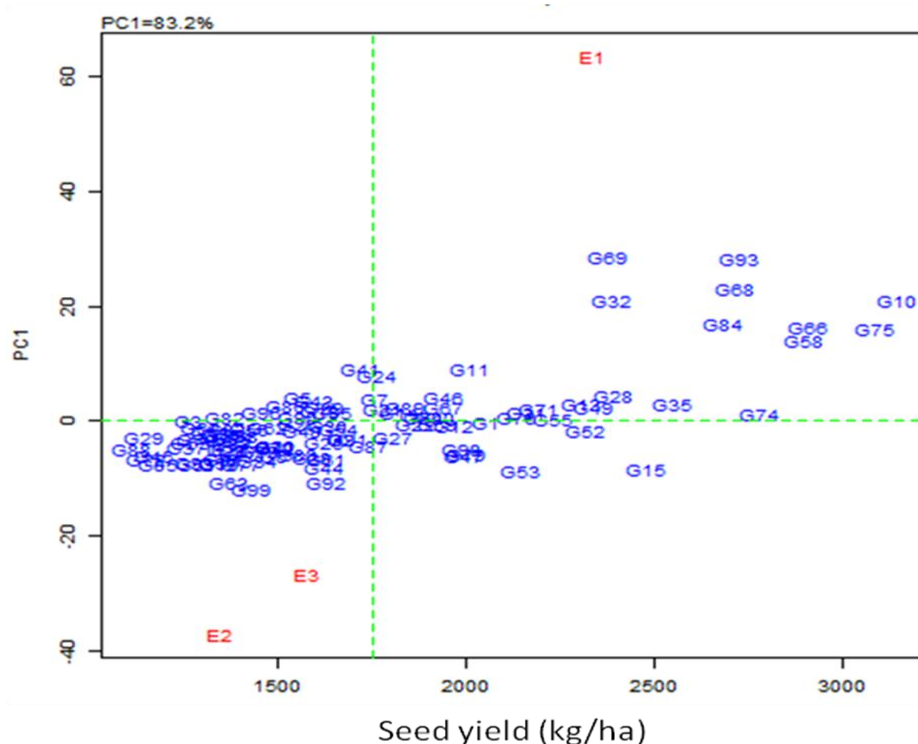


Figure 22: AMMI-1 model biplot for seed yield (kg/ha) presenting the means of ninety nine genotypes (G) and three environments (E) against their corresponding first interaction principal component axis scores (PC1)

Based on additive main effects and multiplicative interaction (AMMI) stability value (ASV) on seed yield of the harvested 99 common bean genotypes across locations, the genotypes were ranked based on least scores, whereby, low score indicates the most stable genotype. ASV ranked ACC 714 as the most stable genotype due to the lowest ASV followed by Bangaya akatebe, Kaempu, Pasi, and SMC 18. Selian 06 was ranked the most unstable genotype due to the highest ASV. The sum of seed yield and AMMI stability rankings also known as Yield Stability Index (YSI) ranked ACC 714 as the highest seed yielding and stable common bean genotypes across sites, followed by Selian 14, Selian 9, Katuku, and Msolini. SUA 90 was ranked the most unstable common bean genotypes based on YSI (Table 8).

The AMMI-1 biplot (Fig. 23), displaying the main effects of common bean genotypes and experimental sites on common bean number of pods. Genotypes placed at or near the mid horizontal line with PC1 score zero are stable, thus they are adapted to a wide range of environments, while those with high PC1 score (positive or negative) values are unstable and adapted to specific sites. The mid vertical line represents the overall mean of number of pods per plant across sites and common bean genotypes found on the right hand side of this line have number of pods greater than the mean. The biplot showed that, most of the planted and

harvested common bean genotypes across sites have pod number per plant below the overall mean. The common bean genotypes identified to be stable and have high pod number per plant included G 35 (kikobe), G21 (Kachele), G27 (Kamosi), G13 (DOR 500) and G58 (Nyeupe kubwa). Experimental site E1 (TARI-Selian) and genotypes G10 (Cheupe), G32 (Katuku2), G93 (Uyole 84) with high positive PC1 score values and experimental site E2 (SUA) with high negative PC1 score values were largely involved in the genotype by environment interaction observed in genotypes for the number of pods per plant.

Figure 23: AMMI1 biplot for the PC1 scores and common bean genotype in three experimental sites (E1 = TARI-Selian, E2 = SUA, and E3 = TARI-Uyole) for number of pods per plant

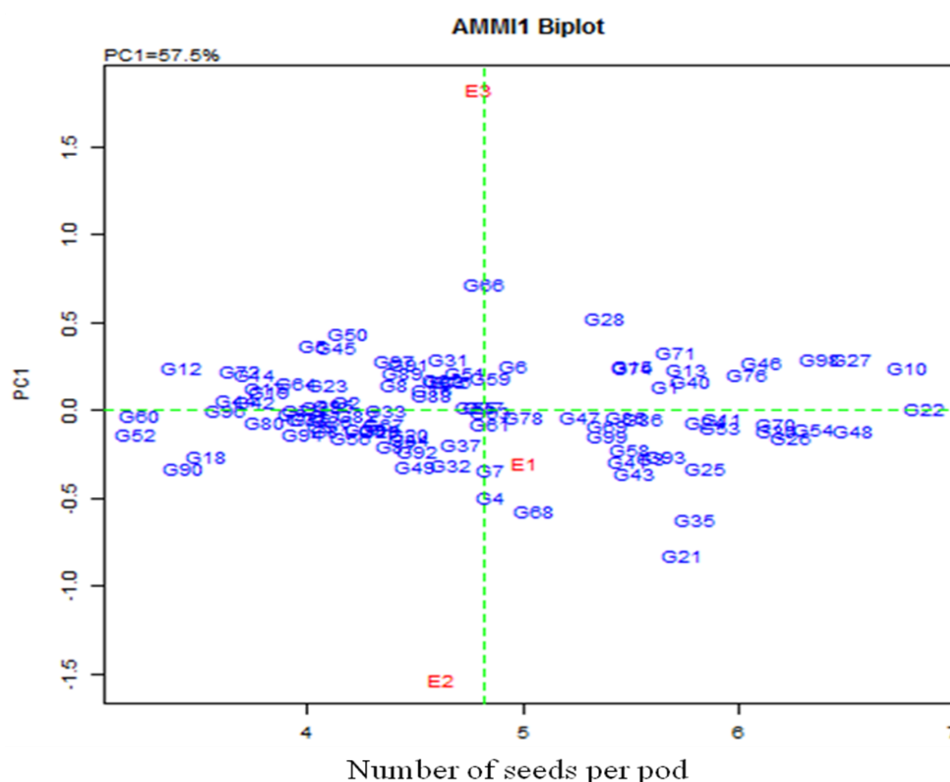


Figure 24: AMMI1 biplot for the PC1 scores and common bean genotype in three experimental sites (E1 = TARI-Selian, E2 = SUA, and E3 = TARI-Uyole) for number of seeds per pod

The AMMI-1 biplot (Fig. 25) presents the main effects of genotypes and environment and the PC1 scores of the common bean genotypes for weight of 100 seeds. Most of the genotypes across sites had weight of 100 seeds greater than the overall mean. The genotypes identified to be stable and have high weight of 100 seeds included G49 (Masusu), G64 (Rosenda), G38 (Kipapi) and G12 (CODMLB 033) as they are found at or closer to PC1 line and located far in the right hand of mid vertical line. Genotypes G89 (Uyole 03), G9 (Calima Uyole), and G8 (CAL 96) have high positive PC1 score while G1 (ACC 714) and G26 (Kamoshi) have high negative PC1 score, thus are the major cause of GxEx.

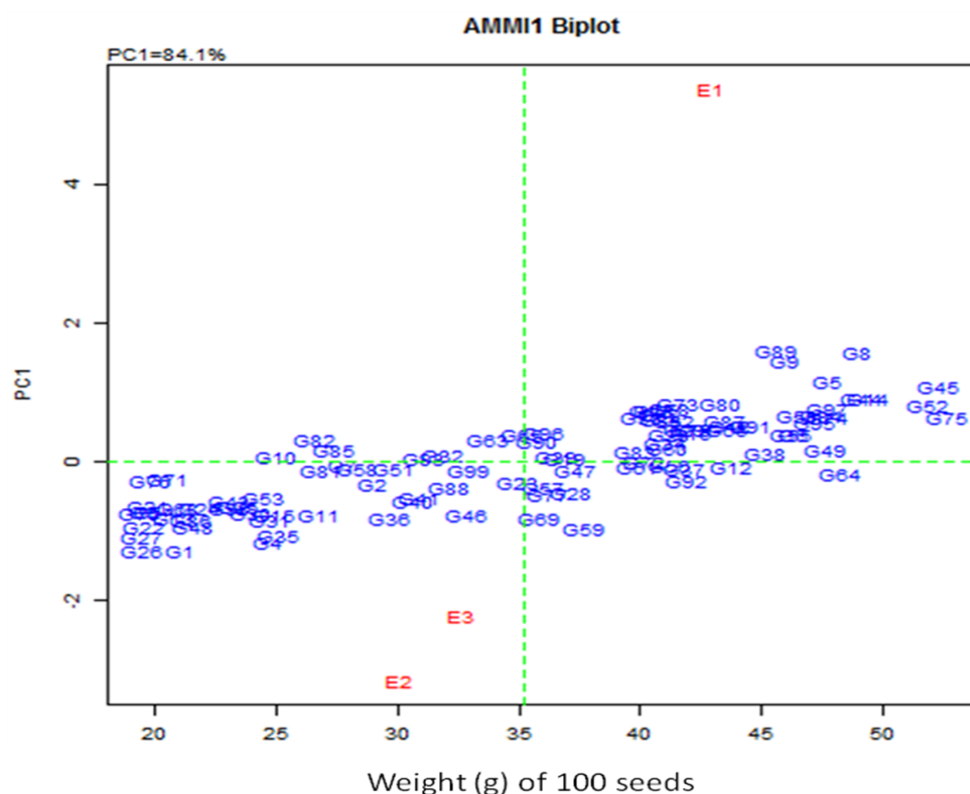


Figure 25: AMMI1 biplot for the PC1 scores and common bean genotype in three experimental sites (E1 = TARI-Selian, E2 = SUA, and E3 = TARI-Uyole) for 100 seed weight

(iii) Experimental sites discriminating power and representativeness on genotypes seed yield and yield components

The GGE biplot (Fig. 26) shows the discriminating power and representativeness of the experimental sites on the seed yield of the common bean genotypes. An experimental site with a longer vector from the origin of the biplot had a larger discriminating ability for superior seed yield genotypes, while those with a shorter vector had low discriminating power. The experimental site vector with a small angle from the average environmental axis (AEA), is described as more representativeness of the common bean seed yield evaluation experiment. Site E1 (TARI-Selian) with a longer vector from the biplot origin had good discriminating ability compared to the other experimental sites, while E3 (TARI-Uyole) with a shorter vector had poor discriminating ability compared to other experimental sites. Site E3 (TARI-Uyole) vector had a small angle with the AEA, thus more representative compared to the other sites, whereas E2 (SUA) had a larger angle with the AEA and therefore the least representative site among the experimental sites.

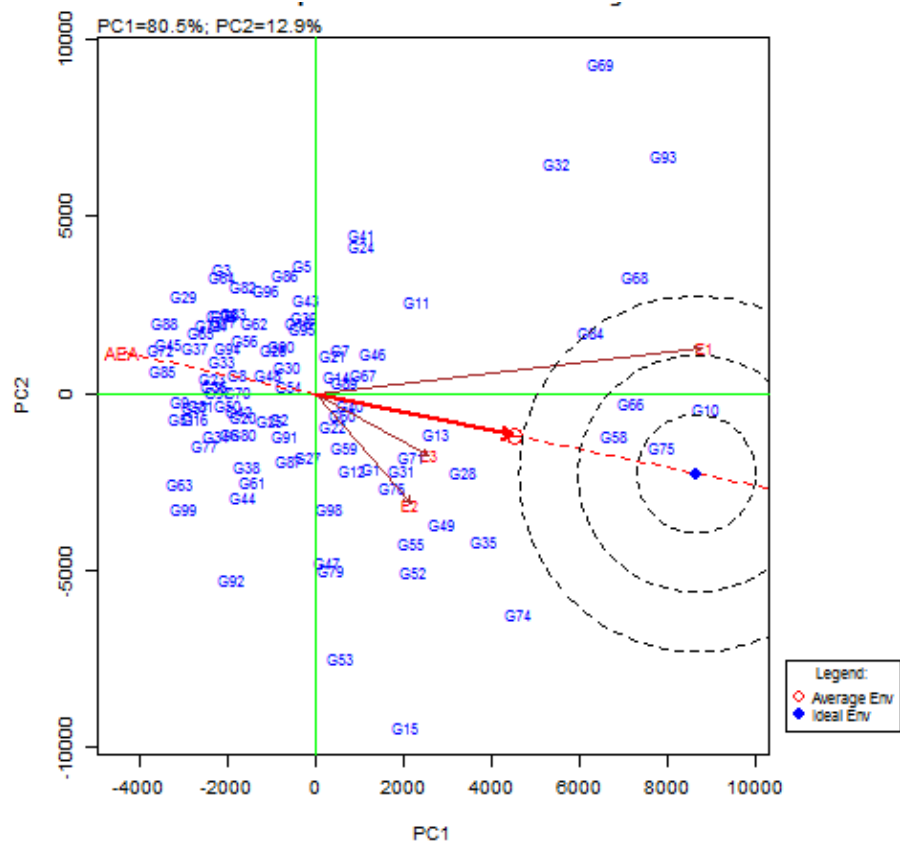


Figure 26: GGE biplot showing experimental sites discriminating power and representativeness on common bean genotypes seed yield

The GGE biplot-Environment view for number of pods per plant of the common bean genotypes showing the relationship among the experimental sites, discriminating (informative) ability and representativeness of the experimental sites in relation to an ideal environment (Fig. 27). The cosine angle between the two environment vectors defines the relationship between the two environments. Small cosine angle indicates a positive correlation while large cosine angle tells weak correlation and right angle means no correlation. Experimental site E2 (SUA) and E3 (TARI-Uyole) have small cosine angle between them, thus they are positively correlated in discriminating the bean genotypes on number of pods per plant. On the other hand, they differ much with E1 (TARI-Selian) in discriminating the common bean genotypes on pod number per plant. The length of the environment vector from the origin of the biplot shows the discriminating ability of the experimental site on genotypes trait of interest. For the number of pods among bean genotypes site E1 was observed as the most discriminating site and E2 the least discriminating site. Representativeness of the experimental site is shown by angle between the experimental site vector and the AEA, experimental site with smaller angle with AEA is more representative of other experimental site, thus E3 is the most representative site though

poor discriminating ability. The ideal experimental site should be most discriminating and also most representative of the other experimental sites. To help visualize the ideal environment, the concentric circles are normally drawn in the positive direction of AEA locating the ideal environment at the centre. Site E1 being in the concentric circles and closer to ideal environment is the best experimental site for selecting common bean genotypes adapted to a wide range of environments.

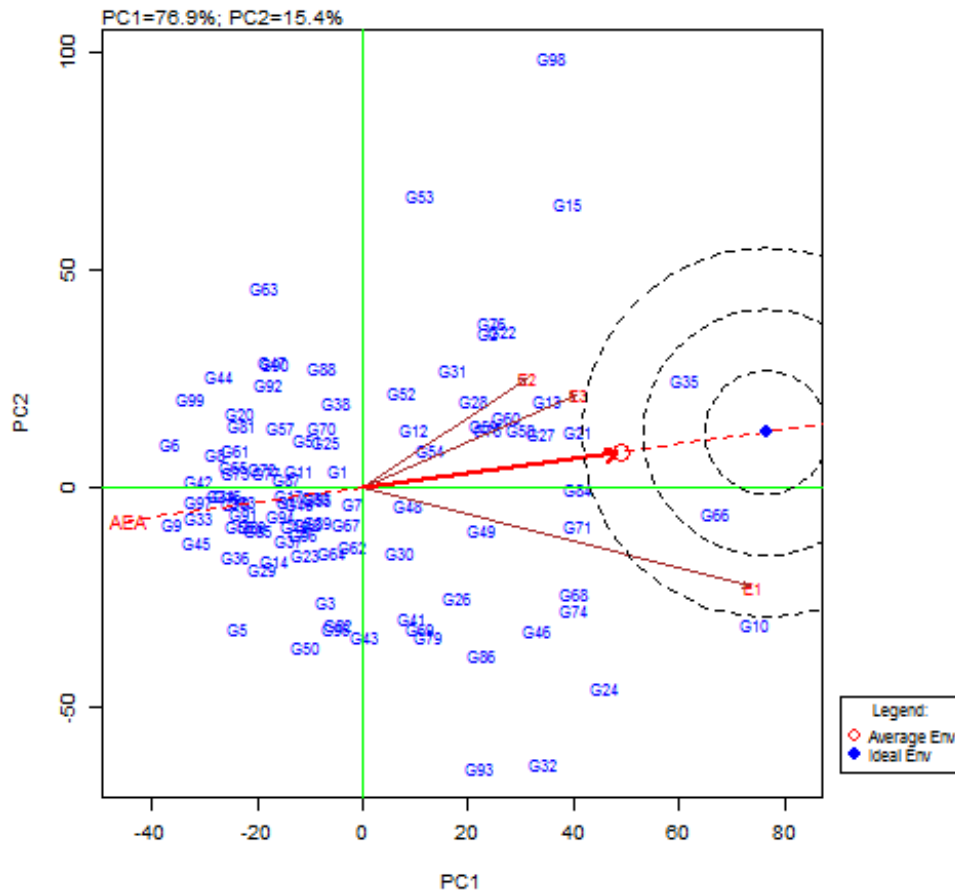


Figure 27: GGE biplot-Environment view for number of pods per plant of common bean genotypes showing discriminating ability and representativeness of the experimental sites in relation to an ideal environment

The GGE biplot (Fig. 28), shows that, site E1 (TARI-Selian) is the most discriminating site and also the most representative of the other experimental site for common bean genotypes number of seeds per pod. As it has the smallest angle with the AEA line and found in the inner most concentric circle closer to the ideal environment. Site E2 (SUA) and E3 (TARI-Uyole) are almost equal and least discriminating and representative sites as compared to E1 (TARI-Selian) due to having large angle with the AEA line and found in the third concentric circle of the ideal environment.

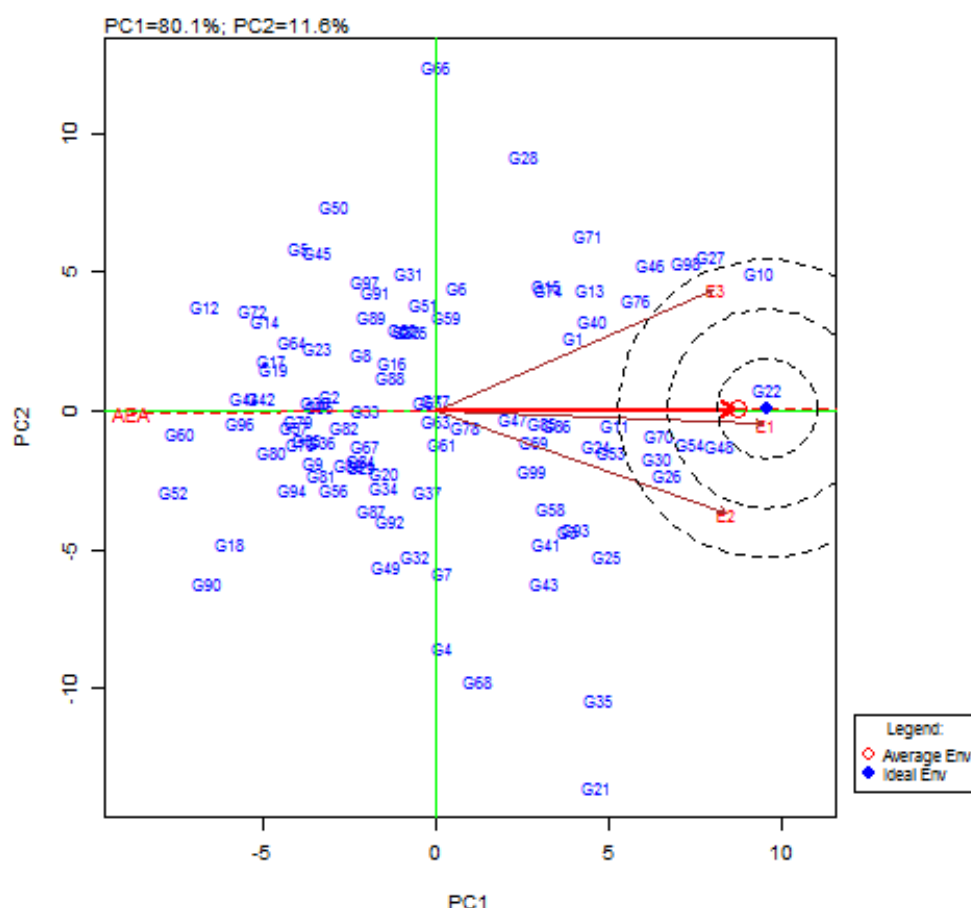


Figure 28: GGE biplot-Environment view for number of seeds per pod of common bean genotypes showing discriminating ability and representativeness of the experimental sites in relation to an ideal environment

Genotype and Genotype by Environment Interaction biplot (Fig. 29) shows that, site E1 (TARI-Selian) is the most discriminating site and also most representative of the other experimental site for common bean genotypes 100 seeds weight, as it is found in the inner most concentric circle and closer to the ideal environment. Site E2 (SUA) and E3 (TARI-Uyole) are almost equal and least discriminating and representative sites as compared to E1 due to having relatively shorter vectors and are found in the third concentric circle of the ideal environment.

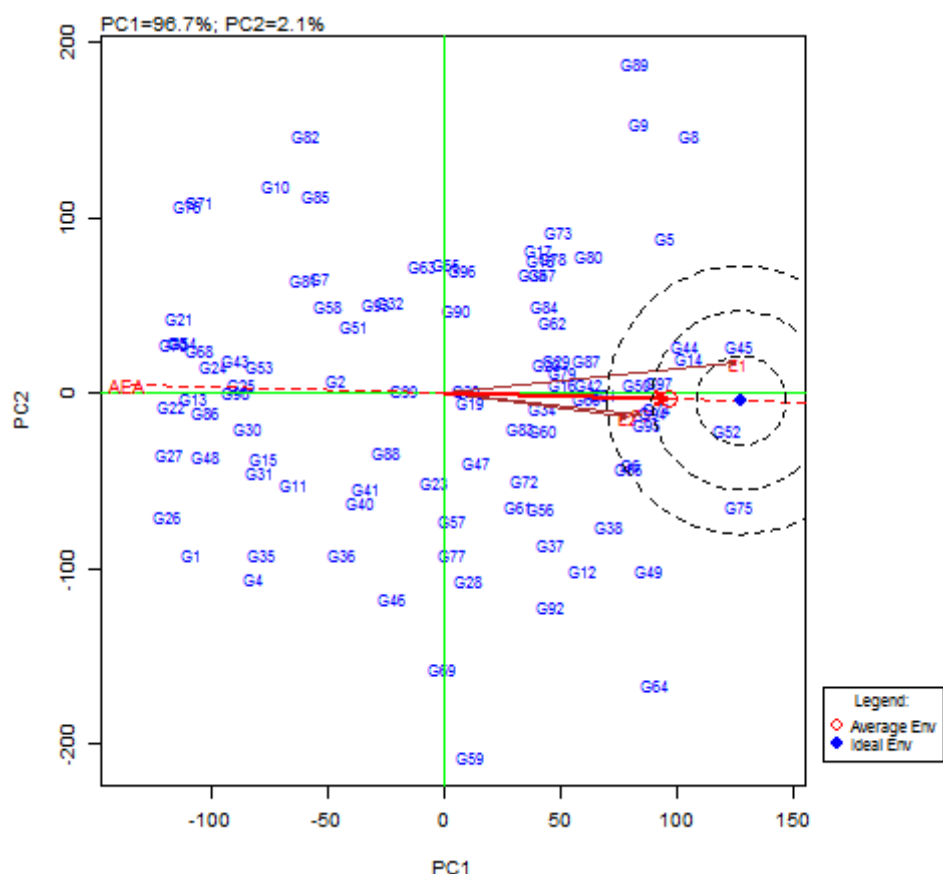


Figure 29: GGE biplot-Environment view for 100 seeds weight of common bean genotypes showing discriminating ability and representativeness of the experimental sites in relation to an ideal environment

(iv) Association between common bean seed yield and yield components with soil chemical properties of the test locations

Pearson correlation analysis revealed that there was a strong positive significant ($r \geq 0.5$, $P \leq 0.001$) relationship between common bean seed yield (kg/ha) with soil available phosphorus, soil pH, soil exchangeable potassium, sodium, and calcium. A strong negative significant ($r \geq -0.5$, $P \leq 0.001$) correlation between seed yield and total soil nitrogen and organic carbon was observed. A weak positive significant ($r = 0.13$, $P \leq 0.001$) correlation between seed yield and soil exchangeable magnesium was observed. A strong positive significant ($r \geq 0.5$, $P \leq 0.001$) relationship was obtained between the number of pods/plant and available soil phosphorus, soil pH, exchangeable soil potassium, sodium, and calcium. A moderate negative significant ($r = -0.3$ to -0.49 , $P \leq 0.001$) relationship between the number of pods/plant with total soil nitrogen, and soil organic carbon was obtained, whereas a weak significant ($r = 0.15$, $P \leq 0.001$) association was observed between the number of pods/plant and soil exchangeable magnesium. A moderate positive significant ($r = 0.3$ to 0.49 , $P \leq 0.001$)

association was observed between 100 seed weight (g) and available soil phosphorus, soil pH, exchangeable soil potassium, sodium, and calcium, whereas exchangeable magnesium had a weak positive significance ($r = 0.18$, $P \leq 0.001$) influence on 100 seed weight. A negative weak significant ($r \leq -0.29$, $P \leq 0.001$) association was observed between 100 seed weight with total soil nitrogen and soil organic carbon (Table 14).

Table 14: Association of common bean seed yield and yield components with soil properties

Soil Property	Seed yield (kg/ha)	Days to 75% flowering	Number of pods/plant	Number of seeds/plant	100 seed weight (g)
Soil N	-0.54***	0.03ns	-0.47***	-0.12***	-0.29***
Soil P	0.71***	-0.15***	0.64***	0.15***	0.45***
Soil OC	-0.52***	0.02ns	-0.45***	-0.12***	-0.28***
Soil K	0.68***	-0.21***	0.63***	0.15***	0.48***
Soil Mg	0.13***	-0.19***	0.15***	0.02ns	0.18***
Soil Na	0.69***	-0.20***	0.64***	0.15***	0.48***
Soil Ca	0.65***	-0.22***	0.61***	0.14***	0.47***
Soil pH	0.62***	-0.23***	0.58***	0.13***	0.46***

*** = significant at $P \leq 0.001$, and ns = not significant ($P > 0.05$)

Pearson correlation analysis for common bean seed yield and yield components (Table 15) showed that there was a strong positive significant ($r = 0.79$, $P \leq 0.001$) relationship between seed yield and number of pods/plant. The number of seeds/pod exhibited a weak positive significant ($r = 0.27$, $P \leq 0.001$) relationship with seed yield, whereas a moderate positive significant ($r = 0.33$, $P \leq 0.001$) association was observed between 100 seed weight and seed yield. No significant ($P > 0.05$) relationship was observed between days to 75% flowering and seed yield and 100 seed weight with the number of pods/plant. Moderate negative significant ($r = -0.3$ to -0.49 , $P \leq 0.001$) associations were observed between 100 seed weight with days to 75% flowering and the number of seeds/pod.

Table 15: Association of common bean seed yield and yield components

Yield component	Days to 75% flowering	Number of pods/plant	Number of seeds/pod	100 seed weight	Seed yield (kg/ha)
Days to 75% flowering	1.00	0.12***	0.27***	-0.34***	-0.003ns
Number of pods/plant	0.12***	1.00	0.43***	0.06ns	0.79***
Number of seeds/pod	0.27***	0.43***	1.00	-0.48***	0.27***
100 seed weight	-0.34***	0.06ns	-0.48***	1.00	0.33***
Seed yield (kg/ha)	-0.003ns	0.79***	0.27***	0.33***	1.00

*** = significant at $P \leq 0.001$, and ns = not significant ($P > 0.05$)

4.1.3 Levels of iron and zinc among common bean genotypes in three bean growing agro-ecological zones of Tanzania

(i) Analysis of variance for seed iron and zinc

Analysis of variance for seed iron and zinc contents at each location showed highly significant difference ($p \leq 0.001$) among the genotypes (Table 16). The range of seed iron content at TARI-Selian was 19.8 – 150.8 mg/kg, whereas it ranged from 13.7 to 142 mg/kg at SUA. On the other hand, seed iron content ranged from 18.4 to 138.4 mg/kg at TARI-Uyole. The content of zinc in common bean seeds ranged from 16.0 to 32.7 mg/kg at TARI-Selian and from 15.3 to 49.9 mg/kg at SUA, and from 15.3 – 64.7 mg/kg at TARI-Uyole. The highest seed iron-containing common bean genotype at TARI-Selian was ACC 714 (150.8 mg/kg) that was closely followed by Malirahinda (145.8 mg/kg), Jabeyila (138.2 mg/kg), CODMLB 033 (125.8 mg/kg) and Kikobe (118.6 mg/kg). At SUA, the highest seed iron-containing common bean genotypes were Mwamikola (142.5 mg/kg), Wifi Nyegela (124.5 mg/kg), Malirahinda (111.9 mg/kg), Selian 94 (97.0 mg/kg), and Urafiki (88.7 mg/kg). Kikobe had 138.4 mg/kg, which was the highest seed iron content among the common bean genotypes at TARI-Uyole, this was closely followed by ACC 714 (114.9 mg/kg), CODMLB 033 (103.1 mg/kg), Kyakaragwe (101.8 mg/kg) and Mwamikola (92.4 mg/kg). The highest zinc-containing genotypes at TARI-Selian, were SMC 18 (32.1 mg/kg), SMC 17 (31.9 mg/kg), Bamgaya akatebe (30.8 mg/kg), Soya (30.4 mg/kg) and Kainja (30.1 mg/kg), while at SUA Kachele recorded the highest seed zinc content of 49.9 mg/kg, followed by Kamoshi (46.6 mg/kg), Ngoma za bahaya (46.4 mg/kg), Jabeyila (38.7 mg/kg), and KAB o6F2-8-35 (45.1 mg/kg). At TARI-Uyole, the highest common bean seed zinc contents were recorded from SMC 18 (64.7 mg/kg), SMC 17 (56.2 mg/kg), KAB o6F2-8-35 (54.6 mg/kg), Lyamungo 90 (53.2 mg/kg), and Bilfa 4 (51.6 mg/kg).

Table 16: Variation in seed iron and zinc contents among common bean genotypes at each test location and across locations

GN	Genotype	Seed iron concentration (mg/kg)				Seed zinc concentration (mg/kg)			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G1	ACC 714	150.8a	81.2def	114.9b	115.6a	21.6n-x	22.2C-G	40.1j-v	27.9E-N
G2	Bagara Ompigize	35.5E-L	53.0o-x	63.3n-u	50.6o-F	22.3l-x	35.2j-s	36.2q-z	31.2p-C
G3	Bangaya Akatebe	57.7m-t	61.1j-r	65.4m-s	61.4j-p	30.8abc	33.1m-v	39.0k-x	34.3i-q
G4	Bilfa 4	35.3E-M	35.0A-G	84.3e-i	51.5o-D	21.6n-x	36.4h-p	51.6bcd	36.5c-k
G5	Bilfa Uyole	29.4J-O	27.2E-I	61.4o-x	39.3D-M	21.3n-y	30.9s-x	42.3f-q	31.5o-C
G6	Buji	51.2r-B	61.9j-r	53.0v-D	55.3k-y	23.4j-v	41.7c-f	42.3f-q	35.8d-m
G7	Burushu	61.5l-q	36.2y-F	70.6k-o	56.1j-x	29.7a-f	34.6k-t	36.5p-z	33.6k-s
G8	CAL 96	61.8l-p	21.9F-I	64.5m-t	49.4o-F	23.4j-v	30.3t-z	25.7F-K	26.5I-Q
G9	Calima Uyole	37.5D-J	20.4GHI	48.4A-H	35.5G-N	22.3l-x	30.9s-x	39.8j-w	31.0r-E
G10	Cheupe	56.2m-u	56.0m-w	46.0C-J	52.7n-C	22.3l-x	32.8n-v	20.4KL	25.2N-T
G11	Chumba Neroza	77.0hij	78.2d-h	74.0j-m	76.4f-i	20.9o-z	36.5h-p	36.5p-z	31.3p-C
G12	CODMLB 033	125.8c	85.7cde	103.1c	104.8abc	27.3b-m	36.4i-p	43.9e-m	35.8d-m
G13	DOR 500	76.0ij	28.5D-I	89.2def	64.5i-n	21.6n-x	27.1w-B	23.9G-K	24.2O-U
G14	Fibea	61.2l-r	49.7p-A	67.7l-q	59.5j-r	28.7a-i	31.4q-w	40.2j-v	33.44k-u
G15	Jabeyila	138.2b	82.4def	85.2e-i	102.0bc	27.6b-l	46.1ab	38.7l-y	37.5c-h
G16	Jesca	26.0K-Q	30.5B-H	20.1NO	25.6N	22.7k-x	29.8u-z	34.9t-B	29.1y-K
G17	KAB o6F2-8-35	84.4f-i	76.1d-j	73.9j-m	78.1fgh	26.2c-o	45.1bc	54.6b	42.0b
G18	KAB o6F2-8-36	53.6n-w	29.3C-I	46.7B-I	43.2x-J	25.9c-p	35.3j-s	37.9m-y	33.0m-u
G19	Kabanima	37.9D-J	21.4F-I	37.2IJK	32.2J-N	19.8r-z	34.7k-t	43.1e-o	32.6n-v
G20	Kabumburi	40.0C-I	43.8u-D	48.1A-H	44.0v-J	23.0j-w	34.6k-t	33.5w-D	30.4t-G
G21	Kachele	60.1l-r	44.6t-C	63.8n-t	56.2j-w	29.0a-h	49.9a	36.8o-y	38.6cd
G22	Kaempu	71.8jk	86.6cde	77.0 i-l	78.5fgh	27.6b-l	37.6f-m	40.4j-v	35.2f-n
G23	Kainja	54.3n-v	61.4j-r	59.0q-y	58.2j-t	30.1a-e	36.2i-p	34.3u-C	33.54k-t
G24	Kaisho kamugole	59.6l-s	40.4w-E	52.4w-E	50.8o-F	29.4a-g	35.7j-r	40.1j-v	35.1g-n
G25	Kakaritusi	42.0x-G	54.7n-w	56.5r-B	51.1o-E	28.3a-j	27.2w-B	27.8D-I	27.8F-N
G26	Kamoshi	51.7p-A	62.6i-q	66.4m-r	60.2j-q	23.7i-u	46.6ab	39.8j-w	36.7c-j
G27	Kamosi	75.8ij	50.3p-A	79.0g-k	68.4g-j	29.4a-g	45.0bc	36.0q-z	36.8c-i
G28	Kanade	36.0E-K	61.4j-r	45.5C-J	47.6q-G	20.9o-z	31.0r-x	29.1A-G	27.0I-P
G29	Kashule	32.0G-N	72.2e-l	65.9m-s	56.7j-v	24.1h-t	38.7e-k	45.00e-l	35.9d-m
G30	Kasukari	110.0de	74.0d-k	57.5r-A	80.5efg	22.7k-x	26.0z-D	35.2s-A	27.9E-N
G31	Katuku	82.5f-i	60.0k-t	53.0v-D	65.2i-n	26.2c-o	30.7s-y	36.5p-z	31.2q-D
G32	Katuku2	29.4J-P	61.4j-r	41.0G-K	44.0v-J	21.3n-y	34.9k-t	21.0K	25.7L-S
G33	Kibugu	41.8A-H	57.2l-u	53.1v-D	50.7o-F	26.6c-n	37.1g-o	45.3e-k	36.3c-l
G34	Kigoma	25.6L-Q	16.8HI	39.0H-K	27.1MN	20.2q-z	24.3B-E	21.9IJK	22.1U
G35	Kikobe	118.6cd	86.6cde	138.4a	114.5ab	25.9c-p	34.3k-u	28.7C-H	29.6v-I

GN	Genotype	Seed iron concentration (mg/kg)				Seed zinc concentration (mg/kg)			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G36	Kilindi	57.5m-t	28.1D-I	44.6C-J	43.4w-J	18.1v-z	18.8F-J	36.9o-y	24.6O-U
G37	Kinyobya	57.0m-u	44.6s-C	56.8r-A	52.8n-B	19.1s-z	36.2i-p	36.0q-z	30.5r-G
G38	Kipapi	33.7F-N	48.8q-A	58.7q-z	47.1r-H	22.0m-x	23.2B-F	40.1j-v	28.4B-M
G39	Kisapuri	53.2n-w	48.8q-A	78.2h-k	60.1j-q	25.1e-r	30.8s-y	38.4m-y	31.5o-C
G40	Kitebe	52.2p-x	53.0o-x	57.2r-A	54.1m-A	27.3b-m	41.2c-g	48.8cde	39.1c
G41	Kituntunu	26.7K-Q	21.3F-I	40.0H-K	29.3K-N	22.0m-x	35.7j-r	33.2x-E	30.3u-H
G42	Kyababikira	86.1fgh	46.0r-B	72.1k-n	68.0g-k	27.6a-l	18.0G-J	40.9h-t	28.8z-L
G43	Kyakaragwe	104.1e	79.7d-g	101.8c	95.2cd	25.1e-r	33.2m-v	34.3u-C	30.9r-F
G44	Lyamungo 85	54.4n-v	36.9y-F	42.5E-J	44.6v-J	22.3l-x	24.9A-E	22.2IJK	23.1R-U
G45	Lyamungo 90	68.4jkl	51.8o-y	45.1C-J	55.1l-z	19.1s-z	15.3J	53.2bc	29.2w-J
G46	Maharage Kamba	115.5d	74.2d-k	61.9o-w	83.9def	17.4xyz	26.9w-B	27.5E-J	23.9Q-U
G47	Maharage Mbeya	69.4jkl	69.8f-n	61.4o-x	66.9h-m	29.4a-g	40.9c-h	41.4h-s	37.2c-i
G48	Malirahinda	145.8ab	111.9b	92.4de	116.7a	25.1e-r	35.4j-s	36.5p-z	32.4n-w
G49	Masusu	51.0r-B	19.3HI	56.8r-A	42.4z-J	22.9k-w	24.9A-E	40.4j-v	29.4w-J
G50	Meupe Uyole	33.9F-N	14.9I	48.8z-H	32.5J-N	23.0j-w	32.5o-v	39.3k-x	31.6o-B
G51	Mshindi	87.7fg	44.6s-C	53.1v-D	61.8j-o	21.8n-x	26.2y-D	21.7JK	23.2R-U
G52	Msolini	47.9 t-C	22.0F-I	58.0q-A	42.6y-J	25.9c-p	34.2k-u	40.6i-u	33.6k-t
G53	Mwami Kola	89.4fg	142.5a	96.9cd	109.6ab	22.3l-x	42.5b-e	34.1v-C	33.0m-u
G54	Ngoma za bahaya	48.2t-C	71.6e-m	54.5t-C	58.1j-t	23.4j-v	46.4ab	29.0B-H	32.9m-u
G55	Ngwakungwaku	37.7D-J	38.7x-E	44.6C-J	40.3B-L	22.3l-x	39.6d-j	35.7r-z	32.6n-v
G56	Njano fupi	43.5w-F	44.6s-C	44.1D-J	44.0v-J	25.5d-q	35.3j-s	30.5z-F	30.4s-G
G57	Njano Uyole	49.3t-C	36.5y-F	41.7F-J	42.5y-J	20.2q-z	31.4q-w	34.1v-C	28.6A-L
G58	Nyeupe Kubwa	51.2r-B	57.2l-u	40.5H-K	49.6o-F	25.9c-p	36.0i-q	35.2s-A	32.3n-x
G59	Nyeupe ndogo	34.4F-N	28.7D-I	56.5r-B	39.9C-M	25.9c-p	32.5o-v	39.5k-x	32.6n-v
G60	Pasi	65.6klm	56.0m-w	48.8z-H	56.8j-v	25.9c-p	26.4x-C	44.2e-m	32.2n-y
G61	Pesa	66.1klm	65.1g-p	72.0k-n	67.8g-l	19.9r-z	27.1w-B	38.4m-y	28.5B-M
G62	Raja	60.8l-r	53.0o-x	62.9n-v	58.9j-s	24.8f-r	43.6bcd	39.0k-x	35.8d-m
G63	Rojo	37.8D-J	67.3f-o	53.0v-D	52.7n-C	20.3q-z	32.0p-v	25.7F-K	26.0K-R
G64	Rosenda	62.9k-o	62.7i-q	69.4k-p	65.0i-n	28.0a-k	21.9D-H	43.6e-n	31.2q-D
G65	Rozikoko fupi	56.7m-u	44.6s-C	53.6u-D	51.6o-D	21.6n-x	38.2e-l	25.6F-K	28.4B-M
G66	Ruondera	49.3t-C	57.2l-u	64.1n-t	56.9j-v	27.3b-m	32.7n-v	35.5r-z	31.8o-z
G67	RWR 2154	65.6klm	46.6r-A	61.4o-x	57.9j-u	24.8f-r	31.4q-w	46.9d-h	34.4h-p
G68	Selian 05	77.7hij	35.9y-F	23.6MNO	45.7t-I	22.3l-x	32.0p-v	37.3n-y	30.5r-G
G69	Selian 06	68.6jkl	49.9p-A	43.6D-J	54.0m-A	16.0z	22.1C-G	15.3L	17.8V
G70	Selian 10	48.1t-C	20.1GHI	20.1NO	29.4K-N	23.0j-w	22.7B-F	41.7g-r	29.1x-J
G71	Selian 11	41.3B-I	50.2p-A	27.8LMN	39.8D-M	21.3n-y	20.7E-I	35.2s-A	25.7L-S
G72	Selian 12	63.2k-n	42.5u-E	44.6C-J	50.1o-F	17.9w-z	24.2B-E	39.5k-x	27.2H-O

GN	Genotype	Seed iron concentration (mg/kg)				Seed zinc concentration (mg/kg)			
		Selian	SUA	Uyole	Mean	Selian	SUA	Uyole	Mean
G73	Selian 13	41.8A-H	41.1v-E	37.6IJK	40.2B-L	24.4g-s	29.2v-A	45.1e-k	32.9m-u
G74	Selian 14	47.0u-D	36.5y-F	51.5x-F	45.0u-J	20.6p-z	26.0z-D	22.2IJK	22.9STU
G75	Selian 15	45.1v-E	36.9y-F	82.9f-j	55.0l-z	20.9o-z	29.2 v-A	33.4w-E	27.9E-N
G76	Selian 9	25.0N-Q	43.8u-D	18.4O	29.0K-N	18.1v-z	26.5x-C	39.5k-x	28.0D-N
G77	Selian 94	91.8f	97.0c	88.0efg	92.3cde	23.0j-w	29.8u-z	43.9e-m	32.2n-y
G78	Selian 97	51.5q-A	21.4F-I	57.0r-A	43.3w-J	20.9o-z	35.3j-s	23.0H-K	26.4J-Q
G79	Selundo	118.1cd	51.6o-z	56.1s-B	75.2f-i	24.4g-s	19.8F-I	20.7K	21.6U
G80	Sinon	31.7H-N	35.6z-G	58.0q-A	41.7A-K	22.3l-x	32.5o-v	40.4j-v	31.7o-A
G81	SMC 17	58.1m-t	70.3f-n	70.9k-o	66.4h-m	31.9ab	26.5x-C	56.2b	38.2c-f
G82	SMC 18	89.3fg	77.7d-i	86.6e-h	84.5def	32.7a	43.5bcd	64.7a	47.0a
G83	Soya	52.2p-y	40.4w-E	32.0KLM	41.5A-K	30.4a-d	38.4e-l	47.7c-g	38.9cd
G84	Soya Mbeya	52.0p-z	57.2l-u	64.6m-t	57.9j-t	28.0a-k	38.2e-l	39.7k-w	35.3e-n
G85	SUA 90	40.8C-I	64.0h-q	45.4C-J	50.1o-F	25.9c-p	34.0l-u	48.3c-f	36.0c-m
G86	Tema	40.6C-I	37.9x-E	60.6p-y	46.4s-I	25.5d-q	41.7c-f	47.7c-g	38.3cde
G87	Tikiumba Nyama	32.5G-N	48.8q-A	55.9s-B	45.7t-I	23.4j-v	37.3f-n	38.2m-y	33.0m-u
G88	Urafiki	88.9fg	88.7cd	77.8h-k	85.1def	20.2q-z	17.4 IJ	38.4m-y	25.3M-T
G89	Uyole 03	28.1J-Q	51.5o-z	36.2JKL	38.6E-M	16.3yz	17.7HIJ	38.4m-y	24.2P-U
G90	Uyole 04	19.8OQ	19.4 HI	44.6C-J	27.9LMN	21.6n-x	23.2B-F	40.4i-v	28.4C-M
G91	Uyole 16	31.3I-N	60.5j-s	42.3F-J	44.7v-J	18.4u-z	19.3F-J	45.3e-k	27.7G-N
G92	Uyole 18	49.6s-C	13.7I	50.7y-G	38.0F-N	21.3n-y	33.1m-v	46.6d-i	33.7j-r
G93	Uyole 84	37.7D-J	40.3w-E	23.6MNO	33.9I-N	18.8t-z	16.5IJ	32.4y-E	22.6TU
G94	Uyole 94	25.3M-Q	57.0l-v	63.3n-u	48.5p-F	24.1h-t	24.5B-E	43.6e-n	30.7r-G
G95	Uyole 96	52.9o-w	42.3u-E	42.3F-J	45.8t-I	27.3b-m	40.5d-i	46.1d-j	37.9c-g
G96	Uyole 98	37.7D-J	20.3GHI	44.9C-J	34.3H-N	18.8t-z	22.7B-F	38.2m-y	26.5I-Q
G97	Wanja	32.5G-N	14.1I	59.1q-y	35.2GN	21.6n-x	30.3t-z	39.0k-x	30.3u-H
G98	Wifi Nyegela	81.3ghi	124.5b	77.8h-k	94.5cd	20.2q-z	36.8g-o	42.8e-p	33.3l-u
G99	Zawadi	47.1u-D	63.7h-q	19.3NO	43.4w-J	21.3n-y	41.3c-g	41.2h-t	34.6h-o
Mean		58.6	51.6	58.2	56.2	23.7	32	37.6	31.1
LSD (0.05)		8.3	12.7	8.1	9.8	4.2	3.8	5.1	4.4
CV (%)		7.1	12.4	7.0	8.5	8.9	5.9	6.8	7.2
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Different letters among genotype values = significant differences by Duncan's new multiple range test (DNMRT) ($p \leq 0.05$); GN = Genotype number; CV % = Coefficient of variation; LSD = Least significance difference; P-value. = F probability

(ii) AMMI analysis and yield stability index for seed iron and zinc

Across locations, there were very highly significant ($P \leq 0.001$) effects of genotypes and genotype by environment interaction on common bean seed iron and zinc contents. Highly significance ($P \leq 0.01$) and very highly significance ($P \leq 0.001$) effect of environment were observed for seed Fe and Zn respectively (Table 17). Mean seed iron content across the three sites ranged from 25.6 to 116.7 mg/kg of dry weight with a grand mean of 56.1 mg/kg. Mean seed zinc content across locations ranged from 15.3 to 64.7 mg/kg of dry weight with a grand mean of 31.1 mg/kg. Additive main effects and multiplicative interaction (AMMI) analysis showed that, the main effects of genotypes and environment accounted for 69.5 % and 1.7 % of seed iron total sum of squares respectively, whereas genotype x environment interaction effect represented 26.3 % of seed iron total sum of squares. The two interaction principle component axes (IPCA 1 and IPCA 2) were both very highly significant ($P \leq 0.001$) for seed iron and accounted for 56.2 and 43.8 % respectively of the genotype by environment interaction for seed iron contents. For seed zinc contents, genotypes contributed 28.6 % of the total sum of squares variation, environment effect contributed 39.7 % and interaction 28.6 % of the total sum of squares variation among the common bean genotypes. The two interaction principle component axes (IPCA 1 and IPCA 2) were both very highly significant ($P \leq 0.001$) and accounted 75.6 and 24.4 % respectively of the genotype by environment interaction for seed zinc contents.

Based on additive main effects and multiplicative interaction (AMMI) stability value (ASV) and Genotype Stability Index (GSI). The harvested 99 common bean genotypes across locations were ranked based on seed iron and zinc content, following the least scores, whereby, low scores indicate the most stable genotype. The sum of mean seed iron contents and AMMI stability value rankings are also known as Genotype Stability Index (GSI) ranked SMC 18 as the most stable high seed iron-containing genotype with 84.5 mg/kg, followed by KAB o6F2-8-35 (78.1 mg/kg), Chumba neroza (76.4 mg/kg), Selian 94 (92.3 mg/kg) and Urafiki (85.1 mg/kg). Genotype Stability Index ranked Uyole 96 (37.9 mg/kg) as the most stable and high seed zinc-containing genotype, closely followed by CODMLB 033 (35.8 mg/kg), Soya (38.9 mg/kg), Kaisho kamugole (35.1 mg/kg) and Kitebe (39.1 mg/kg) (Table 18).

Table 17: AMMI analyses of variance for seed iron and zinc contents of the common bean genotypes planted across three locations

Source of Variation	Seed iron content							Seed zinc content					
	DF	SS	MS	F	P-value	%TSS	%GEISS	SS	MS	F	P-value	%TSS	%GEISS
Total	593	355608	600					48893	82.4				
Treatments	296	346568	1171	46.9	<0.001	97.5		47401	160.1	33.1	<0.001	96.9	
Genotypes	98	246986	2520	101.0	<0.001	69.5		14006	142.9	29.5	<0.001	28.6	
Environments	2	6067	3033	5.4	<0.01	1.7		19426	9712.9	424.1	<0.001	39.7	
Block	3	1700	567	22.7	<0.001	0.5		69	22.9	4.7	<0.01	0.1	
Interactions	196	93516	477	19.1	<0.001	26.3		13969	71.3	14.7	<0.001	28.6	
IPCA 1	99	52513	530	21.3	<0.001		56.2	10565	106.7	22.1	<0.001		75.6
IPCA 2	97	41003	423	16.9	<0.001		43.8	3403	35.1	7.3	<0.001		24.4
Error	294	7340	25					1423	4.8				

DF = degree of freedom, SS = sum of square, MS = mean sum square, F = F value, P-value = F probability, %TSS = percentage of total sum square and %GEISS = percentage of genotype by environment interaction sum square

Table 18: Across locations seed iron and zinc contents and ranking of 99 common bean genotypes based on AMMI stability value (ASV), and genotype stability index (GSI)

GN	Genotype	Seed iron concentration (mg/kg)						Seed zinc concentration (mg/kg)					
		Mean	ASV	RASV _i	RM _i	GSI _i	RGSI _i	Mean	ASV	RASV _i	RM _i	GSI _i	RGSI _i
G1	ACC 714	115.6	4.47	97	2	99	47	27.9	3.12	75	75	150	79
G2	Bagara Ompigize	50.6	2.07	67	54	121	65	31.2	1.11	27	50	77	35
G3	Bangaya Akatebe	61.4	0.77	18	27	45	9	34.3	0.76	14	25	39	11
G4	Bilfa 4	51.5	3.19	88	50	138	81	36.5	3.17	79	13	92	49
G5	Bilfa Uyole	39.3	2.08	68	85	153	91	31.5	1.71	44	47	91	48
G6	Buji	55.3	1.33	42	41	83	32	35.8	1.42	38	19	57	19
G7	Burushu	56.1	1.81	59	40	99	48	33.6	1.29	34	27	61	23
G8	CAL 96	49.4	2.68	82	58	140	82	26.5	2.93	71	82	153	80
G9	Calima Uyole	35.5	1.32	41	88	129	75	31.0	0.95	22	53	75	34
G10	Cheupe	52.7	1.02	29	48	77	29	25.2	4.90	94	88	182	98
G11	Chumba Neroza	76.4	0.73	16	16	32	4	31.3	1.40	36	49	85	42
G12	CODMLB 033	104.8	2.40	78	5	83	31	35.8	0.61	10	17	27	2
G13	DOR 500	64.5	3.67	94	25	119	63	24.2	2.62	63	90	153	81
G14	Fibea	59.5	0.70	15	30	45	10	33.4	0.86	17	30	47	15
G15	Jabeyila	102.0	4.00	95	6	101	49	37.5	3.23	80	9	89	46
G16	Jesca	25.6	1.11	33	99	132	77	29.1	0.25	3	66	69	31
G17	KAB o6F2-8-35	78.1	0.65	14	15	29	3	42.0	2.13	48	2	50	17
G18	KAB o6F2-8-36	43.2	1.24	36	75	111	60	33.0	0.77	15	32	47	16
G19	Kabanima	32.2	0.74	17	93	110	59	32.6	1.40	37	38	75	33
G20	Kabumburi	44.0	0.79	19	70	89	38	30.4	1.70	43	59	102	57
G21	Kachele	56.2	0.83	20	39	59	17	38.6	4.70	93	5	98	55
G22	Kaempu	78.5	1.58	53	14	67	21	35.2	0.71	12	21	33	9
G23	Kainja	58.2	1.01	28	32	60	18	33.5	2.24	50	29	79	37
G24	Kaisho kamugole	50.8	0.88	23	52	75	27	35.1	0.51	8	22	30	4
G25	Kakaritusi	51.1	1.44	47	51	98	46	27.8	2.25	51	77	128	68
G26	Kamoshi	60.2	1.35	43	28	71	24	36.7	3.09	74	12	86	43
G27	Kamosi	68.4	1.57	52	18	70	23	36.8	3.73	85	11	96	53
G28	Kanade	47.6	2.34	75	60	135	78	27.0	1.99	45	80	125	65
G29	Kashule	56.7	3.44	91	38	129	73	35.9	0.94	21	16	37	10
G30	Kasukari	80.5	3.32	89	13	102	52	27.9	0.91	20	74	94	52
G31	Katuku	65.2	1.85	61	23	84	34	31.2	0.48	7	51	58	22
G32	Katuku2	44.0	2.81	83	71	154	92	25.7	5.15	95	85	180	97
G33	Kibugu	50.7	1.59	54	53	107	57	36.3	0.91	19	14	33	8
G34	Kigoma	27.1	1.01	27	98	125	69	22.1	2.54	58	97	155	82

GN	Genotype	Seed iron concentration (mg/kg)						Seed zinc concentration (mg/kg)					
		Mean	ASV	RASV _i	RM _i	GSI _i	RGSI _i	Mean	ASV	RASV _i	RM _i	GSI _i	RGSI _i
G35	Kikobe	114.5	2.83	85	3	88	37	29.6	3.12	76	62	138	72
G36	Kilindi	43.4	1.60	55	72	127	72	24.6	3.16	78	89	167	92
G37	Kinyobya	52.8	0.43	4	46	50	13	30.5	1.50	39	57	96	54
G38	Kipapi	47.1	1.86	63	61	124	67	28.4	2.85	69	71	140	74
G39	Kisapuri	60.1	1.66	57	29	86	35	31.5	0.53	9	48	57	20
G40	Kitebe	54.1	0.56	9	44	53	15	39.1	1.13	28	3	31	6
G41	Kituntunu	29.3	0.89	24	95	119	64	30.3	2.01	46	61	107	59
G42	Kyababikira	68.0	2.36	76	19	95	43	28.8	4.30	89	67	156	83
G43	Kyakaragwe	95.2	1.32	40	7	47	12	30.9	1.26	32	54	86	44
G44	Lyamungo 85	44.6	0.87	22	68	90	39	23.1	2.75	66	94	160	86
G45	Lyamungo 90	55.1	1.42	45	42	87	36	29.2	8.41	99	64	163	90
G46	Maharage Kamba	83.9	3.49	92	12	104	54	23.9	1.32	35	92	127	67
G47	Maharage Mbeya	66.9	0.93	25	21	46	11	37.2	1.26	33	10	43	13
G48	Malirahinda	116.7	3.34	90	1	91	40	32.4	1.15	29	40	69	30
G49	Masusu	42.4	2.15	69	78	147	88	29.4	2.50	56	63	119	63
G50	Meupe Uyole	32.5	1.67	58	92	150	90	31.6	0.42	6	46	52	18
G51	Mshindi	61.8	2.82	84	26	110	58	23.2	3.09	73	93	166	91
G52	Msolini	42.6	1.89	64	76	140	84	33.6	0.25	2	28	30	5
G53	Mwami Kola	109.6	4.51	98	4	102	51	33.0	3.49	83	33	116	62
G54	Ngoma za bahaya	58.1	2.22	71	33	104	55	32.9	5.78	97	36	133	69
G55	Ngwakungwaku	40.3	0.61	11	81	92	41	32.6	2.36	53	39	92	50
G56	Njano fupi	44.0	0.61	10	69	79	30	30.4	2.82	68	58	126	66
G57	Njano Uyole	42.5	0.51	6	77	83	33	28.6	0.73	13	68	81	39
G58	Nyeupe Kubwa	49.6	1.46	48	57	105	56	32.3	1.69	42	41	83	41
G59	Nyeupe ndogo	39.9	1.49	49	83	132	76	32.6	0.36	4	37	41	12
G60	Pasi	56.8	1.05	30	37	67	22	32.2	3.07	72	43	115	61
G61	Pesa	67.8	0.48	5	20	25	2	28.5	1.56	40	69	109	60
G62	Raja	58.9	0.21	1	31	32	6	35.8	2.53	57	18	75	32
G63	Rojo	52.7	2.60	79	47	126	70	26.0	3.12	77	84	161	87
G64	Rosenda	65.0	0.54	8	24	32	5	31.2	4.03	88	52	140	73
G65	Rozikoko fupi	51.6	0.37	3	49	52	14	28.4	4.67	92	70	162	88
G66	Ruondera	56.9	1.19	35	36	71	25	31.8	1.01	23	44	67	28
G67	RWR 2154	57.9	0.87	21	35	56	16	34.4	2.67	64	24	88	45
G68	Selian 05	45.7	3.53	93	65	158	94	30.5	0.16	1	56	57	21
G69	Selian 06	54.0	1.54	50	45	95	44	17.8	3.59	84	99	183	99
G70	Selian 10	29.4	1.91	65	94	159	95	29.1	3.39	81	65	146	78
G71	Selian 11	39.8	1.81	60	84	144	85	25.7	2.21	49	86	135	70

GN	Genotype	Seed iron concentration (mg/kg)						Seed zinc concentration (mg/kg)					
		Mean	ASV	RASV _i	RM _i	GSI _i	RGSI _i	Mean	ASV	RASV _i	RM _i	GSI _i	RGSI _i
G72	Selian 12	50.1	1.26	38	55	93	42	27.2	2.61	62	79	141	76
G73	Selian 13	40.2	0.65	13	82	95	45	32.9	2.70	65	35	100	56
G74	Selian 14	45.0	0.52	7	66	73	26	22.9	2.81	67	95	162	89
G75	Selian 15	55.0	2.64	81	43	124	66	27.9	0.39	5	76	81	40
G76	Selian 9	29.0	2.23	72	96	168	99	28.0	2.09	47	73	120	64
G77	Selian 94	92.3	1.07	32	9	41	7	32.2	2.30	52	42	94	51
G78	Selian 97	43.3	2.02	66	74	140	83	26.4	4.65	91	83	174	95
G79	Selundo	75.2	4.79	99	17	116	61	21.6	2.59	61	98	159	85
G80	Sinon	41.7	1.65	56	79	135	79	31.7	0.78	16	45	61	25
G81	SMC 17	66.4	1.39	44	22	66	20	38.2	6.13	98	7	105	58
G82	SMC 18	84.5	0.33	2	11	13	1	47.0	4.53	90	1	91	47
G83	Soya	41.5	1.24	37	80	117	62	38.9	1.03	24	4	28	3
G84	Soya Mbeya	57.9	0.99	26	34	60	19	35.3	1.04	25	20	45	14
G85	SUA 90	50.1	2.24	73	56	129	74	36.0	2.42	54	15	69	29
G86	Tema	46.4	1.28	39	62	101	50	38.3	1.10	26	6	32	7
G87	Tikumba Nyama	45.7	1.86	62	64	126	71	33.0	1.18	31	34	65	27
G88	Urafiki	85.1	1.07	31	10	41	8	25.3	3.87	86	87	173	93
G89	Uyole 03	38.6	2.20	70	86	156	93	24.2	3.90	87	91	178	96
G90	Uyole 04	27.9	1.57	51	97	148	89	28.4	2.93	70	72	142	77
G91	Uyole 16	44.7	2.60	80	67	147	87	27.7	5.31	96	78	174	94
G92	Uyole 18	38.0	2.32	74	87	161	97	33.7	2.48	55	26	81	38
G93	Uyole 84	33.9	1.43	46	91	137	80	22.6	2.57	60	96	156	84
G94	Uyole 94	48.5	3.06	86	59	145	86	30.7	3.43	82	55	137	71
G95	Uyole 96	45.8	0.63	12	63	75	28	37.9	0.65	11	8	19	1
G96	Uyole 98	34.3	1.14	34	90	124	68	26.5	2.55	59	81	140	75
G97	Wanja	35.2	2.37	77	89	166	98	30.3	0.88	18	60	78	36
G98	Wifi Nyegele	94.5	4.02	96	8	104	53	33.3	1.15	30	31	61	24
G99	Zawadi	43.4	3.13	87	73	160	96	34.6	1.62	41	23	64	26
Mean		56.1	1.75	50	50	100	50	31.1	2.29	50	50	100	50

GN = Genotype number, IPC1 and IPC2 are interaction principal component 1 and 2 respectively, ASV = AMMI Stability Value, RASV = rank of the genotype across environments based on AMMI Stability Value, GSI = Genotype Stability Index, RM = rank of the genotype across environments based on mean seed mineral (iron and zinc) contents across environments, RGSI = rank of the genotype based on Genotype Stability Index

(iii) Ideal, stable and high seed iron and zinc-containing bean genotypes by GGE

The genotype main effect plus genotype by-environment interaction (GGE) biplot explained 89.3 % of the total genotype effects and genotype by environment interaction. Based on GGE biplot, G48 (Malirahinda) was located into the center of the concentric circles closer to the ideal genotype, thus it was the most stable and high seed iron-containing bean genotype with 116.7 mg/kg. The other stable and high seed iron-containing bean genotypes situated in the next concentric circles, includes G35 (Kikobe) that had 114.5 mg/kg, and G12 (CODMLB 033) with 104.8 mg/kg, followed by G1 (ACC 714) with 115.6 mg/kg, G15 (Jabeyila) had 102.0 mg/kg, G53 (Mwamikola) had 109.6 mg/kg, G43 (Kyakaragwe) had 95.2 mg/kg, and G77 (Selian 94) with 92.3 mg/kg, which were placed in the third concentric circle. Other stable and high seed iron beans, situated in the fourth concentric circle included G46 (Maharage kamba) which had 83.9 mg/kg, G88 (Urafiki) had 85.1 mg/kg, G82 (SMC 18) had 84.5 mg/kg, G30 (Kasukari) had 80.5 mg/ha, and G98 (Wifi nyegela) which had 94.5 mg/ha. Most genotypes were stable but low seed iron contents, thus were not desirable and placed far outside the concentric circles around the ideal genotypes though very close to the average environment axis (AEA) (Fig. 30).

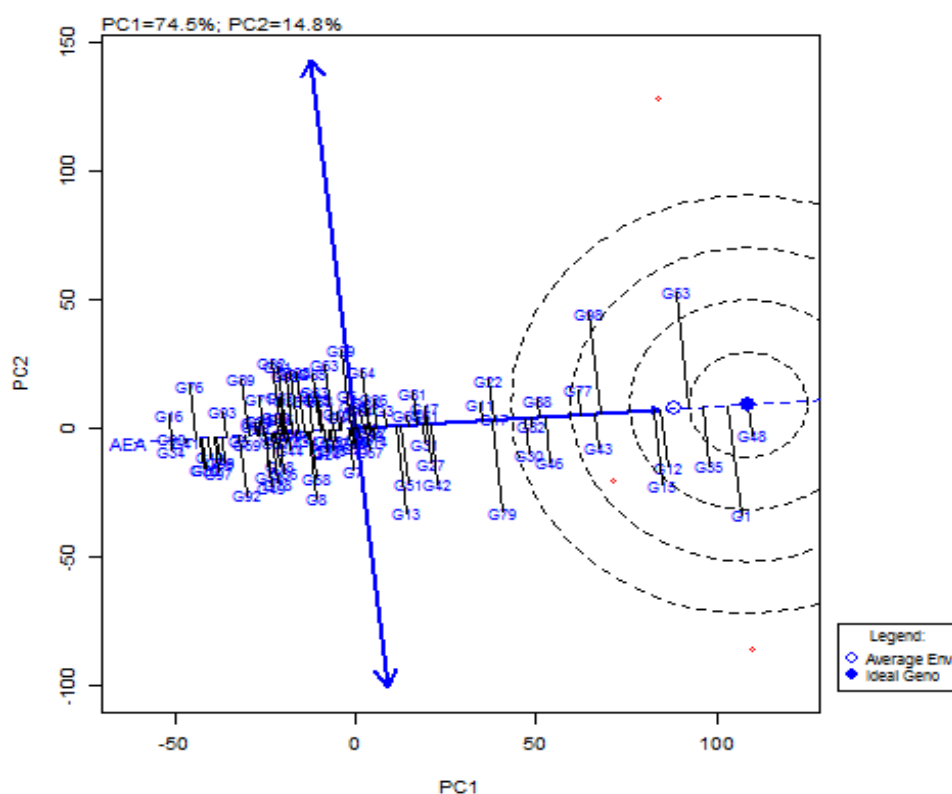


Figure 30: GGE biplot displaying the ranking of common bean genotypes relative to an ideal genotype based on seed iron content (the center of the concentric circles)

The genotype main effect plus genotype by-environment interaction (GGE) biplot explained 94.1 % of the total genotype effects and genotype by environment interaction. Using GGE biplot, G17 (KAB o6F2-8-35) was identified as the most stable and high zinc-containing common bean genotype with 42.0 mg/kg as it was located into the second concentric circle closer to the ideal genotype at the centre of concentric circles. The second stable and high seed zinc-containing bean genotype, which was placed in the third concentric circle was G82 (SMC 18) had 47.0 mg/kg. Other stable and high seed zinc-containing bean genotypes situated in the fourth concentric circle, includes and G40 (Kitebe) which had 39.1 mg/kg, followed by G83 (Soya) with 39.9 mg/kg, and G95 (Uyole 96) with 37.9 mg/kg. Most genotypes were stable but low seed zinc contents, thus were not desirable and placed far outside the concentric circles around the ideal genotypes though very close to the average environment axis (AEA) (Fig. 31).

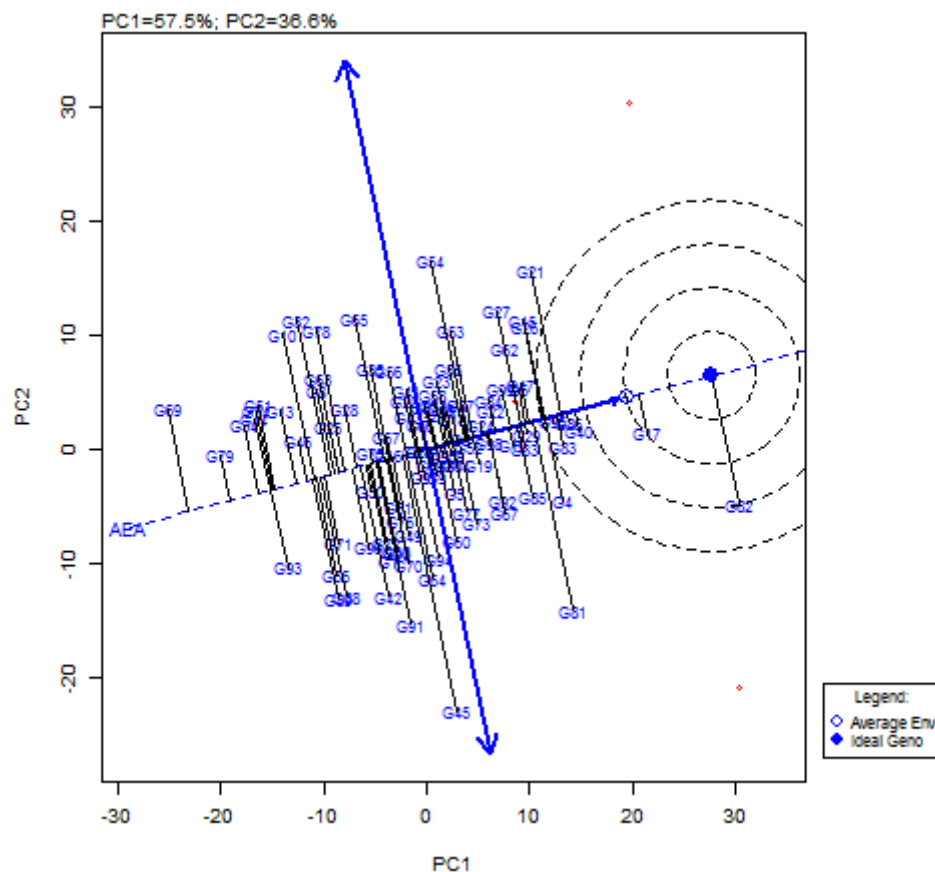


Figure 31: GGE biplot displaying the ranking of common bean genotypes relative to an ideal genotype based on seed zinc content (the center of the concentric circles)

(iv) Experimental sites discriminating power and representativeness on genotypes seed iron and zinc

Test location E1 (TARI-Selian) and E3 (TARI-Uyole) had small cosine angle between them, and thus were more related in discriminating common bean genotypes in terms of seed iron contents. Having long vectors from the biplot origin, test locations E1 (TARI-Selian) and E2 (SUA), had higher discriminating power on genotypes seed iron content compared to E3 with shorter vector. Test location E3, having a small angle with the average environmental axis (AEA) compared to the rest sites, was more representative of the other sites. The GGE biplot, assisted in visualization of the best discriminating and representative test location, by drawing concentric circles around the ideal environment. Site E1 (TARI-Selian) and E3 (TARI-Uyole) both fall into the third concentric circle of the ideal environment, but E1 was placed more closer to the ideal environment, thus, E1 (TARI-Selian) was the most discriminating and representative test location among others on common bean genotypes seed iron contents (Fig. 32).

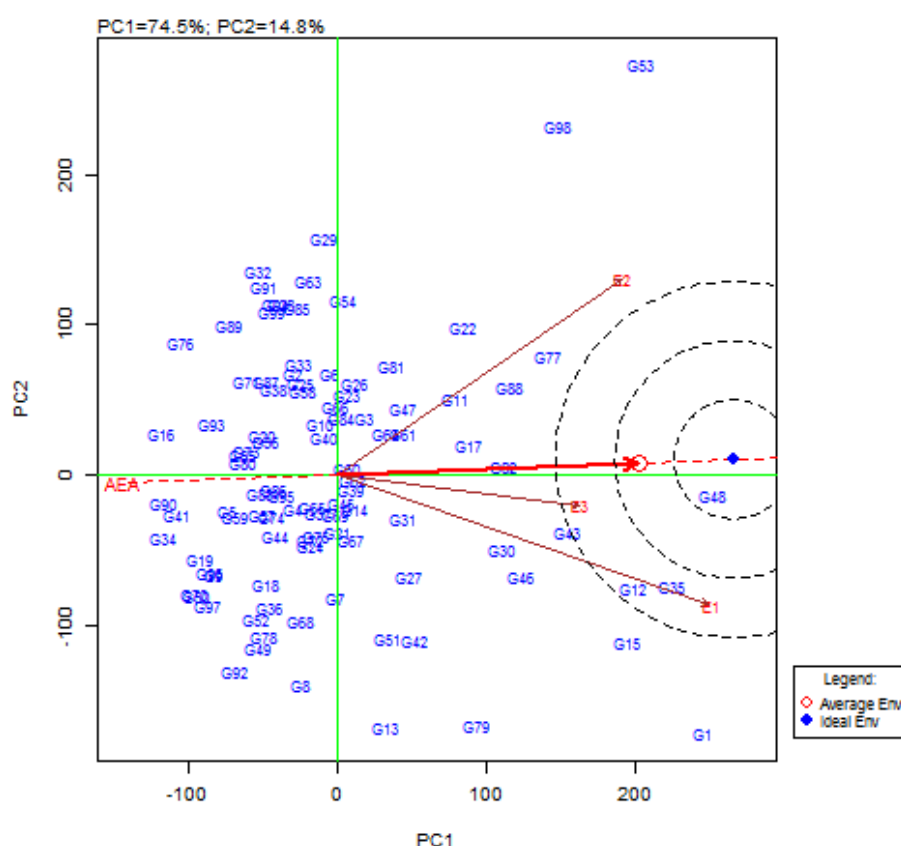


Figure 32: GGE biplot showing the ranking of the experimental sites (E1 = TARI-Selian, E2 = SUA, and E3 = TARI-Uyole) based on discriminating ability and representativeness for common bean genotypes seed iron contents

Having longer vectors from the biplot origin, test locations E2 (SUA) and E3 (TARI-Uyole), had higher discriminating power on genotypes seed zinc content compared to E1 (TARI-Selian) with shorter vector. Test location E1 (TARI-Selian), having a small angle with the average environmental axis (AEA) compared to the rest sites, was more representative of the other sites on bean genotypes seed zinc contents. There was no test location that was placed in the concentric circles of the ideal environment by GGE biplot graphic, thus there was no best test location with combined discriminating ability and representativeness of the test location on bean genotypes seed zinc contents (Fig. 33).

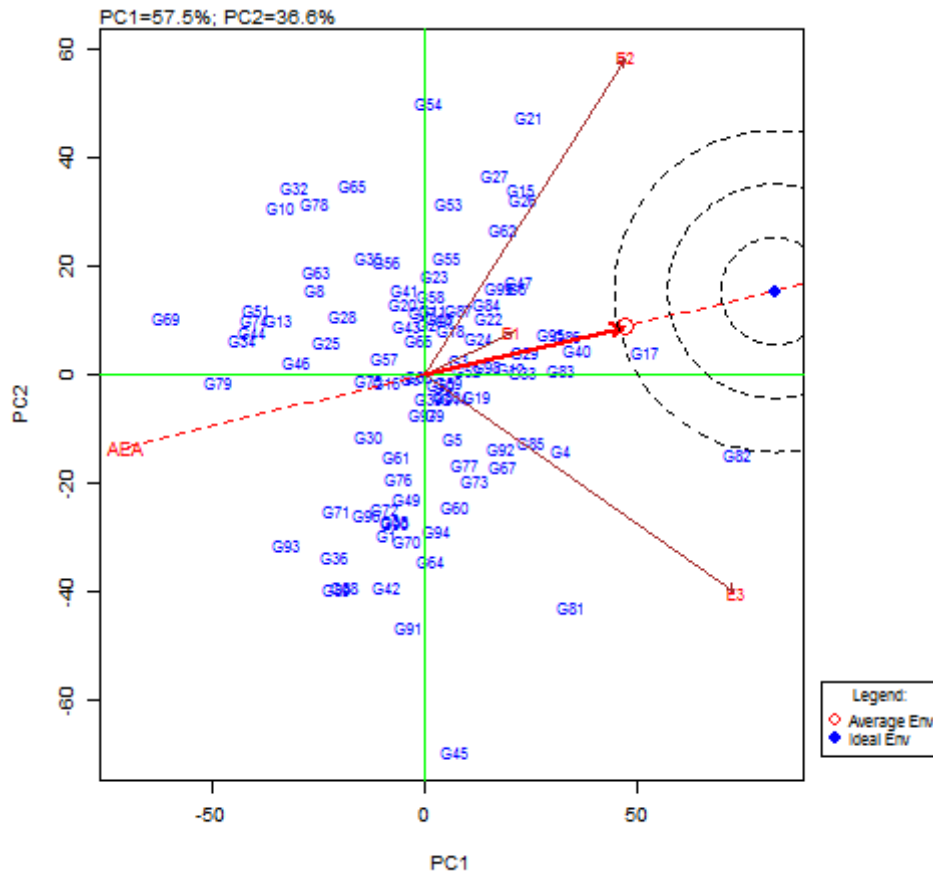


Figure 33: GGE biplot showing the ranking of the experimental sites (E1 = TARI-Selian, E2 = SUA, and E3 = TARI-Uyole) based on discriminating ability and representativeness for common bean genotypes seed zinc contents

(v) Relationship between common bean seed Fe and Zn with weather and soil properties

Association of common bean seed iron and zinc with weather and soil parameters varied from non-significance to highly significance ($P \leq 0.001$) and weak to strong positive and negative. Pearson correlation analysis revealed that, total rainfall received during growth, flowering,

pod setting and filling to physiological maturity strongly positively and significantly ($r = 0.93$, $P \leq 0.001$) associated with common bean seed iron contents, while it was strongly negatively and significantly ($r = -0.51$, $P \leq 0.05$) associated with seed zinc contents. Mean temperature during growth, flowering, pod setting and filling to physiological maturity had a strong negative and significant relationship ($r = -1.0$, $P \leq 0.001$). No relationship between mean temperatures recorded during bean growth to physiological maturity with seed zinc content was observed.

Physical soil properties, silt and sand had strong and positive significant relationship ($r = 1.0$, $P \leq 0.001$ and $r = 0.99$, $P \leq 0.001$) with seed iron content. Soil clay had strong and negative significant association ($r = -1$, $P \leq 0.001$) with seed iron contents, while no relationship between measured soil physical properties with common bean seed zinc content was observed. Among the measured soil chemical properties, iron, available phosphorus and exchangeable potassium had strong positive and significant relationship ($r = 0.83$, $P \leq 0.001$, $r = 0.89$, $P \leq 0.001$ and 0.63 , $P \leq 0.01$) with seed iron contents, while strong negative and significant association was obtained between these soil chemical properties with seed zinc content. Soil nitrogen, organic carbon, and manganese, were negatively and significantly related ($r = -0.99$, $P \leq 0.001$, $r = -0.99$, $P \leq 0.001$ and $r = -0.77$, $P \leq 0.001$) to seed iron content. Strong positive and significant ($r = 0.75$, $P \leq 0.001$) relationship between soil manganese and seed zinc content was observed, soil nitrogen and organic carbon were not significantly related to seed zinc content. A moderate positive and significant relationship ($r = 0.44$, $P \leq 0.001$) between soil pH and seed iron content was observed, while a strong negative and significant association ($r = -0.96$, $P \leq 0.001$) was observed between soil pH and seed zinc content (Table 19).

Table 19: Pearson correlations of common bean seed iron (Fe) and zinc (Zn) contents with soil and weather properties

Soil and weather parameters	Seed Fe (mg/kg)	Seed Zn (mg/kg)
Total rainfall from planting to maturity	0.932***	-0.509*
Mean temperature from planting to maturity	-1.000***	0.132ns
Soil pH	0.442*	-0.957***
% Clay	-1.000***	0.190ns
% Silt	0.999***	-0.116ns
% Sand	0.992***	-0.284ns
Copper (Cu) (mg/kg)	-0.043ns	-0.979***
Zinc (Zn) (mg/kg)	-0.318ns	-0.884***
Manganese (Mn) (mg/kg)	-0.773***	0.751***
Iron (Fe) (mg/kg)	0.832***	-0.682**
Total Nitrogen (TN) %	-0.991***	0.025ns
Soil Organic Carbon (OC) %	-0.989***	0.014*
Available Phosphorus (P) (mg/kg)	0.892***	-0.589**
Exchangeable Potassium (K ⁺) (CmolKg ⁻¹)	0.633**	-0.866***

*, **, and *** = significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ respectively, and ns = not significant ($P \leq 0.05$)

4.1.4 Levels of phytic acid, P, Mg and, Mn among common bean genotypes grown in Tanzania

(i) Variation for seed phytic acid and minerals

The mean common bean seed phytic acid (PA), phosphorus (P), magnesium (Mg), and Manganese (Mn) contents are presented in table 18. Seed PA contents ranged from 711.8 to 1914.6 mg/100 g with a grand mean of 1175.3 mg/kg, phosphorus ranged from 3307.0 to 7037.0 mg/kg with overall mean of 5371.0 mg/kg, while magnesium had a range of 1511.4-2503.0 mg/kg with a mean of 1827.8 mg/kg and manganese had a range of 10.2-38.8 mg/kg with a mean of 17.4 mg/kg. Most of the common bean genotypes had PA content between 900 and 1500 mg/100 g, whereas few genotypes had PA below 900 mg/100 g and above 1500 mg/100 g (Fig. 34A). For the case of seed phosphorus contents, most of the bean genotypes had phosphorus between 4500 and 6500 mg/kg, with very few bean genotypes having seed phosphorus content below 4500 mg/kg and above 6500 mg/kg (Fig. 34B). Majority of the tested bean genotypes had seed magnesium content between 1250 and 2250 mg/kg, whereas very few had seed magnesium content above 2250 mg/kg (Fig. 34C). Most of the tested bean genotypes had seed manganese content between 10 and 20 mg/kg, while few bean genotypes were observed to have seed manganese content above 20 mg/kg (Fig. 34D).

Analysis of variance revealed highly significance variability ($P \leq 0.001$) among the common bean genotypes for seed PA, P, Mg, and Mn. The lowest seed PA-containing common bean genotype was Fibea (712 mg/100 g) that was closely followed by Selian 06 (734 mg/100 g), Maharage kamba (746 mg/100 g), DOOR 500 (760 mg/100 g), and katuku2 (840 mg/100 g). The highest seed PA contents was recorded by SMC 17 (1915 mg/100 g). The highest seed P-containing common bean genotype was SMC 18 (7037 mg/kg), which was closely followed by SMC 17 (6942 mg/kg), Kyakaragwe (6334 mg/kg), Selian 94 (6260 mg/kg), and Urafiki (6200 mg/kg), whereas Katuku2 (3307 mg/kg) recorded the lowest seed P content. Bilfa 4 (2503 mg/kg) was the highest seed Mg-containing genotype followed by Kikobe (2278 mg/kg), ACC 714 (2241 mg/kg), Malirahinda (2219 mg/kg), and Jabeyila (2143 mg/kg). The lowest seed Mg-containing common bean genotype was Bagara ompigize (1511 mg/kg). The highest seed Mn-containing common bean genotype was ACC 714 (38.8 mg/kg), closely followed by Bilfa 4 (30.8 mg/kg), Kikobe (30.8 mg/kg), Malirahinda (30.0 mg/kg), and Selian 94 (29.7 mg/kg), whereas Katuku2 recorded the lowest seed Mn content (28.4 mg/kg) (Table 20).

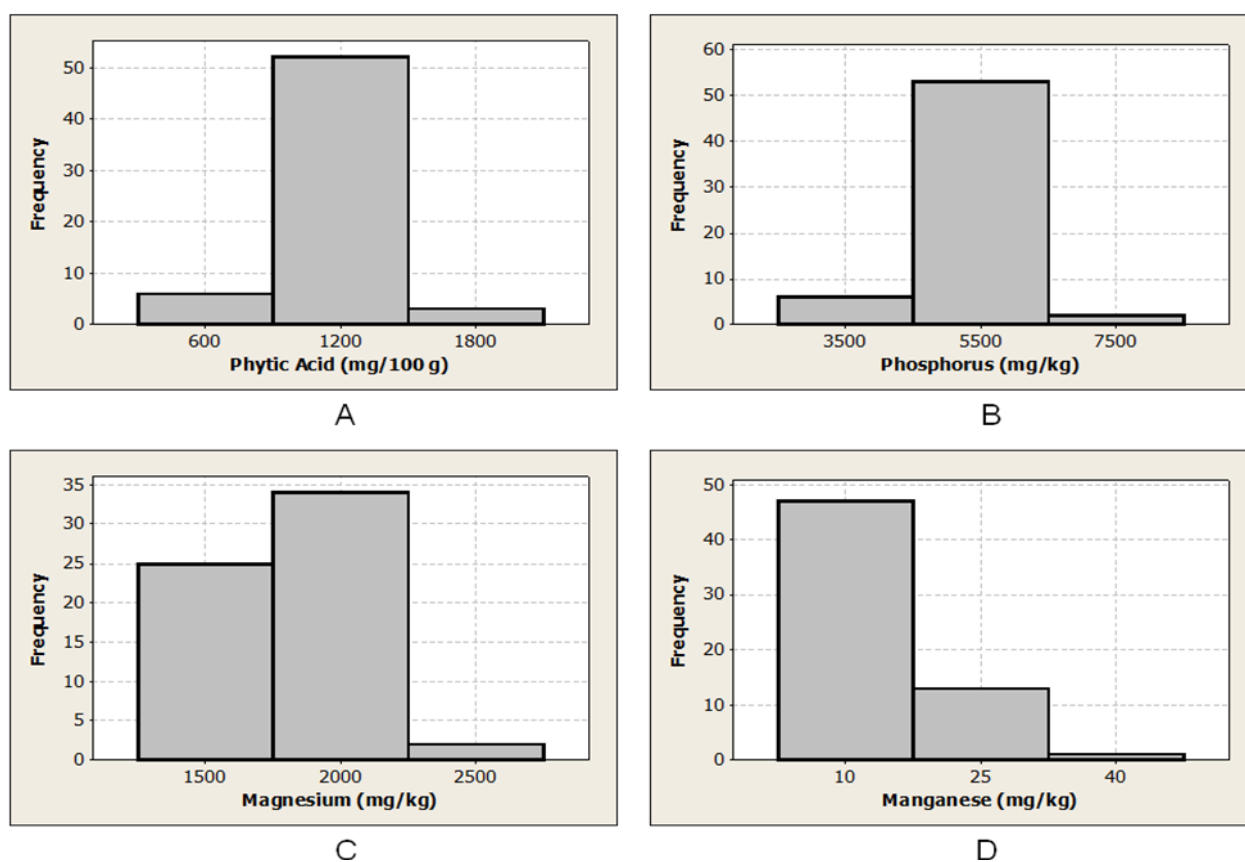


Figure 34: Distribution of 61 common bean genotypes seed phytic acid and minerals; (A) Phytic acid; (B) Phosphorus; (C) Magnesium; (D) Manganese

Table 20: Variation in seed phytic acid and minerals among the tested common bean genotypes

GN	Genotype	Phytic acid (mg/100g)	Phosphorus (mg/kg)	Magnesium (mg/kg)	Manganese (mg/kg)
G1	ACC 714	1141f-o	5023mno	2241bc	38.8a
G2	Bagara Ompigize	1196h-s	5505f-n	1511y	14.1s-A
G3	Bangaya Akatebe	1370rst	5535f-n	1835i-q	15.4j-u
G4	Bilfa 4	1188h-s	5621e-k	2503a	30.8b
G6	Buji	1239k-t	5470f-n	1707n-x	13.3v-B
G7	Burushu	1163f-q	5183j-o	1917f-l	13.2w-B
G8	CAL 96	1156f-p	5562f-m	1541xy	16.3i-r
G11	Chumba Neroza	1174h-r	5441f-n	2057d-g	24.7f
G12	CODMLB 033	1203h-s	5239i-o	1792k-t	26.3ef
G13	DOR 500	760ab	4315pq	2128b-e	16.9h-n
G14	Fibea	712a	4705op	1683n-y	14.5q-A
G15	Jabeyila	1235j-t	6157b-e	2143b-e	26.8de
G16	Jesca	1213i-t	5438f-n	1816j-r	13.2w-B
G17	KAB o6F2-8-35	1330o-t	5872b-g	1659p-y	16.5i-q
G21	Kachele	1199h-s	5218i-o	1918f-l	14.1s-A
G22	Kaempu	1127f-o	5213i-o	1687n-y	16.8h-o
G24	Kaisho kamugole	1287l-t	5084k-o	1816j-r	12.8y-B
G26	Kamoshi	1031c-j	5150j-o	1967e-k	14.6p-z
G27	Kamosi	1199h-s	5213i-o	1992e-j	15.5j-u
G30	Kasukari	1304n-t	5830b-h	1846i-p	16.2i-r
G31	Katuku	1311n-t	5548f-n	1963e-k	15.0n-x
G32	Katuku2	840abc	3307r	2119b-e	10.2C
G34	Kigoma	1203h-s	5542f-n	1676o-y	15.7j-t
G35	Kikobe	968c-g	5844b-h	2278b	30.0bc
G38	Kipapi	1292m-t	5682d-j	1638r-y	13.5u-A
G41	Kituntunu	1316o-t	5224i-o	1629s-y	14.8o-y
G42	Kyababikira	1082e-l	5144j-o	1549wxy	17.0h-m
G43	Kyakaragwe	1413tu	6334b	2009e-i	17.4hijk
G45	Lyamungo 90	1208h-s	5571f-m	1841i-p	28.3cd
G46	Maharage Kamba	746ab	3631r	1800k-s	25.5ef
G47	Maharage Mbeya	1167g-r	5153j-o	1707n-x	16.6i-p
G48	Malirahinda	1036d-k	5519f-n	2219bcd	29.7bc
G49	Masusu	1229i-t	5446f-n	1703n-x	14.6p-z
G50	Meupe Uyole	1196h-s	5418f-n	1635r-y	14.8o-y
G51	Mshindi	1025c-i	5470f-n	1698n-x	11.3BC
G52	Msolini	1194h-s	5340g-n	1580v-y	13.0x-B
G53	Mwami Kola	1359p-t	5614f-l	1903g-m	20.8g
G54	Ngoma za bahaya	1575uv	5778c-i	1855i-o	15.0n-x
G59	Nyeupe ndogo	921b-e	4283pq	1771l-t	15.3l-w
G60	Pasi	1062e-k	5332g-n	1964e-k	13.0x-B
G61	Pesa	1220i-t	5445f-n	1584u-y	14.4r-A
G63	Rojo	1313n-t	5962b-f	1629s-y	13.6t-A
G64	Rosenda	1383st	5556f-m	1899g-m	13.8s-A
G67	RWR 2154	1200h-s	5808b-h	1629s-y	13.6u-A
G68	Selian 05	1004c-h	4191q	1555wxy	15.2m-w
G69	Selian 06	734a	3347r	1830i-q	17.3hijkl
G72	Selian 12	1092e-m	5100k-o	1711n-x	18.7h
G73	Selian 13	1364q-t	5776c-i	1653q-y	15.9j-s
G76	Selian 9	1332o-t	5828b-h	2091c-f	14.7p-y
G77	Selian 94	1269l-t	6260bc	1864h-n	28.4cd
G79	Selundo	854a-d	5048l-o	1817j-r	16.3i-r
G80	Sinon	1189h-s	5556f-m	1765l-v	14.0s-A
G81	SMC 17	1915w	6942a	2040e-h	16.3i-r
G82	SMC 18	1729v	7037a	1914f-m	20.6g
G83	Soya	1131f-o	5233i-o	1751l-v	17.5hij
G88	Urafiki	1235j-t	6200bcd	1732m-w	18.2hi

GN	Genotype	Phytic acid (mg/100g)	Phosphorus (mg/kg)	Magnesium (mg/kg)	Manganese (mg/kg)
G89	Uyole 03	1158f-p	5456f-n	1556wxy	12.5AB
G90	Uyole 04	1034c-k	5292h-n	1766l-u	12.4AB
G92	Uyole 18	1095e-m	5201j-o	1606t-y	14.8o-y
G94	Uyole 94	963c-f	5479f-n	1679n-y	12.6zAB
G98	Wifi Nyegela	1107e-n	4987no	2132b-e	15.3k-v
Mean		1175.3	5371	1827.8	17.4
LSD (0.05)		165.4	454.9	151.2	1.7
CV %		7	4.2	4.1	4.9
P-value		< 0.001	< 0.001	< 0.001	< 0.001

GN = Genotype number; Different letters among genotype values = significant differences by Duncan's new multiple range test (DNMRT) ($p \leq 0.05$); CV % = Coefficient of variation; LSD = Least significance difference; P-value. = F probability

(ii) Common bean genotypes seed phytic acid to mineral molar ratio

Phytic acid to minerals (Fe, Zn, Mg, and Mn) molar ratios for the common bean genotypes are presented in table 19. The phytic acid to iron molar ratio ranged from 5.47 to 45.18 with a mean of 18.94, phytic acid to zinc molar ratio ranged from 26.21 to 75.95 with mean of 49.71, while phytic acid to magnesium molar ratio had a range of 0.13 - 0.35 with a mean of 0.24 and phytic acid to manganese molar ratio had a range of 24.32 - 97.73 with a mean of 60.77. Most of the common bean genotypes had phytic acid to iron molar ratio between 10 and 30, whereas few genotypes had phytic acid to iron molar ratio below 10 and above 40 (Fig. 35A). Most of the bean genotypes had seed phytic acid to zinc molar ratio between 32.5 and 57.5, with very few bean genotypes having phytic acid to zinc molar ratio below 32.5 and above 57.5 (Fig. 35B). Majority of the tested bean genotypes had phytic acid to magnesium molar ratio between 0.15 and 0.35, whereas very few had seed magnesium content above 0.15 (Fig. 35C). Most of the tested bean genotypes had seed phytic acid to manganese molar ratio between 45 and 75, while few bean genotypes were observed to have phytic acid to manganese ratio below 45 and above 75 (Fig. 35D).

Among the tested common bean genotypes, Maharage kamba recorded the lowest phytic acid to iron molar ratio of 5.47, which was closely followed by Malirahinda (6.01), ACC 714 (6.40), Kikobe (6.91), and Jabeyila (7.56). The highest phytic acid to iron molar ratio was recorded from Selian 9 (45.18). For the case of phytic acid to zinc molar ratio, Fibe recorded the lowest value of 26.21 among the bean genotypes, followed by Nyeupe ndogo (34.37), Soya (34.80), Kikobe (35.63), and DOOR 500 (36.02). The highest phytic acid to zinc molar ratio was observed from Selian 9 (75.95). Regarding phytic acid to magnesium molar ratio, the genotypes DOOR 500 (0.13), Katuku2 (0.15), Maharage kamba (0.15), Selian 06 (0.15),

and Kikobe (0.16) had the lowest values, whereas SMC 17 (0.35) recorded the highest phytic acid to magnesium molar ratio. The lowest phytic acid to manganese molar ratio was calculated from the following bean genotypes; Maharage kamba (24.32), ACC 714 (24.47), Malirahinda (29.01), Kikobe (26.83), and 32.08) whereas SMC 17 (97.73) had the highest phytic acid to manganese molar ratio (Table 21).

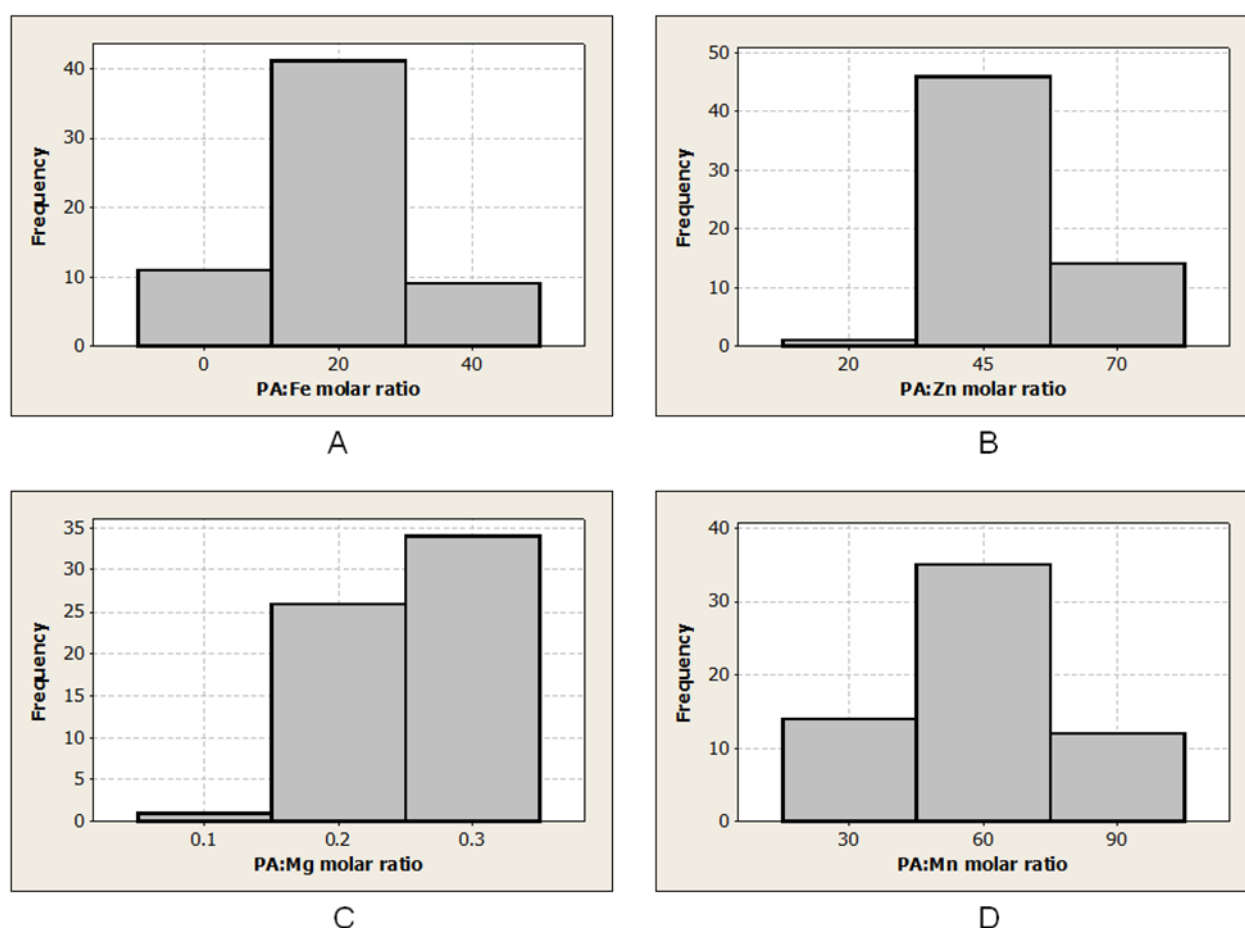


Figure 35: Distribution of 61 common bean genotypes seed phytic acid to minerals molar ratio; (A) PA:Fe molar ratio; (B) PA:Zn molar ratio (P); (C) PA:Mg molar ratio; (D) PA:Mn molar ratio

Table 21: Variation in seed phytic acid to minerals molar ratios among the tested common bean genotypes

GN	Genotype name	Phytic acid to iron molar ratio	Phytic acid to zinc molar ratio	Phytic acid to magnesium molar ratio	Phytic acid to manganese molar ratio
G1	ACC 714	6.40	56.95	0.19	24.47
G2	Bagara Ompigize	28.53	54.81	0.29	70.61
G3	Bangaya Akatebe	20.09	41.25	0.27	74.02
G4	Bilfa 4	28.48	53.58	0.17	32.08
G6	Buji	20.50	48.82	0.27	77.6
G7	Burushu	16.00	40.68	0.22	73.09
G8	CAL 96	15.82	45.55	0.28	59.1
G11	Chumba Neroza	12.9	58.63	0.21	39.56
G12	CODMLB 033	8.09	44.88	0.25	38.15
G13	DOR 500	8.46	36.02	0.13	37.34
G14	Fibea	9.85	26.21	0.16	40.97
G15	Jabeyila	7.56	48.67	0.21	38.38
G16	Jesca	39.51	52.95	0.25	76.61
G17	KAB o6F2-8-35	13.33	52.41	0.3	67.15
G21	Kachele	16.88	36.9	0.23	71.02
G22	Kaempu	13.27	37.11	0.25	55.8
G24	Kaisho kamugole	18.27	45.58	0.26	83.53
G26	Kamoshi	16.89	46.52	0.19	58.75
G27	Kamosi	13.39	39.04	0.22	64.37
G30	Kasukari	10.04	59.78	0.26	66.85
G31	Katuku	13.44	47.64	0.25	72.99
G32	Katuku2	24.16	37.9	0.15	68.29
G34	Kigoma	39.72	58.01	0.26	63.91
G35	Kikobe	6.91	35.63	0.16	26.83
G38	Kipapi	32.40	58.18	0.29	79.51
G41	Kituntunu	41.76	64.57	0.3	73.98
G42	Kyababikira	10.65	40.38	0.26	52.87
G43	Kyakaragwe	11.49	60.81	0.26	67.7
G45	Lyamungo 90	14.94	59.22	0.24	35.47
G46	Maharage Kamba	5.47	48.45	0.15	24.32
G47	Maharage Mbeya	14.22	39.31	0.25	58.4
G48	Malirahinda	6.01	46.74	0.17	29.01
G49	Masusu	20.39	51.3	0.27	69.97
G50	Meupe Uyole	29.83	49.91	0.27	67.35
G51	Mshindi	9.88	46.21	0.22	75.33
G52	Msolini	21.11	46.41	0.28	76.64
G53	Mwami Kola	12.86	55.91	0.26	54.44
G54	Ngoma za bahaya	27.64	72.21	0.31	87.73
G59	Nyeupe ndogo	22.64	34.37	0.19	50.27
G60	Pasi	13.69	37.61	0.2	67.81
G61	Pesa	15.61	60.66	0.28	70.66
G63	Rojo	29.37	67.71	0.3	80.28
G64	Rosenda	18.61	47.79	0.27	83.25
G67	RWR 2154	15.47	55	0.27	73.55
G68	Selian 05	10.93	41.89	0.24	54.96
G69	Selian 06	9.06	49.97	0.15	35.32
G72	Selian 12	14.61	64.87	0.23	48.7
G73	Selian 13	27.59	53.02	0.3	71.66
G76	Selian 9	45.18	75.95	0.23	75.39
G77	Selian 94	11.69	56.32	0.25	37.2
G79	Selundo	6.12	36.2	0.17	43.51
G80	Sinon	31.78	51.18	0.25	70.57
G81	SMC 17	27.91	57.02	0.35	97.73
G82	SMC 18	16.39	52.37	0.33	70.01
G83	Soya	18.34	34.8	0.24	53.95

GN	Genotype name	Phytic acid to iron molar ratio	Phytic acid to zinc molar ratio	Phytic acid to magnesium molar ratio	Phytic acid to manganese molar ratio
G88	Urafiki	11.76	59.55	0.26	56.39
G89	Uyole 03	34.94	67.42	0.27	77.01
G90	Uyole 04	44.22	45.17	0.22	69.33
G92	Uyole 18	18.68	49.4	0.25	61.66
G94	Uyole 94	32.2	37.4	0.21	63.54
G98	Wifi Nyegela	11.52	51.6	0.19	60.08
Mean		18.94	49.71	0.24	60.77
Minimum		5.47	26.21	0.13	24.32
Maximum		45.18	75.95	0.35	97.73

(iii) Relationship among common bean seed minerals and phytic acid

Association of common bean seed phytic acid with seed minerals varied from non-significance to highly significance ($P \leq 0.001$) and weak ($r \leq 0.29$) to strong ($r \geq 0.5$) positive and negative. Pearson correlation (r) analysis revealed that, seed phytic acid was strongly positively and significantly ($r = 0.75$, $P \leq 0.001$) associated with common bean seed phosphorus contents, while no relationship between common bean seed phytic acid with seed iron, zinc, magnesium and manganese content was observed. Seed iron had strong and positive significant relationship ($r = 0.93$, $P \leq 0.001$ and $r = 0.59$, $P \leq 0.001$) with seed manganese and magnesium content respectively, while no relationship between seed iron with common bean seed zinc, phytic acid and phosphorus content was observed. Strong positive and significant ($r = 0.54$, $P \leq 0.001$) relationship between seed manganese and seed magnesium content was observed (Table 22).

Table 22: Correlation among common bean seed minerals and phytic acid

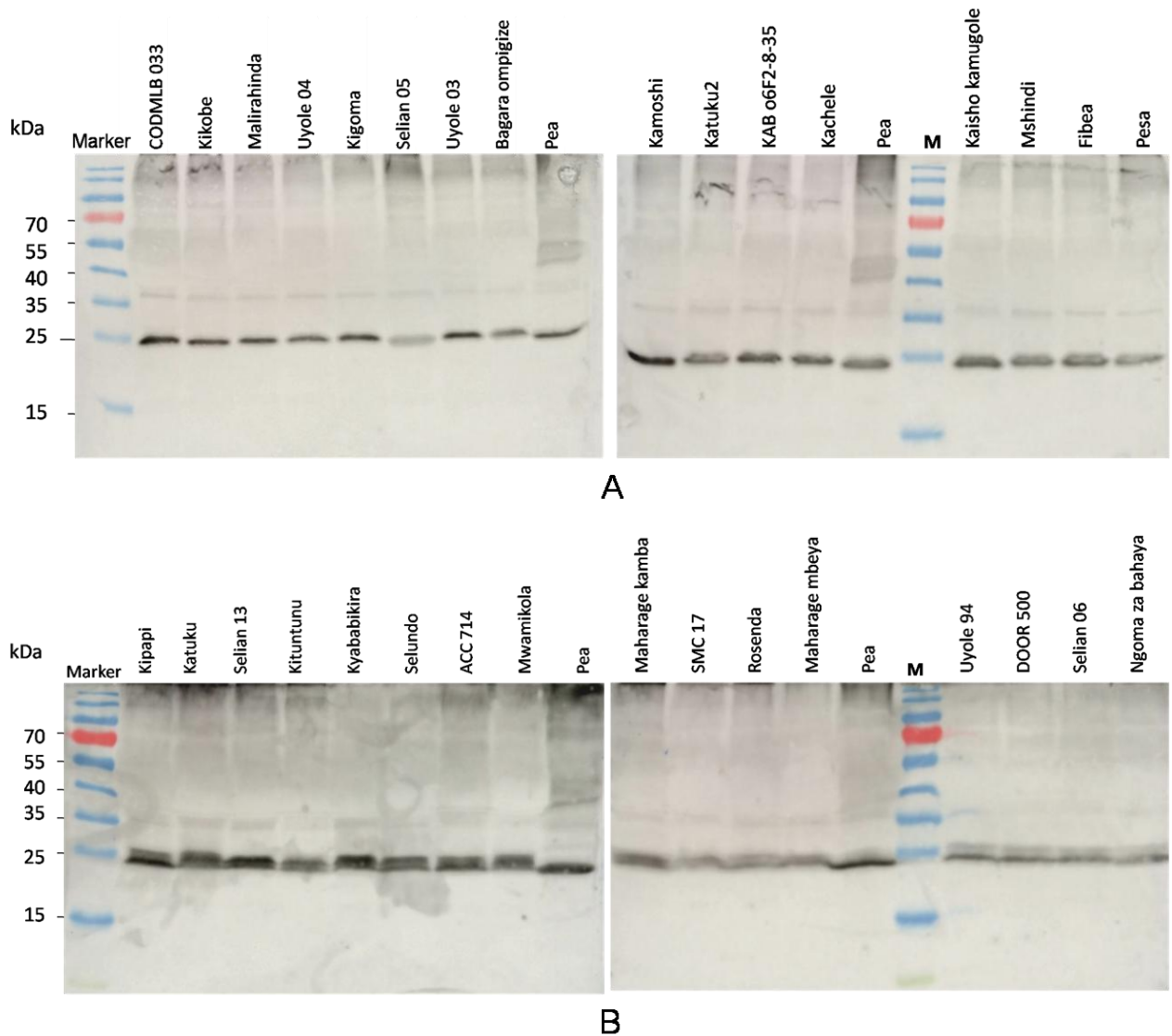
Parameter	Iron (mg/kg)	Magnesium (mg/kg)	Manganese (mg/kg)	Phytic acid (mg/100g)	Phosphorus (mg/kg)	Zinc (mg/kg)
Iron (mg/kg)	1.00					
Magnesium (mg/kg)	0.59***	1.00				
Manganese (mg/kg)	0.93***	0.54***	1.00			
Phytic acid (mg/100g)	-0.05ns	0.01ns	-0.02ns	1.00		
Phosphorus (mg/kg)	0.14ns	0.06ns	0.13ns	0.75***	1.00	
Zinc (mg/kg)	-0.07ns	-0.06ns	-0.06ns	0.08ns	0.11ns	1.00

*** = Significance relationship at $P \leq 0.001$ and ns = non-significance relationship at $P \leq 0.05$

4.1.5 Levels of ferritin protein using Western blot analysis

Ferritin levels among common bean genotypes were detected by western blot analysis as bands with molecular mass of approximately 25 kiloDaltons (kDa) (Fig. 36A - D). The levels of ferritin as immunosignal values of ferritin bands were determined by image J software.

The normalized common bean genotypes ferritin values greatly varied among the genotypes, with a range of 0.04 - 2.93 signal intensity relative to pea (*Pisum sativum*), and a mean of 0.97 signal intensity relative to pea. The highest ferritin levels among the tested bean genotypes were observed for Bilfa 4 (2.93), followed by Kasukari (2.25), Kaempu (2.06), Jabeyila (1.94), and CODMLB 033 (1.82). The lowest ferritin levels were recorded from Maharage Mbeya (0.04), Rosenda (0.21), SMC 17 (0.22), Selian 06 (0.34), and DOOR 500 (0.38) (Fig. 37).



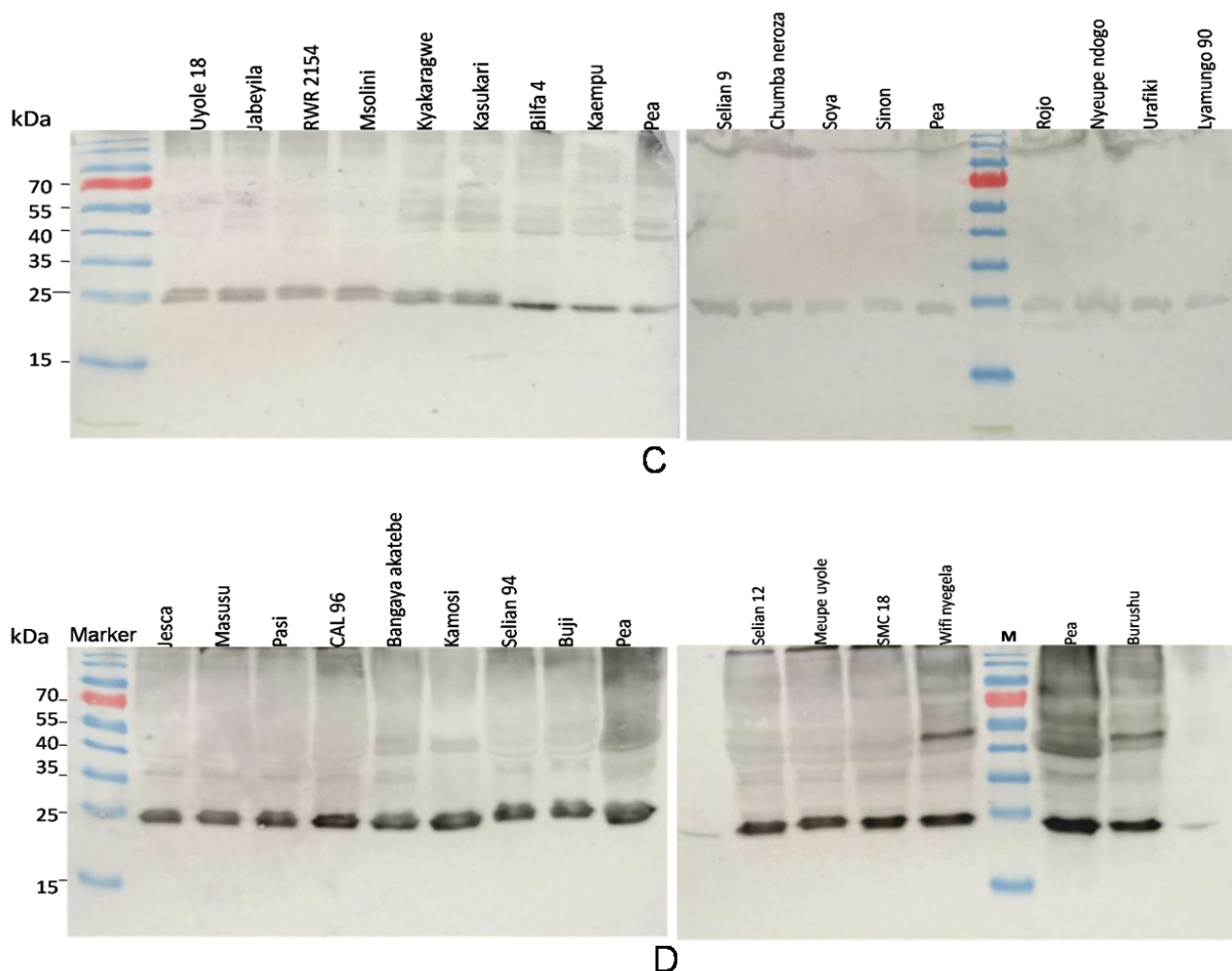


Figure 36: A – D ferritin monomers at 25 kDa as detected by western blot analysis

Pearson correlation analysis (Table 23) revealed that, there was a positive significance ($r = 0.29$, $P \leq 0.001$) correlation between ferritin levels and bean seed manganese, whereas, a weak positive but non-significance ($r \leq 0.29$, $P \leq 0.05$) relationship was observed between bean ferritin levels with seed iron, magnesium and phosphorus. A weak negative but non-significance ($r \leq -0.29$, $P \leq 0.05$) relationship was observed between bean ferritin levels with seed zinc and phytic acid.

Table 23: Relationship between common bean ferritin levels with seed phytic acid and minerals

Parameter	Ferritin	
	Pearson Correlation (r)	P-value
Iron (Fe)	0.13	0.32
Zinc (Zn)	-0.12	0.34
Magnesium (Mg)	0.24	0.06
Manganese (Mn)	0.29	0.03
Phosphorus (P)	0.12	0.35
Phytic acid (PA)	-0.04	0.74

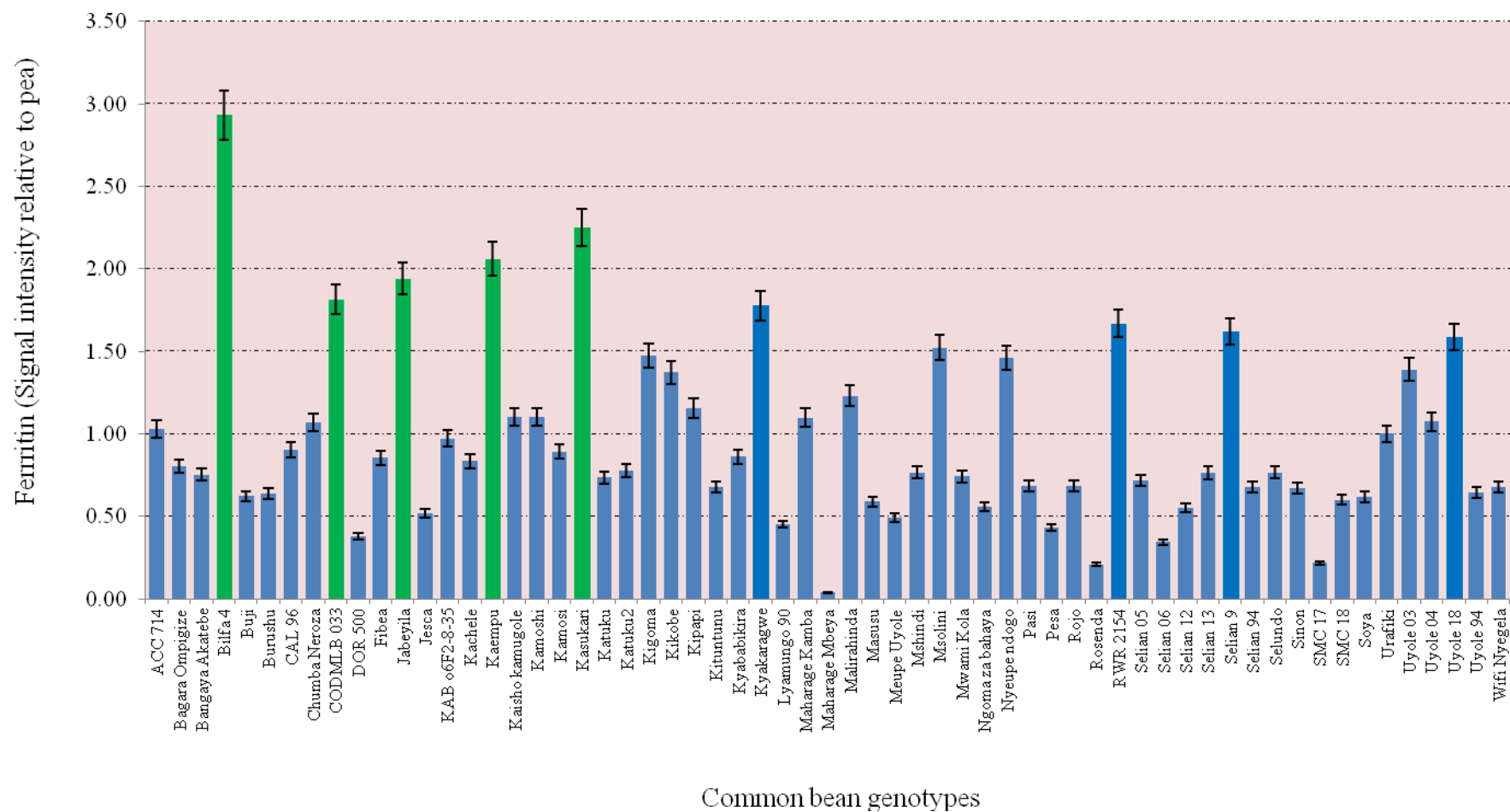


Figure 37: Ferritin levels variations among common bean genotypes grown in Tanzania with those genotypes containing high ferritin levels highlighted in green

4.1.6 F2 population of common bean crosses obtained by crossing widely consumed yellow bean varieties with a high and stable seed iron content cultivar

About 121 flowers from 30 bean plants of a low seed iron and zinc containing genotype (Kigoma) were hand pollinated with pollens from a high seed iron and zinc containing breeding line (CODMLB 033). Forty-nine hand pollinated flowers from 26 plants were successful, and their seeds were later planted as F1. After maturity, the 26 F1 seeds were harvested and advanced to F2 for segregation in seed iron and zinc contents. For the case of Njano Uyole and CODMLB 033 crossing, about 77 flowers from 25 plants of Njano Uyole, a low seed iron and zinc containing variety were hand pollinated with pollens from CODMLB 033. Out of 77 flowers, 15 flowers from 9 plants were successful, and their seeds planted as F1. After maturity, the pods from individual 9 F1 plants were harvested and advanced to F2 for segregation on seed iron and zinc contents.

The results for second filial generation (F2) seed iron, and zinc contents, and percentage increase in these minerals are presented in Table 24. F2 lines developed from crossing Kigoma and CODMLB 033 had a range of seed iron contents from 42.2 to 92.4 mg/kg while zinc contents ranged from 27.3 to 42.3 mg/kg. F2 lines developed from crossing Njano Uyole and CODMLB 033 had a range of seed iron contents from 64.7 to 83.7 mg/kg while zinc contents ranged from 32.1 to 40.1 mg/kg. Seed iron contents increased by 12.5 - 146.4 % in F2 lines developed from Kigoma and CODMLB 033 from a baseline of 37.5 mg/kg in a widely consumed landrace (Kigoma), whereas seed zinc increased by -0.7 – 53.1 % from a baseline of 27.5 mg/kg in Kigoma. The content of seed Fe in F2 lines developed from Njano Uyole and CODMLB 033 increased by 42.5 - 83.7 % whereas seed zinc content had an increase of -2.5 - 53.1 % from seed iron and zinc baseline of 45.4 and 33.0 mg/kg respectively in Njano Uyole variety. For the case of seed color, out of 26 lines developed from Kigoma/CODMLB 033, 17 lines were red mottled, 5 were brown, 3 were yellow, and 1 had orange color. Among 9 lines developed from Njano Uyole/CODMLB 033, 4 lines had red mottled seed color, 3 had orange, and 2 were yellow in seed color.

Table 24: Variations in seed iron, zinc, and percentage increase in seed iron and zinc and 100 seed weight among F2 generation

Genotype	Pedigree	Seed color	Fe (mg/kg)	Zn (mg/kg)	Fe % increase	Zn % increase
BF01	Kigoma/CODMLB 033	Red mottled	88.6 bc	30.0 k-p	136.3	9.1
BF02	Kigoma/CODMLB 033	Red mottled	52.0 mno	29.9 l-p	38.7	8.7
BF03	Kigoma/CODMLB 033	Red mottled	42.2 pq	29.9 k-p	12.5	8.7
BF04	Kigoma/CODMLB 033	Yellow	84.1 cd	42.1 b	124.3	53.1
BF05	Kigoma/CODMLB 033	Yellow	88.5 bc	37.1 c-g	136.0	34.9
BF06	Kigoma/CODMLB 033	Red mottled	92.4 b	40.0 bc	146.4	45.5
BF07	Kigoma/CODMLB 033	Light yellow	52.2 mno	28.8 m-p	39.2	4.7
BF08	Kigoma/CODMLB 033	Red mottled	69.8 f-j	32.1 h-m	86.1	16.7
BF09	Kigoma/CODMLB 033	Red mottled	50.5 no	36.6 c-g	34.7	33.1
BF10	Kigoma/CODMLB 033	Red mottled	79.0 de	39.1 bcd	110.7	42.2
BF11	Kigoma/CODMLB 033	Red mottled	68.0 g-j	31.9 h-n	81.3	16.0
BF12	Kigoma/CODMLB 033	Light brown	68.8 g-j	35.3 d-i	83.5	28.4
BF13	Kigoma/CODMLB 033	Red mottled	71.9 e-i	32.1 h-m	91.7	16.7
BF14	Kigoma/CODMLB 033	Red mottled	58.9 klm	30.0 k-p	57.1	9.1
BF15	Kigoma/CODMLB 033	Brown	65.7 h-k	31.1 i-p	75.2	13.1
BF16	Kigoma/CODMLB 033	Red mottled	71.0 e-i	33.5 g-l	89.3	21.8
BF17	Kigoma/CODMLB 033	Light brown	54.3 mn	34.6 e-j	44.8	25.8
BF18	Kigoma/CODMLB 033	Brown	45.1 op	31.8 i-o	20.3	15.6
BF19	Kigoma/CODMLB 033	Red mottled	49.3 nop	34.3 f-k	31.5	24.7
BF20	Kigoma/CODMLB 033	Red mottled	46.2 op	27.7 nop	23.2	0.7
BF21	Kigoma/CODMLB 033	Red mottled	50.0 nop	27.3 p	33.3	-0.7
BF22	Kigoma/CODMLB 033	Light orange	77.5 def	36.3 c-h	106.7	32.0
BF23	Kigoma/CODMLB 033	Brown	62.2 jkl	35.4 d-i	65.9	28.7
BF24	Kigoma/CODMLB 033	Red mottled	77.7 def	40.4 bc	107.2	46.9
BF25	Kigoma/CODMLB 033	Red mottled	75.7 efg	42.3 b	101.9	53.8
BF26	Kigoma/CODMLB 033	Red mottled	55.1 lmn	30.3 j-p	46.9	10.2
BF27	Njano Uyole/CODMLB 033	Orange	73.2 e-h	32.1 h-m	61.2	-2.7
BF28	Njano Uyole/CODMLB 033	Red mottled	67.7 g-j	38.3 b-f	49.1	16.1
BF29	Njano Uyole/CODMLB 033	Yellow	71.5 e-i	36.9 c-g	57.5	11.8
BF30	Njano Uyole/CODMLB 033	Orange	65.2 h-k	39.0 b-e	43.6	18.2
BF31	Njano Uyole/CODMLB 033	Red mottled	78.6 de	34.1 f-l	73.1	3.3
BF32	Njano Uyole/CODMLB 033	Orange	71.0 e-i	40.1 bc	56.4	21.5
BF33	Njano Uyole/CODMLB 033	Red mottled	75.0 efg	36.8 c-g	65.2	11.5
BF34	Njano Uyole/CODMLB 033	Yellow	64.7 ijk	38.3 b-f	42.5	16.1
BF35	Njano Uyole/CODMLB 033	Red mottled	83.7 cd	38.2 b-f	123.2	38.9
Parents						
CODMLB 033		Red mottled	123.5a	47.1a		
Kigoma		Yellow	37.5q	27.5op		
Njano Uyole		Yellow	45.4op	33.0g-m		
Mean			67.2	34.76		
CV %			5.0	5.3		
LSD (0.05)			6.85	3.72		
P-value			<0.001	<0.001		

Different letters among genotype values = significant differences by Duncan's new multiple range test (DNMRT) ($p \leq 0.05$); BF = Biofortified F2 generation; CV % = Coefficient of variation; LSD = Least significance difference; P-value. = F probability

Analysis of variance revealed highly significance variability ($P \leq 0.001$) among the F2 lines for seed iron and zinc contents. For the F2 lines developed from Kigoma/CODMLB 033, the highest seed iron containing line was BF06 (92.4 mg/kg), followed by BF01 (88.6 mg/kg), BF05 (88.5 mg/kg), BF04 (84.1 mg/kg), and BF10 (79.0 mg/kg). The highest seed zinc containing F2 lines among the lines developed from Kigoma/CODMLB 033 were BF25 (42.3 mg/kg), BF04 (42.1 mg/kg), BF24 (40.4 mg/kg), BF06 (40.0 mg/kg), and BF10 (31.1 mg/kg). For the F2 lines developed from Njano uyole/CODMLB 033, the highest seed iron containing line was BF35 (83.7 mg/kg), followed by BF31 (78.6 mg/kg), BF33 (75.0 mg/kg), BF27 (73.2 mg/kg), and BF29 (71.5 mg/kg). The highest seed zinc containing F2 lines among the lines developed from Njano Uyole/CODMLB 033 were BF32 (40.1 mg/kg), BF30 (39.0 mg/kg), BF28 (38.3 mg/kg), BF34 (38.3 mg/kg), and BF35 (38.2 mg/kg).

A strong positive and significant ($r = 0.68$, $P \leq 0.001$) relationship between F2 lines on seed iron and seed zinc content was observed (Table 25).

Table 25: Association between F2 generation seed iron and zinc contents

Parameter	Seed iron (Fe) (mg/kg)	
	Pearson Correlation (r)	P-value
Seed Zinc (Zn) (mg/kg)	0.68	<0.001

4.2 Discussion

4.2.1 Seed yield and yield components of common bean genotypes grown in Tanzania

This study reported that seed yield and yield components of common bean genotypes were strongly influenced by the genetic makeup, environmental conditions of the sites, and their interactions. The influence of genotype, environment, and genotype by environment interaction has been also reported by other studies (Barili *et al.*, 2015). Common bean genotypes particularly the landraces which were high yielding in specific sites can be used for improving varieties specific for locations where they have performed better. The highest seed yield was recorded at TARI-Selian followed by TARI-Uyole and lastly SUA-Morogoro, this may have been caused by well-distributed rainfall and soil properties. The high variations in the performance of common bean genotypes within location form the basis for selection on the respective bean traits (Acquaah, 2013).

The study has found that common bean genotypes largely determine seed yield (39.3%) compared to environmental conditions that accounted for 31.4 % of the variation in seed yield. This indicated that the genotypes and experimental sites used were diverse and good for specific and general genotype adaptability studies. Similarly, Horn *et al.* (2017), determined a large contribution of cowpeas genotypes (38.0%) in seed yield compared to environmental effects (5.0%), and (Balakrishnan *et al.*, 2016) reported 41.3 % genotype main effect on rice seed yield compared to the environmental main effect (31.9%). In contrast to this study, Tadesse *et al.* (2018) reported a larger contribution of environmental effect (78.2%) compared to the genotype main effect (6.5%). The difference in genotype main effect reported by this study may be due to a difference in the number of common bean genotypes and location used, whereby the current study used 99 diverse bean genotypes while (Tadesse *et al.*, 2018) used 14 all white bean genotypes. Due to nearly equal environmental influence and genotype main effect on seed yield, this trait selection needs to be done in several environments to have a genotype that can be grown across several agro-ecological zones and perform more or less the same. Days to 75% flowering, number of pods/plant, number of seeds/pod, and 100 seed weight were observed to be largely influenced by genotypes than environment and genotype by environment interaction, thus these traits are easy to select and breed for compared to seed yield.

There are several adaptabilities and stability analysis procedures that are used by plant breeders in the selection of plant genotypes that performs more or less similar across environments (Barili *et al.*, 2015; Horn *et al.*, 2017). Additive main effects and multiplicative interaction (AMMI) stability value (ASV) is one of the modern methods used for the identification and selection of plant genotypes that are stable across environments. Plant genotypes with low ASV closer to zero are thought to be more stable whereas those with great values are influenced by environmental effects (Horn *et al.*, 2017). Some of the bean genotypes that were ranked as stable by ASV had very low yield, this is because stability doesn't care about high or low yielding genotypes (Rono *et al.*, 2016). Thus yield stability index (YSI) was used to identify high seed yielding and stable bean genotypes, as it combines both stability and high yielding traits into a single index, that is used in the selection of genotypes (Adjebeng-danquah *et al.*, 2017; Milioli *et al.*, 2018). Genotypes with lower YSI are more useful as they have high mean yield and stability traits (Bose *et al.*, 2014). A number of high seed yield and stable common bean genotypes were identified in this study based on YSI.

The concentric circles help in the visualization of the ideal experimental site, which has both high discriminating ability of superior genotypes and representativeness of the experimental sites (Tena *et al.*, 2019). Experimental site E1 (TARI-Selian), has both the high discriminating ability of superior common bean genotypes and representativeness of other experimental sites, thus it is an ideal site for a selection of the widely adapted common bean genotypes, as this site provided more information on seed yield performance of the tested genotypes. The experiment can be further conducted into other sites to provide more information on this, as this was a one-season field experiment. Mare *et al.* (2017) used GGE biplot to determine the discriminating power and representativeness of the experimental sites on sorghum genotypes yield.

The influence of individual soil properties on common bean performance indicated a strong positive effect of available soil phosphorus on seed yield and number of pods per plant also moderate 100 seed weight. Thus available soil phosphorus was the most important soil-plant nutrient to increase bean productivity and therefore needs to be considered carefully when growing beans. Seed yield at TARI-Selian which had optimum available soil phosphorus level were higher compared to TARI-Uyole and SUA which had low soil available phosphorus. The phosphorus influence and limiting factor for common bean seed yield was also been reported (Mourice & Tryphone, 2012). Soil available phosphorus enhances the ability of root nodules to fix atmospheric nitrogen and increases the number of pods/plant, seed weight and eventually seed yield (Mourice & Tryphone, 2012). Soil available phosphorus is an important element to consider when producing common beans for increased seed yield of the beans.

Total soil nitrogen and soil organic carbon influenced common bean seed yield negatively compared to the study of Chekanai *et al.* (2018), where soil organic carbon and nitrogen influenced seed yield in common bean positively. The negative influence of total soil nitrogen on common bean seed yield and its components may be due to low rainfall at SUA which recorded higher total soil nitrogen compared to other sites. Nie *et al.* (2017) reported that, total soil nitrogen availability is positively influenced by precipitation, thus the availability of the measured total high soil nitrogen at SUA prior-planting may have been decreased by low rainfall during bean growing season. In addition, atmospheric nitrogen fixation into soils is negatively influenced by low soil moisture (rainfall) and thus making the fixed nitrogen by the rhizobia low and thus low absorption by the bean plants, leading to low

yield (Reinprecht *et al.*, 2020). Tamagno *et al.* (2018) reported reduction in yield due to low biological nitrogen fixation as a result of reduced photosynthesis. In all sites, soil organic carbon was optimum, though it was higher at SUA, compared to the other sites, therefore its influence on bean seed yield maybe it is the function of other soil and weather parameters. Availability of soil organic carbon has been reported as a function of soil moisture (Zhou *et al.*, 2020), thus lower rainfall during plant growth, results into low soil organic carbon availability. Furthermore, Lei *et al.* (2019) reported positive influence of soil available phosphorus and exchangeable potassium on availability of soil organic carbon. Sokoine University of Agriculture (SUA) had lower available soil phosphorus and exchangeable potassium compared to the other sites, thus this condition may have caused low soil organic carbon available to bean plants and thus unexpected negative soil carbon with seed yield. In all experimental sites, soil exchangeable potassium, magnesium, and sodium were adequate for common bean growth, and the highest levels of these were recorded at TARI-Selian, whereas soil exchangeable calcium was adequate and highest at TARI-Selian and low in other sites. All the measured exchangeable bases were positively and strongly correlated with seed yield.

4.2.2 Seed iron and zinc contents of common bean genotypes grown in Tanzania

This study has found great variations in seed iron and zinc contents among the common bean genotypes in all the three experimental sites. Similarly, Blair *et al.* (2009) reported variations in seed iron and zinc contents among 87 recombinant inbred lines, while Yeken and Akpolat (2018) reported variations in seed iron and zinc contents for 22 random population common bean genotypes. The variation in seed iron and zinc contents among common bean genotypes, forms basis for high seed iron and zinc contents selection and biofortification of the widely consumed bean varieties in the country. Due to differences in environmental conditions such as soil properties and rainfall among the experimental sites, TARI-Selian recorded the highest mean seed iron compared to the other two sites, while SUA recorded the lowest mean seed iron. Variation of mean seed iron and zinc had also been reported in the US (Zacharias *et al.*, 2018), while Phuke *et al.* (2017) found variations in mean seed iron and zinc content of sorghum due to different environmental conditions. The highest seed iron and zinc containing common bean genotypes at each experimental site, can be used for specific environmental breeding purposes, as they are well adapted to that specific environment (Caligari & Forster, 2015).

The concentration of iron and zinc in common bean seeds were influenced by genotypes, environmental conditions and their interactions, similarly Bohn *et al.* (2004) and Chekanai *et al.* (2018) reported influence of the main effects of genotypes, environment and their interaction on common bean seed iron and zinc. The variation in the influence of genotypes, environments and interactions of the common bean genotypes with environments in accumulating iron and zinc into seeds, gives a room for conducting selection. In this study, common bean seed iron content variations was more influenced by genotypes (69.5 %) compared to environmental conditions (1.7 %), while seed zinc concentration was more influenced by environmental conditions (39.7 %) than genotypic effect (28.6 %). Thus, selection of high seed iron-containing bean genotypes is not much complicated exercise compared to high seed zinc containing genotypes. It is also easy to improve seed zinc than iron contents by modifying the growing environmental conditions. Genotype by environment interaction on seed iron (26.3 %) and zinc (28.6 %) indicated that, the experimental sites were diverse and thus influenced differently the rankings of bean genotypes in terms of seed iron and zinc contents.

Genotype by environmental interaction complicated selection of high seed iron and zinc containing bean genotypes, thus the use of AMMI stability value (ASV) and genotype stability index (GSI) in selecting stable and high seed iron-containing bean genotypes is recommended. Based on ASV, bean genotypes with low ASV, are selected as stable genotypes and those with high are considered unstable (Bocianowski *et al.*, 2019). The most five stable bean genotype by ASV in terms of seed iron contents with exception of SMC 18, the rest had low seed iron contents. Therefore selecting stable genotypes based on ASV alone would result in selecting even those with low seed iron or zinc contents only if they had consistently more or less similar mineral contents across sites (Adjebeng-danquah *et al.*, 2017). The use of GSI is emphasized as it combines both stability and high performance of the genotypes into a single index (Akter *et al.*, 2014). The GSI adds the genotype ranking through ASV to that of its mean performance. Genotypes with lowest GSI are ranked most stable and high performance for the trait evaluated (Bose *et al.*, 2014). Based on GSI, SMC 18 was ranked as the most stable and high seed iron-containing genotype, followed by KAB o6F2-8-35, Chumba neroza, Selian 94, Urafiki, Kyakaragwe, Kaempu, CODMLB 033, Kikobe, Malirahinda, ACC 714, Jabeyila, Mwamikola, Kasukari, Wifi nyegela and Selundo. Stable and high seed zinc-containing bean genotypes were Uyole 96 followed by CODMLB

033, Soya, Kaisho kamugole, Kitebe, Tema, Kibugu, Kaempu, Kashule, Maharage Mbeya, Soya Mbeya others were KAB o6F2-8-35, SUA 90, Kamoshi, Jabeyila and SMC 18.

Genotype and genotype by environment interaction biplot analysis, enable visualization of the genotypes and environments association through biplot and show genotypes adaptability and stability across environments (Ukalski & Klisz, 2016). An ideal genotype according to average-environment axis (AEA) is the one found on longest vector of the AEA towards the direction of high performance and at the center of the first concentric circle (Horn *et al.*, 2017). The ideal genotype for seed iron contents should have high and more or less similar seed iron content across experimental sites. It is difficult to have such a genotype. Thus concentric circles surrounding the ideal genotypes are normally drawn to help visualize genotypes closer to the ideal genotype. Thirteen bean genotypes were observed to be stable and high seed iron-containing. Among them, one bean genotype G 48 (Malirahinda) was found closer to the center of the first concentric circle and thus it was the ideal genotype followed by other desirable 12 bean genotypes which were placed within the next concentric circles of the GGE biplot. The use of GGE biplot analysis enabled selection of fastest cooking bean genotypes from several varieties tested (Cichy *et al.*, 2019). The variation in seed iron and or zinc contents among common bean genotypes can be used to select those with high in these minerals and use them in breeding programs for improving these traits through genetic iron or zinc biofortification.

Total rainfall during bean growth to physiological maturity significantly influenced positively the concentration of iron in common bean seed, while mean temperature significantly negatively influenced bean seed iron contents. Thus bean genotypes grown in drought prone or during intermittent or terminal drought condition will have less seed iron contents compared to those grown in areas or season with adequate and well distributed rainfall. It is important to select high seed iron containing for drought prone areas. Climate change, through reduced rainfall and global warming will have negative influence on bean seed iron content. Similarly, Ghanbari *et al.* (2013) and Hummel *et al.* (2018) reported negative influence by drought on seed iron contents in beans, while (Smith *et al.*, 2019) reported negative influence of drought to some genotypes and no effect to most of the tested bean genotypes on seed iron contents due to drought. Seed zinc contents was negatively influenced by total amount of rainfall received during growth to physiological maturity, indicating that,

zinc concentration in common beans increases with increase in drought. Similarly, Pereira *et al.* (2014) reported increase of zinc concentration in bean seeds due to drought.

Soil available phosphorus, exchangeable potassium and iron, significantly and positively influenced the concentration of seed iron, whereby seed zinc concentration in beans was negatively influenced by these minerals. The increase in seed iron content may be due to the function of soil P in root growth and development and promoting nutrient uptake and seed production in legumes (Mitran *et al.*, 2018). The concentration of Fe in bean seeds was negatively affected by soil total nitrogen and organic carbon, while these soil parameters had no effect on seed zinc contents, this may be due to carbon fixation by photosynthesis in leaves resulted from high nitrogen uptake from the soils. No effect to reduction of rice grain iron and zinc contents due to increased soil nitrogen was also reported Handel *et al.* (2010) and Gu *et al.* (2015). The positive and significant association between soil pH and bean seed iron content, may be due to fact that soil pH at slightly acidic to slightly basic (6.5 – 7.5) is more favorable to plant root growth and available soil phosphorus which enhance nutrient uptake from the soils, the soil pH of the experimental sites ranged from 5.8 to 7.2 (McCauley *et al.*, 2017).

The environmental conditions may be favorable for zinc uptake from the soils into the plant and within different parts of the plant, but uptake may be challenged by competition from other positively charged divalent ion for example iron. In some cases ion transporters can transport more than one mineral ion, for example iron-regulated transporter (IRT) and nicotianamine (NA), transports Fe, Mn, Cu and Zn (Bindraban *et al.*, 2015; Connorton *et al.*, 2017). Thus some soils or weather parameters which would have been expected to influence positively bean seed iron or zinc contents could negatively influence.

4.2.3 Phytic acid, P, Mg and, Mn among common bean genotypes grown in Tanzania

The study determined how bean genotypes varied in seed phytic acid (PA) (711.8 - 1914.6 mg/100 g). The findings of this study on phytic acid supports the previous study which reported significance different in phytic acid among the bean genotypes (Guzman-Maldonado *et al.*, 2000). The range of PA obtained by this study is in correspondence with those reported by Petry *et al.* (2013) 400 – 2600 mg/100 g and Golam *et al.* (2011) 125.2 – 3164.2 mg/100g. Understanding the contents of seed phytic acid among bean genotypes is of much importance as it inhibits absorption of divalent minerals in the human gut and the inhibition is dose

dependent. A study conducted by Han *et al.* (2019) showed that, the increase in phytic acid content varied inversely proportion with mineral absorption in intestinal. The large variation in seed phytic acid contents among the tested bean genotypes allows selection of genotypes with lower grain PA contents for use in improving the common bean seed quality particularly mineral bioavailability in human gut.

The finding of this study demonstrated that, bean seed phosphorus (P), magnesium (Mg), and manganese varied significantly among the genotypes. The current study supports the previous studies on these minerals, which found significance variation among these minerals in common bean seeds (Golam *et al.*, 2011; Silva *et al.*, 2012). In humans, phosphorus is important for growth and repair of body cells and tissues, magnesium is used as a cofactor in many enzymes that are involved in different body biochemical reactions, while manganese is used in bone formation and for amino acids, cholesterol, and carbohydrates metabolism (WHO & FAO, 2004; Soetan *et al.*, 2010). The levels of phosphorus (3307.0 - 7037.0 mg/kg), magnesium (1511.4 - 2503.0 mg/kg) and manganese 24.32 - 97.73 mg/kg obtained in the present study corresponds with the P contents reported by Beebe *et al.* (2009) phosphorus seed content of up to 7095 mg/kg with mean of 3684 mg/kg, Mg (1057.52 – 2654.18 mg/kg) and Mn (12.16 – 96.54 mg/kg) seed content reported by Rasool *et al.* (2019). Common bean is an important source of essential minerals and it is among crops with high contents of seed P, Mg and Mn. Thus knowing seed concentration of these essential minerals among the common bean genotypes grown in Tanzania is of much importance for improvement of these minerals in widely consumed varieties.

A genetic variation in phytic acid to mineral molar ratios were observed among common bean genotypes in the present study and thus corresponds with other studies by Guzman-Maldonado *et al.* (2000), which reported variation in phytic acid to mineral molar ratios among common bean genotypes. Mineral bioavailability in human gut is much influenced by phytic acid to mineral ration, as it increases with decrease in phytic acid to mineral molar ratio. Therefore phytic acid to mineral ratio predicts mineral bioavailability in human gut (Hoppler *et al.*, 2014). In the present study all of the tested common bean genotypes had phytic acid to mineral molar ratio above the critical values recommended for good mineral (Fe, Zn, Mg, and Mn) absorption, likewise most of the studies (Guzman-Maldonado *et al.*, 2000; Aparecida *et al.*, 2011; Teshome & Emire, 2012) obtained higher molar ratio values than the recommended values. This demonstrates that, there is negative influence of the

phytic acid in mineral (Fe, Zn, Mg, and Mn) absorption from the tested bean genotypes, though the influence varies inversely proportional with the phytic acid to mineral (Fe, Zn, Mg, and Mn) molar ratios. The recommended levels of phytic acid to iron and zinc molar ratios suitable for absorption of iron and zinc in human gut are 1.0 and 15.0 respectively (Hailu & Addis, 2016; FAO/IZiNCG, 2018). For magnesium absorption in human gut (Bohn *et al.*, 2004) reported absorption of 38 % at phytic acid to magnesium molar ratio of 0.4. Due to variation in phytic acid to mineral (Fe, Zn, Mg, and Mn) molar ratios it is possible to select genotypes with low molar ratios for use in common bean breeding programs to increase bioavailability of these minerals (Fe, Zn, Mg, and Mn).

The present study found positive relationships between common bean seed iron, magnesium and manganese and between phytic acid and phosphorus. Similarly, Paredes *et al.* (2009) reported positive and significance association between iron and manganese and a positive relationship between magnesium and manganese in common bean seeds, whereas a non-significance relationship between phosphorus with iron, magnesium and manganese was reported like in the present study. The similar results on iron and zinc non significance relationship was reported by Golam *et al.* (2011), while Celmeli *et al.* (2018) reported a strong and positive relationship between seed iron and zinc contents of common beans. The different relationship between seed iron and zinc obtained may have been caused by the number of genotypes used in study, as the present study used 61 genotypes versus 15 genotypes that were used by Celmeli *et al.* (2018). No significance relationship between phytic acid and minerals (Fe, Zn, Mg, and Mn) explains that, high seed mineral (Fe, Zn, Mg, and Mn) with low phytic acid seed contents bean genotypes can be selected.

4.2.4 Ferritin levels of the common bean genotypes grown in Tanzania

The presence of ferritin protein in the tested bean genotypes were confirmed by using western blot analysis procedures, in line (Cvitanich *et al.*, 2010) used western blotting technique to confirm the presence of ferritin protein in beans. Common bean seed iron bioavailability is enhanced by levels of ferritin protein, which is localized in amyloplasts of mature seeds. It is the major iron storing protein in seeds, whereas inside ferritin protein core, iron is stored in the form of insoluble Fe^{3+} state and released as soluble hydrated Fe^{2+} when in need (Marinova & Vladimirova, 2009). A great genetic variability in ferritin levels among bean genotypes grown in Tanzania had been demonstrated by this study, similarly, Lukac *et al.* (2009) reported variation in common bean ferritin concentration among bean genotypes. It is

important to determine the levels of ferritin in beans, as 13 – 42 % of total seed iron in beans is bound to ferritin (Cvitanich *et al.*, 2010; Hoppler *et al.*, 2014). Variation in seed ferritin among the tested genotypes, gives a room for improvement of this important traits in beans. The genotypes identified to contain high levels of ferritin can be good candidates for use in iron biofortification, as the iron bound to ferritin is readily available for absorption in human gut. Ferritin bound iron is reported to strongly positively related to ferritin levels (Kate1 *et al.*, 1997), thus genotypes found with high ferritin levels can be having high ferritin bound iron compared to those with low levels of ferritin.

In this study, common bean ferritin levels were positively related to total seed iron, thus it is possible to select a genotype with high ferritin levels and total seed iron. Hoppler *et al.* (2014) reported a positive relationship between ferritin bound iron and total seed iron in common beans. A negative association between phytic acid content and ferritin levels was reported by this study, which means that, genotypes with high levels of ferritin in most cases have low phytic acid and thus can be used in common bean seed iron biofortification for improved iron bioavailability in human gut.

4.2.5 Common bean F2 lines seed iron and zinc contents

Reffering to hybridization results, the rate of successful hybridization for Kigoma and CODMLB 033 crossing was 40.5 %, whereas the success in Njano Uyole and CODMLB 033 crossing was 19.5 %. The rate of successful in hand pollination revealed by this study is comparable to hand pollination success of 2.3 – 22.5 % in beans reported by Bhanu *et al.* (2018). Similarly, Merin *et al.* (2019) reported rate of successful in bean hand pollination of 23.8 %, while Vollmann *et al.* (1992) reported rate of successful in hand pollination of beans below 18 %. The low pod setting of the hand pollinated flowers in beans is reported to be due to failure of the pollen tubes to penetrate the stigma and in some cases, fertilization occurs, but embryo abortion takes place due high temperature above 23 °C, genetic response of the parents and relative humidity below 45 or higher than 55 (Bhanu *et al.*, 2018). In Arusha, the temperature and relative humidity in January and February, during when the crosses were made are higher than the favorable temperature and relative humidity for pod setting for intra and inter specific crosses in beans. This might have contributed to lower success in pod setting of the hand pollinated flowers in this study. Thus for a high rate of success in hand pollination in beans, it is better to conduct in May to August, during when the temperatures are low and use many plants.

A large variation in seed iron (45.1 - 92.4 mg/kg) and zinc (27.7 - 53.1 mg/kg) contents among the developed F2 lines was determined by this study. The findings of this study is in line with the previous study which reported significance different in seed iron (30 - 130 mg/kg) and zinc (10 - 60 mg/kg) contents among the bean F2 lines (Kimani & Warsame, 2019). A study by Blair *et al.* (2011) reported significance variation in common bean seed iron (28.0 – 95.1 mg/kg) and zinc (16.9 – 56.8 mg/kg) contents for recombinant inbred line (RIL) population developed from high iron (98.0 mg/kg) and zinc (41.2 mg/kg) containing genotype (G21242) with low iron (35.0 mg/kg) and zinc (27.3 mg/kg) containing genotype (G21078). Seventeen F2 lines out of 35 developed in this study had higher seed iron content ≥ 70 mg/kg and zinc content ≥ 30 mg/kg that includes, BF01, BF04, BF05, BF06, BF08, BF10, BF13, BF16, BF22, BF24, BF25, BF27, BF29, BF31, BF32, BF33, and BF35 will be advanced to further generation for more selection aimed at developing a yellow bean variety high in both seed iron and zinc contents.

The percentages increase in F2 lines seed iron contents of up to 146.4 %, and zinc content up to 53.1 % attained in this study are comparable with those attained by Blair *et al.* (2011), who reported an increase of up to 180 % in common bean seed iron content and 89.0 % increase in seed zinc content. Likewise, Zemolin *et al.* (2016) reported increase in F2 lines seed iron content in common bean by 109.8 % and seed zinc content by 121.0 % compared to the low mineral containing parents (Kigoma and Njano Uyole). Increase in seed iron and zinc contents by 3-folds and zinc content by 2-folds through artificial hybridization, explains that, it is possible to increase mineral contents in widely consumed common bean varieties using conventional breeding, if the parents have contrasting seed mineral contents. This study also demonstrated that, there was a significant and positive association between seed iron and zinc contents of the F2 lines. Similarly, Amongi *et al.* (2018) and Giuberti *et al.* (2019) reported a significant and positive relationship between seed iron and zinc contents in common bean lines. Positive and significant relationship between bean seed iron and zinc contents particularly in a segregating population from two parents indicates that, the two minerals co-segregate and thus there is high chance of selecting a genotype with high seed iron and zinc contents and a possibility of identifying loci that control both high seed iron and zinc contents.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study determined highly significant effects of bean genotypes, environments, and genotype by environment interaction in seed yield and yield related traits. However, the study was able to identify high and stable seed yielding genotypes across test locations that could produce more or less similar seed yield quantity across locations, these includes ACC 714, Selian 14, Selian 9, Katuku and Msolini.

In terms of yield related traits the present study identified genotypes with highest number of pods/plant across test locations, these includes Cheupe, Ruondera, Kikobe, Wifi nyegela, and Jabeyila. Genotypes with highest number of seeds/pod identified included Kaempu, Cheupe, Malirahinda, Kamosi, and Wifi nyegela. Moreover, bean genotypes with highest 100 seed weight across locations were identified, these includes Selian 15, Lyamungo 90, Msolini, Fibea, and Lyamungo 85. The identified bean genotypes high in yield related traits can be used in improving these traits in beans.

The study also revealed highly significant effects of bean genotypes, environments, and genotype by environment interaction for both seed iron and zinc contents. Seed iron content variations was more influenced by genotypes (69.5 %) compared to environmental conditions (1.7 %), while seed zinc concentration was more influenced by environmental conditions (39.7 %) than genotypic effect (28.6 %), thus agronomic biofortification can be more effective in seed zinc increase compared to seed iron increase in common beans. Furthermore, the study identified the most stable and high seed iron-containing genotypes that includes SMC 18, KAB o6F2-8-35, Chumba neroza, Selian 94, and Urafiki. The most stable and high seed zinc-containing genotypes identified were Uyole 96, CODMLB 033, Soya, Kaisho kamugole, and Kitebe. The identified stable and high seed mineral-containing bean genotypes can be grown in different agro-ecologies producing more or less similar amount of seed iron and or zinc contents.

The study identified common bean genotypes with low PA, which includes Fibea, Selian 06, Maharage kamba, DOOR 500, and katuku2. The study also identified high Mg-containing

genotypes that include Bilfa 4, Kikobe, ACC 714, Malirahinda, and Jabeyila, whereas ACC 714, Bilfa 4, Kikobe, Malirahinda, Selian 94, were identified as high seed Mn-containing genotypes. Furthermore, the study identified, genotypes containing low PA to Fe molar ratio that include Maharage kamba, Malirahinda, ACC 714, Kikobe, and Jabeyila. Those with low PA to Zn molar ratio include Fibea, Nyeupe ndogo, Soya, Kikobe, and DOOR 500. Regarding PA to Mg molar ratio, the genotypes DOOR 500, Katuku2, Maharage kamba, Selian 06 and Kikobe had the lowest values. The lowest PA to Mn was recorded from the following bean genotypes; Maharage kamba, ACC 714, Malirahinda, and Kikobe.

This study identified bean genotype with high levels of ferritin, which include Bilfa 4, Kasukari, Kaempu, Jabeyila, CODMLB 033. This study revealed a non-significance association between ferritin and total seed iron contents. Thus there is low possibility of selecting bean genotypes with high in total seed iron and ferritin, this suggests that, high seed iron and high ferritin levels in common bean seeds can be combined in one genotype through breeding.

The present study has developed common bean F2 lines from widely consumed yellow bean varieties with increased seed iron and zinc contents. Among the developed F2 lines, seventeen F2 lines were identified containing high in both seed iron content ≥ 70 mg/kg and zinc content ≥ 30 mg/kg these include BF01, BF04, BF05, BF06, BF08, BF10, BF13, BF16, BF22, BF24, BF25, BF27, BF29, BF31, BF32, BF33, and BF35. The F2 generation with high in both seed iron and zinc contents will be advanced into further generations for more selection aimed at developing a yellow bean variety high in these essential minerals

5.2 Recommendations

- (i) The bean genotypes (those not yet released) with high and stable seed iron and zinc contents and those with high seed yield across locations are recommended for further testing in many more other bean-growing areas involving farmers and other common bean stakeholders for participatory variety selection, recommendation, and release. The genotypes are also recommended for different breeding purposes in different agro-ecologies of Tanzania.
- (ii) The F2 lines developed in this study are recommended for quantification of PA and ferritin levels for QTL analysis and identification of molecular markers linked to these traits.

- (iii) The F2 lines could be forwarded to develop recombinant inbredlines for identification of lines with high seed iron and zinc contents using molecular markers.
- (iv) There is a need of using modern molecular techniques like Genome Wide Association Study (GWAS) in studying genetic variability underlying Fe, Zn, PA, P, Mg, Mn, ferritin contents, and PA to mineral (Fe, Zn, Mg, Mn) molar ratios in common bean as it is the current powerful tool in identification of candidate genes associated with traits of interest.
- (v) There is a need of determining the content of common bean seed PA, P, Mg and Mn in a multilocation trial so as to understand the influence of genotypes, environments and genotype by environment interaction on the levels of these traits.
- (vi) Since this study determined the levels of ferritin in common beans grown in Tanzania, there is a need for quantification of the concentration of iron loaded in ferritin in these genotypes though it is reported to be positively and significantly related to the levels of ferritin.
- (vii) The technique used by this study in improving common bean mineral (Fe and Zn) contents is recommended for improving these minerals in other important crops grown in Tanzania for improved human iron and zinc intake. PA and Ferritin determination need to be done in other crops grown in Tanzania for improved bioavailability of iron and zinc in human gastrointestinal tract.
- (viii) There is a need of conducting zinc agronomic biofortification using identified common bean genotypes with high and low seed iron and zinc contents so as to see the mineral amount increase and the cost of agronomic biofortification using both mineral and foliar fertilizers.
- (ix) There is a need of doing cost effective study on genetic iron and zinc biofortification against food fortification and supplementation in Tanzania, since most of these study have been done in Asia and South America

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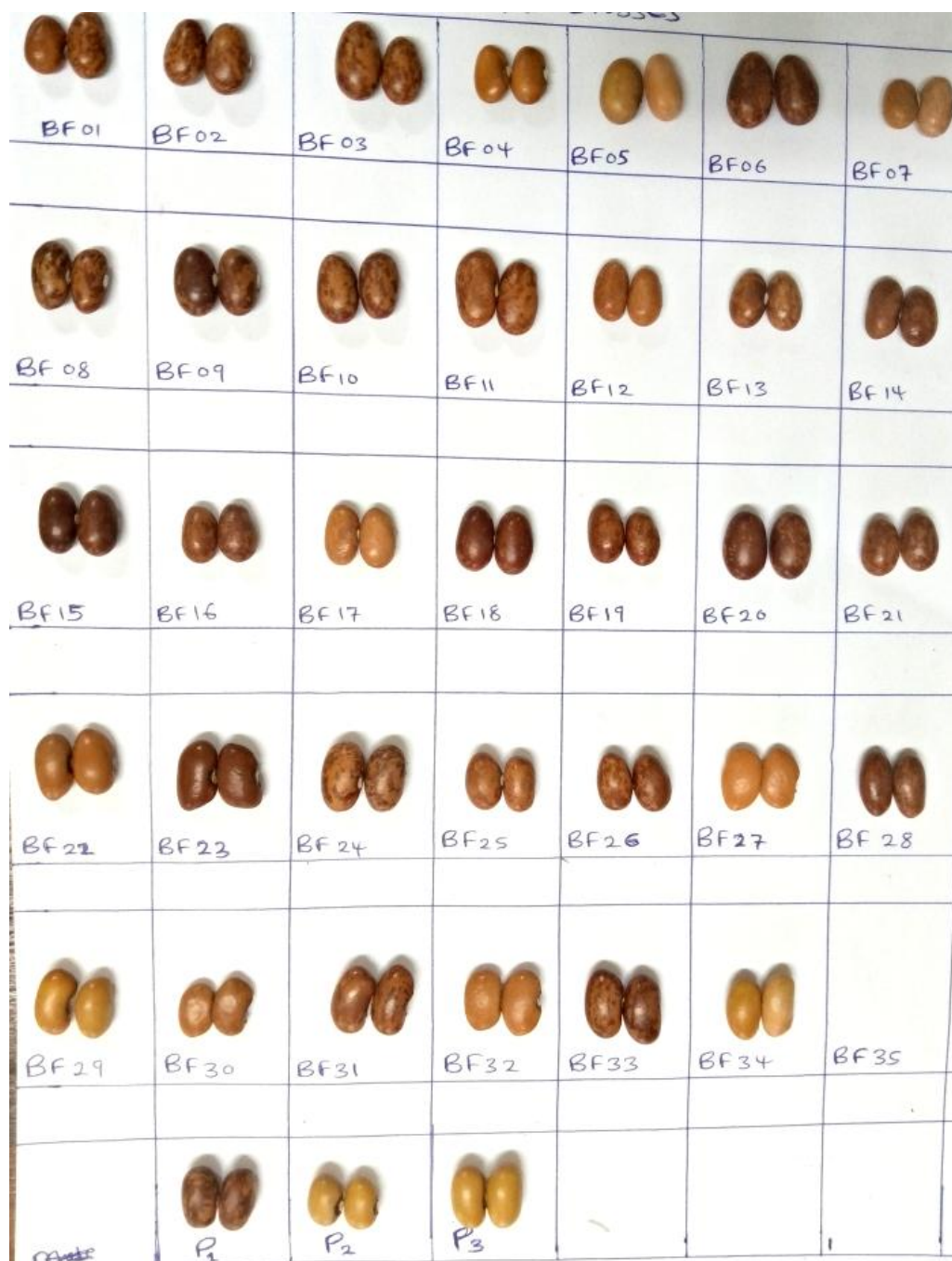
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APPENDICES

Appendix 1: F2 lines seeds physical appearance

The developed F2 lines varied in physical appearance in terms of seed shape, size and seed color, most of the F2 lines had red mottled seed color, whereas few had yellow, light yellow, brown, light brown and orange (Appendix 1).



RESEARCH OUTPUTS

Published Articles

- Philipo, M., Ndakidemi, P. A., & Mbega, E. R. (2020). Environmental and genotypes influence on seed iron and zinc levels of landraces and improved varieties of common bean (*Phaseolus vulgaris* L.) in Tanzania. *Ecological Genetics and Genomics*, 15, 1-12.
- Philipo, M., Ndakidemi, P. A., & Mbega, E. R. (2021). Environmentally stable common bean genotypes for production in different agro-ecological zones of Tanzania. *Heliyon*, 7(1), 1-12.
- Philipo, M., Ndakidemi, P. A., & Mbega, E. R. (2020). Multilocation dataset on seed Fe and Zn contents of bean (*Phaseolus vulgaris* L.) genotypes grown in Tanzania. *Data in Brief*, 31, 1-12.
- Philipo, M., Ndakidemi, P. A., & Mbega, E. R. (2021). Importance of common bean genetic zinc biofortification in alleviating human zinc deficiency in sub-Saharan Africa. *Cogent Food and Agriculture*, 7(1), 1-22.

Poster Presentation

Research output 2: High seed iron and zinc-containing F2 generation

The current study has developed 17 common bean F2 crosses high in both seed iron ≥ 70 mg/kg and zin ≥ 30 mg/kg through crossing widely consumed yellow bean varieties with high seed iron and zinc-containing genotype.