PARTICIPATORY BREEDING, ASSOCIATION MAPPING AND INHERITANCE OF IRON AND ZINC ACCUMULATION IN COMMON BEAN (*Phaseolus vulgaris* L.)

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DECLARATION

This thesis is my original project and has not been presented for award of a degree in any other University or institution of higher learning.

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To my children Ariane Kataliko Adel and Victoire Kataliko Bénédicte.

ABSTRACT

In the Democratic Republic of Congo (DRC), particularly in North-Kivu province, the prevalence of iron deficiency affects over 36% of pre-school children and at the national level, 71% and 53% of pre-school and pregnant women. Iron biofortified common beans with high yield potential is a promising strategy to address mineral deficiencies in this region where cereal/bean-based diets are the most important.

The objectives of this study were to : i) determine farmers' varietal preferences, production constraints and perceptions of biofortified (iron and zinc) common bean in DRC ; ii) evaluate the cooking time and quality of cooked beans of the new high-performing second-generation biofortified common beans ; iii) assess the genetic variability and yield stability of iron and zinc biofortified common bean genotypes ; iv) determine the mode of inheritance of seed iron and zinc concentration and reduction of polyphenols in common bean ; and v) identify quantitative trait loci (QTLs) associated with high seed iron and zinc contents in common bean accessions and local varieties cultivated in DRC.

In the first study, results from 180 and 140 farmers surveyed in Lubero and Beni territories in North-Kivu province indicated that farmers plant a mixture of traditional and improved varieties. Average bean yield in the two territories is less than 800 kg ha^{-1}. Farmers do not use fertilizers or pesticides. High yield is the primary attribute farmers expect from improved seeds followed by earliness in Lubero territory where the crop takes four to six months to mature. In Beni territory, a part from the yield, priority trait is resistance to abiotic stresses. Major constraints to common bean productivity in these territories include lack of high yielding varieties resistant/tolerant to pests and diseases, low soil fertility and adapted to climatic changes and variability, wandering of animals that decimate common bean crops and small land sizes. Respondents were not aware that there are common bean varieties with high mineral concentration. However, a half of the respondents knew that iron is involved in blood structure in reference to the tablets women use during pregnancy. They indicated that no biofortified common beans are grown by farmers but they wished to test performance of the new iron and zinc dense beans.

In the second study, from 285 second-generation biofortified common bean lines, farmers selected 124 performing genotypes which had from 14.2 to 26.8 pods per plant compared to local varieties whose pods varied from 8.7 to 13.0 per plant. These genotypes had yellow and lighter seed coats. Hydration coefficient varied from 1.4 to 3.4 among biofortified types and from 1.7 to 2.0 among local lines. Cooking time varied from 73 to 170 minutes among

biofortified genotypes with an average of 118 minutes and from 120 to 144 minutes among local lines with an average of 134 minutes. However, the percentage of beans remaining whole after cooking was high (93.0 to 99.8%) for local lines than for biofortified lines (21.3 to 100%). From the study on genetic variability and yield stability involving 160 iron and zinc dense lines, yield and iron and zinc levels were high for biofortified genotypes than for the local varieties. Three biofortified genotypes, G4-24A, BF08-01-47B and RWV 2359 had significantly higher grain yields (4699.7, 2818.3 and 2690.9 kg ha^{-1}) compared to the checks.

Three biofortified genotypes RK 11, BF08-07-22 and BF08-26-162 combined iron and zinc concentration significantly higher than those for the high iron and zinc checks. Broad-sense heritability and genetic gain under selection were high for pod length (95.3 and 63.57%). Heritability was moderate for 100-seed mass (57.2%) and seed iron (68.3%) and genetic gain was low for all other traits. Yield stability assessment showed no ideal genotype across locations. However, biofortified genotypes BF08-14-96B, BF08-7-19B and BF08-14-51C, were the most stable and had more than 1,800 kg ha^{-1} of grain yield. Genotype G4-24A, which was the best yielding genotype, was the most unstable.

In the fourth experiment on inheritance of iron, zinc and polyphenols in common bean, broad sense heritability was important for all the study characters (>0.70) indicating the importance of genetic effects controlling the inheritance of characters. No significant GCA effects were observed among the testers and lines for iron, zinc and total polyphenols. However, among hybrids, significant negative SCA effects were observed for hybrid RW582x041/1 for total polyphenols. On the other hand, significant positive SCA effects were observed for hybrid RW547x720/12 for iron.

The fifth experiment aimed to identify QTLs associated with seed iron and zinc using high density DArTSeq SNP markers on 183 dense iron and zinc common bean introductions and five local genotypes. Typically, the study population can be structured into four subpopulations. Two QTLs associated with seed iron were found on chromosomes 1 and 3 and two other QTLs associated with zinc were found on chromosomes 6 and 9. Thirteen genes were proposed as candidate genes controlling iron and zinc in common bean. One gene was located on chromosome 1, seven on chromosome 3, three on chromosome 6 and two on chromosome 9. These genes belong to four families : NHE, ABC, ZIP and MATE genes.

It is possible to alleviate mineral deficiencies and increase farmers' returns using candidate common bean genotypes with high yield potential and high iron and zinc amounts in Lubero and Beni territories in North-Kivu province.

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ABC : ATP-binding cassette transporter

ALS : Angular leaf spot

AMMI : Additive main effect and multiplicative interaction

ANTHR : Anthracnose

APROMA : Association des Producteurs de Manioc

BLINK : Bayesian-information Linkage-disequilibrium Iteratively Nested Keyway

CGIAR : Consultative Group on International Agriculture

CIAT : International Center for Tropical Agriculture

CIM : Composite interval mapping

CIMMYT : International Maize and Wheat Improvement Center

CREA : Research Centre for Genomics and Bioinformatics

DArTSeq : Diversity Arrays Technology Sequencing

DNA : Deoxyribo nucleic acid

DRC : Democratic Republic of Congo

ECABREN : Eastern and Central African Bean Research Network

FRO : Ferric reduction oxydase

GAE : Gallic acid equivalent

GEI : Genotype by Environment Interaction

GGE : Genotype main effect plus genotype x environment interaction

GPS : Global positioning system

GWAS : Genome-Wide Association Studies

ICP : Inductively coupled plasma technique

IDA : Iron Deficiency Anaemia

INERA : Institut National pour l'Etude et la Recherche Agronomique

IRRI : International Rice Research Institute

Lf : Lectin-free

Lpa : Low phytic acid

MATE : Multi drug and toxic compound extrusion

NA : nicotianamine

NRAMP : Natural resistance associated macrophage protein

PABRA : Pan Africa Bean Research Alliance

PBB : Participatory plant breeding

PC : principal component

PVS : Participatory varietal selection

QTL : Quantitative Trait Loci

RR : Root rots

SNP : Single nucleotide polymorphisms

UCG : Université Catholique du Graben

UNE : *Una Norma Española*

UNILUK : Université Adventiste de Lukanga

UoN : University of Nairobi

WSC : White seed coat

ZIP : Zinc/Iron-regulated transporter-related protein

CHAPTER 1

1. GENERAL INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a major legume providing important nutrients such as proteins and minerals including zinc and iron (Broughton *et al*., 2003). The crop is widely grown because it is suitable for various environments (Broughton *et al.*, 2003). It is cultivated in altitudes varying from 500 to 2,500 meters above sea level (masl) (Farrow & Muthoni-Andriatsitohaina, 2020). Common bean is a highly marketable crop and popular amongst consumers (Bouis and Welch, 2017; Carrasco-Castilla *et al.*, 2012). In addition, its consumption has been reported to be involved in reduction of colon and breast cancer and heart diseases (Buruchara *et al*., 2011).

All plant parts are consumed as food or feed, except the roots (Waluse, 2012). Common bean is the third most important legume crop globally, after soybean and peanut. A high annual per capita intake of 13 to 40 kg of dry beans has been reported in developing countries, particularly in Eastern and Central Africa, especially within families with low-income in rural and urban areas (Mbikayi *et al*., 2018). HarvestPlus (2014) reported a daily consumption of 123 g of common beans among households in Rwanda and Democratic Republic of Congo (DRC). In these countries, common beans are primarily used as a source of proteins in diets.

Among the four main legumes cultivated in DRC, common bean ranks the first and it is used by 83.8% of consumers, followed by groundnut (8.1%), soybeans (6.3%) and peas (0.9%) (Mbikayi *et al*., 2018). Production volumes are lower than the demand and hence there are imports from Rwanda particularly in South-Kivu province (Vwima, 2014).

In DRC and several African countries, millions of people suffer from malnutrition due to micronutrient deficiencies, especially deficiencies of iron and zinc (Kimani *et al*., 2012). Iron deficiency causes anaemia which is associated with functional impairment resulting in chronic

heart failure, inflammatory bowel disease, cancer and kidney disease (Rocha *et al*., 2018). Zinc deficiency leads to growth failure and development of infections (Caproni *et al*., 2020).

In several provinces in DRC, particularly North and South-Kivu, the prevalence of iron deficiency is high with attendant high iron deficiency anaemia among pre-school children and pregnant women (Mbikayi *et al*., 2018). However, common bean is regarded as the near-perfect food in the country.

A well-established bean biofortification breeding programme is needed and a low-cost approach designed to develop high iron and zinc varieties. The target is to ultimately improve human deficiencies toward these micronutrients and deliver economic benefit through the supply and value chains. There should be promotion through participatory breeding approaches for rapid adoption to overcome public health problems such as iron deficiencies in diets of poor people and to contribute to food and nutrition security.

Common bean production per unit area in Eastern and Central Africa is low. While expected yield is 1.5 t ha^{-1} and more in intensive cropping systems, Hillocks *et al*. (2006) and Batumike (2018) have reported yields less than 0.5 t ha^{-1} in Eastern Africa. Amongi *et al*. (2018) reported yields of 0.8 t ha^{-1} in DRC, while the expected yield potential ranges from 1.35 to 2.0 t ha^{-1}. FAOSTAT (2023) database showed a yield of 0.54 t ha^{-1} for common bean yield in DRC. Additionally, most of African countries grow unimproved common bean varieties with poor resistance to several important diseases in tropical regions. Kimani & Warsame (2019) reported the importance of leaf spot, root rots and anthracnose in tropical regions.

Most of the available varieties have low iron and zinc concentrations and require longer cooking times, which can lead to a loss of nutritional value. Their yield is poor and unstable and often they lack some important traits preferred by farmers and consumers. This is because some of

the value chain actors, particularly farmers are not adequately involved in development of new varieties.

There is a need to develop suitable iron and zinc biofortified common bean genotypes through participatory approach, among growers in DRC to reduce the prevalence of mineral deficiency. These genotypes should have high yields with the low antinutritional factors. In addition, they should cook fast. Muroki et al. (2023) evaluated cooking times for soaked beans, ranging from 37 to 60 minutes for biofortified varieties and from 45 to 90 minutes for traditional varieties. Biofortified varieties included MAC 44 and RWV 1129. Talwana (2025) reported cooking time varying from 50 to 70 minutes and from 80 to 120 minutes among biofortified and traditional common bean varieties. According to Panzeri *et al*. (2011), antinutritional factors such as polyphenolics, phytates and phaseolin chelate minerals and reduce their absorption at intestinal level. Digested by humans and other monogastric animals, they reduce nutritional value of seeds chelating and reducing availability of important minerals including iron, zinc, calcium and magnesium. A part from polyphenols, Campion *et al*. (2013) reported that phytates as well as protease inhibitors and lectins exert serious antinutritional effects reducing iron and zinc bioavailability/absorption making them less available.

It is therefore important to exploit genetic variability among the available germplasm to select high yielding and dense iron and zinc genotypes that cook fast. It is also important to understand the mode of inheritance of iron and zinc in common bean in the production environments of DRC. Furthermore, association mapping of diverse common bean genotypes grown under these conditions should be conducted to accelerate delivery of high seed iron and zinc common bean lines to reduce the prevalence of micronutrient deficiencies in DRC.

Over 45 micronutrient-dense common bean lines have already been identified and distributed for evaluation and release to over 25 African countries as the fast-track nursery (Kimani, 2009;

Kimani *et al*., 2005). These varieties cook faster and have higher water absorption rates compared to all major varieties grown in Kenya (Kimani *et al*., 2016). In addition, seven biofortified bean varieties have formally been released in Kenya after independent validation by the regulatory authority (Kimani *et al*., 2016). These beans have marketable grain types that are comparable to popular commercial varieties and they have 80% more iron, 60% more zinc, a high yield potential (>50%), as well as tolerance to major bean diseases under the study conditions. However, very few of these cultivars have been comprehensively evaluated in terms of stability, performance and productivity over a range of field environments outside of Kenya.

High yielding lines tolerant to major biotic and abiotic stresses in Eastern and Central Africa and with high iron and zinc contents have been developed by the University of Nairobi. These varieties are referred to as the "second-generation" biofortified lines. Eighty-four $F_{4:7}$ recombinant inbred lines (RIL) with 66 to 136 ppm and 10 to 60 ppm of iron and zinc contents respectively, have been advanced at the University of Nairobi (Kimani & Warsame, 2019).

For these new varieties to make meaningful impact on food security, supply and value chain, resilience and ultimately consumer nutrition in Eastern DRC, yield stability analysis and genotype by environment interaction should be conducted followed by their screening along with selected checks. Validation of these lines in breeding programmes through mapping QTLs associated with high iron and zinc should also be conducted as well as participatory selection with common bean growers in Eastern DRC. Association mapping analysis is critical to estimate the structure of population and linkage disequilibrium connecting variation observed in the genome with the phenotype of the plant species or varieties (Diapari *et al*., 2014).

Working on 383 accessions from North and South-Kivu provinces in Eastern DRC through HarvestPlus programme functioning in Bukavu, Mbikayi *et al*. (2018) reported a positive and significant Pearson correlation ($r=0.94$; $P<0.001$) between iron and zinc contents suggesting

that selection for high iron results in an increase of zinc and vice versus. Other studies have revealed similar findings (Mulambu *et al*., 2017; Blair *et al*., 2009 ; Welch & Graham, 2004 ; Beebe *et al*., 2000). However, iron and zinc contents significantly fluctuated with environments suggesting a need for further studies to better understand the genetic basis of iron and zinc accumulation and to identify suitable stable high performing genotypes with farmer and consumer preferred traits.

Normal iron and zinc contents in common beans vary from 14.6 to 44.2 ppm for zinc and from 18.8 to 82.4 ppm for iron (Costa *et al*., 2006; Freire, 1997). Concentrations varied between 75 to 100 ppm and more for iron and from 30.1 to 45.6 ppm and more for zinc in biofortified bean varieties (Kimani & Warsame, 2019). The use of improved high iron and zinc bean lines with low antinutritional factors as a fast-track type to increase the bioavailability of these minerals and related-benefits is sustainable, relatively cheap and the promising management strategy.

The development and utilization of improved common bean lines with higher yields (more than 1.5 t ha^{-1}) and higher levels of bioavailable micronutrients is a sustainable, effective and potentially long-lasting alternative towards reducing deficiencies of micronutrients such as wasting, stunting, and anaemia (Kimani *et al*., 2012) particularly in Eastern and Central Africa where common bean is a leading staple food after maize (Amongi *et al*., 2018). Development of such cultivars will ensure wide availability of iron and zinc, regular access and at low cost and culturally appropriate.

The success of this study will enhance the adoption rate of suitable common bean genotypes, increase access to high bioavailable seed with iron and zinc in common bean lines. It will also improve the understanding of genetic mechanisms underlying the absorption of iron and zinc in common bean and to identify future directions for breeding programmes in Eastern and Central Africa.

The broad objective of this research is to contribute to food and nutrition security in DRC through development of stable and high yielding common bean genotypes with enhanced bioavailable seed iron and zinc.

The specific objectives were to :

(i) determine farmers' varietal preferences, production constraints and perceptions of biofortified (Fe and Zn) common bean in DRC ;

(ii) evaluate the cooking time and quality of high performing second-generation biofortified common beans ;

(iii) assess the genetic variability and yield stability of new high iron and zinc common bean genotypes in Eastern DRC ;

(iv) determine the mode of inheritance of seed iron and zinc concentration and reduction of polyphenols in common bean; and

(v) identify QTLs associated with high seed iron and zinc concentration in accessions and local common bean varieties cultivated in DRC.

CHAPTER 2

2. LITERATURE REVIEW

2.1.Domestication of common bean

Common bean (2n=2x=22) belongs to the order of Fabales, family of Fabaceae, the genus *Phaseolus*. This genus counts around 80 cultivated and wild species. Common bean (*Phaseolus vulgaris* L.) is highly diversified throughout the world. The crop was domesticated in two regions and led to two gene pools, the Andean gene pool in South America and the Mesoamerican gene pool in the highlands of Central America (Chacon *et al*., 2005; Gichangi *et al*., 2012 ; Rocha *et al*., 2018). Andean lines are large-seeded and exhibit low yields. Mesoamerican lines, however, are small and medium-seeded types and tend to be associated with high yields. In addition, Islam *et al*. (2002) reported that Andean lines have likely more mineral amounts than Mesoamerican lines.

Beeble *et al*. (2014) observed that domestication of common bean has visibly changed its plant morphology and phenology. Changes are observed on the growth habit, seed retention, maturity and seed size. Oshone *et al*. (2014) explained that some of the differences observed between the cultivated and wild common bean genotypes are related to pod and seed size (low size associated with wild type) and the edible parts such as immature and dry pods.

2.2.Production, economic importance and nutritional value of common bean

Common bean is cultivated throughout the world. Recent figures showed that world bean production is 28.5 million tons on 37.7 million ha (FAOSTAT, 2023). Asia contributes 50% of the total production followed by Africa and America that contribute 25% and 24%, respectively. Europe contributes only 1% of this production (Nadeem *et al*., 2021).

India, Brazil, Myanmar, China and the USA are the top five producing countries worldwide in the respective order. According to FAOSTAT (2023) database, over the global production of 28.5 million t, they produce about 14.583 million tons.

In Africa, common beans are produced in Eastern and Central, Southern and Western Africa. These three regions produce 86.7% of the total African production (FAOSTAT, 2023). Eastern and Central Africa contributes 68.5% to the overall African bean production, while Central and Southern Africa contributes to 17.22 and 0.96%. Out of a total cultivated area of 10.5 million ha (FAOSTAT, 2023), common bean occupies 22%, 7.4% and 70.6% of the total area in Southern, Western and Eastern and Central Africa respectively (Farrow & Muthoni-Andriatsitohaina, 2020).

Eastern, Central and Southern Africa produce 6.13, 1.18 and 0.056 million tons on 7.86, 1.77 and 0.058 million ha of cultivated land, while the total African production is 8.32 million tons on 10.5 million ha of land (FAOSTAT, 2023). Amongi *et al*., (2018) have also reported that common bean production is mainly concentrated in the Eastern and Central African regions.

Most of common bean is produced at higher altitudes (>1,500 masl) and at mid-altitudes (between 1,000 and 1,500 masl) (Farrow & Muthoni-Andriatsitohaina, 2020). Recent production figures available on FAOSTAT (2023) show that Tanzania is the leading country producing 1.484 million tons on 1.080 million ha followed by Uganda and Kenya with 0.865 and 0.861 million tons of dry beans produced on 1.16 and 1.22 million ha respectively. Democratic Republic of Congo (DRC) produces 0.276 million tons on 0.505 million ha (FAOSTAT, 2023).

Common bean increases the smallholder farmers' potential incomes and particularly when biofortified beans combining high micronutrient qualities and good agronomic

performance and particularly high yielding biofortified beans are used. Funes *et al*. (2019) reported that biofortified bush beans, in Rwanda, increase the farmer incomes by 24% equivalent to $75/ha compared to traditional bush beans. They also reported an increase of 25% equivalent to $103/ha associated with biofortified climbing beans.

Common beans represent an important source of accessible and low-cost proteins, carbohydrates and fibers. Chemonics International reported that common bean grains contain 20 to 28% of proteins, 32% of carbohydrates and 56% of fibers (Amongi *et al*., 2018).

Common beans are an important source of nutrients for billions of people living in regions where problems of undernourishment have been reported. These regions include Sub-Saharan Africa particularly Eastern Africa and the Caribbean where cereal/bean-based diets are the most important. Compared to cereals such as rice, wheat and maize containing respectively 6.3 to 24.4 µg/g, 25 to 56µg/g and 9.6 to 63.2µg/g of iron ; the content of this nutrient varies from 35 to 90 µg/g in common bean (Beebe *et al*., 2000). Relative zinc content in this species ranges from 21 to 54 µg/g. Deficiencies of these nutrients are sources of problems such as anaemia, decreased immunity, growth and development of organisms. These deficiencies are addressed through the promotion of biofortification programmes to enhance the dietary intake of iron as well as zinc through conventional plant breeding and modern biotechnology methods to increase nutrients density and bioavailability (Caproni *et al*., 2020).

Rocha *et al*. (2018) reported, however, 1 to 3% of phytate of the total seed weight in common beans. Panzeri *et al*. (2011) contrasted this range and reported that dry beans contain 0.6% to 2.4% of phytic acid. Accumulation of phytate reduces the bioavailability of minerals including iron and zinc.

2.3.Constraints to common bean productivity in Eastern Africa

There are several constraints affecting common bean production worldwide. These constraints have biotic and abiotic causes and include common bacterial blight, anthracnose, angular leaf spots, bean stem maggot, rust, aphid, bean common mosaic virus, bruchid, web blight, halo blight, Pythium, N deficiency, P deficiency, mid- and late-season drought (Farrow & Muthoni-Andriatsitohaina, 2020). The diseases can induce 80 to 100% of yield losses. The level of losses depends on the stage and the severity of the infection.

Angular leaf spot, anthracnose, common bacterial blight and root rots are the top four common diseases in Eastern and Central Africa (Farrow & Muthoni-Andriatsitohaina, 2020 ; Worrall & Wamonje, 2015). Favored by warm (16 – 28°C with 24°C optimum) and wet conditions, angular leaf spot induces 50 to 100 kg ha^{-1} of yield losses, a consequence of premature defoliation. It affects all aerial parts of a diseased plant. These parts include stems, leaves, petioles and pods even though recognized symptoms are present on leaves (Saettler, 1991).

Anthracnose is devastating between the range of 13 to 26°C (optimum 20°C) and around 100% of humidity. Winds and splashing rains exacerbate the spread of the infection. Losses associated with this disease are more important compared to those associated with angular leaf spot (Smithson, 1990).

Common bacterial blight is favored under warm conditions particularly in low to mid altitudes (<1,500 masl). A number of races of the pathogen exist; however, according to Fourie & Herselman (2011) ; Beebe & Andersson (2014), only the race 2 of *Xanthomonas bacteria* is the most common.

Root rots have different causes varying from soil fungi to various species of oomycetes that include *Sclerotium*, *Pythium*, *Rhizoctonia* and *Fusarium*. These pathogens induce considerable effects in intensive common bean production areas and particularly in case of low fertility (Saettler, 1991).

Major abiotic stresses affecting common bean production include low soil fertility and climate stresses. The last refers to risk of drought and already affects about 73% of total area in Eastern, Central and Southern Africa under common bean cultivation (Farrow & Muthoni-Andriatsitohaina, 2020). Heat and higher temperatures, frequent in lowlands represent serious drawbacks to common bean production.

Low African soil fertility is due to depletion, low rate of use of fertilizer, soil acidity due to aluminum or magnesium excess and low supply of bases. Aluminum toxicity and phosphorous deficiency affect 20% and 75% of common bean production (Rao *et al*., 2016). To reduce the effects associated with these drawbacks, three alternatives can be applied: the use of nutrients to enhance soil fertility, soil conservation methods and the use of tolerant lines (Polania *et al*., 2016).

Genetic diversity toward tolerance to drought and other abiotic constraints exists in common bean. This tolerance is associated with mechanisms such as early maturity, deep rooting and a greater ability to partition dry matter to grain (Polania *et al*., 2016).

2.4.Micronutrient malnutrition problem

Micronutrient problems arise when the food intake or supplements are not sufficient to meet the body demand either in the case of poor absorption or in case of increased loss and important utilization.

In humans, adult males and females, iron content respectively approximates 35 and 45mg kg^{-1} of wet body weight. About 70% of this amount is involved in the structure of hemoglobin whose function is to carry oxygen around the body. This is why growth retardation and impairment of cognitive ability are associated with deficiency of iron. Zinc is involved in immune system, cell growth and division, hydrolysis of energetic nutrients such that deficiency is harmful and reduces immunity, growth and development (Philipo *et al*., 2020).

Malnutrition is an overall problem due to nutrient imbalance in the human consumption. According to FAO (2017), around 1.9 billion people are affected by malnutrition, while 462 million are underweight. Further, 29% or 528 million of women are affected by anaemia that would be subjected to supplementation with iron.

However, micronutrient malnutrition, described as hidden hunger is less obvious. Its effects are not easily expressed and are a consequence of lack of food diversity among poor people. Staple food is popular as diet in low-income households. Important source of calories, the staple food, often provide small amounts of minerals as well as vitamins. Cultural reasons are other factors limiting nutritional intake even when nutritious foods are available in a specific zone.

Quaim *et al*. (2007) and Funes *et al*. (2019) reported that the high at-risk groups affected by micronutrient deficiencies are children, women and particularly pregnant and breast-feeding. Ugen *et al*. (2012) reported a high prevalence of malnutrition in Eastern DRC

among children under five years with 5-10% being moderately malnourished and over 20% at risk of malnutrition. They suggested that this malnutrition is due mainly to protein and iron deficiencies exacerbated by an important dependence on starchy-carbohydrates foods representing poor sources of proteins and micronutrients.

Severe health consequences are associated with micronutrient deficiencies. These problems include increased susceptibility to infectious diseases, mental and physical impairments and therefore increased mortality. Further, Funes *et al*. (2019) reported that 1.3 billion people are at risk of iron deficiency and consequently more susceptible to viral infections.

2.5.Strategies for fighting hidden hunger

2.5.1. Food diversification

Food diversification involves consuming a wide range of food types instead of relying on a single commodity. It ensures access to essential micronutrients that are often lacking in monotonous diets.

Diversification of food diets is a sustainable approach and improves the nutritional intake. It is unfortunately impeded by poor income particularly in low-income households leading to a novel intervention through biofortification.

2.5.2. Food fortification

Food fortification is a strategy toward fighting hidden hunger that consists of adding micronutrients to foodstuffs during processing. This occurs when the food is deficient in micronutrients such as minerals and vitamins important for development and growth (Kimani & Warsame, 2019). The major deficiencies around the world include iron, iodine and vitamin A, important particularly for pregnant women and children (Nilson & Pizza, 1998).

Staple food mainly starchy containing low levels of micronutrients are complemented with fortifying food such as milk and oil in order to tackle anaemia and other drawbacks associated with mineral deficiencies.

2.5.3. Mineral supplementation

Mineral supplementation refers to a strategy of distributing mineral as well as vitamin capsules at regular intervals. It represents an early strategy toward prevention of micronutrient deficiency that would lead to some abnormalities. For instance, according to Maric *et al*. (2014), early supplementation with folates during pregnancy can prevent up 70% of all neutral tube defects, while vitamin D supplementation is critical for management of elderly and postmenopausal women.

Nilson & Pizza (1998) recognized vitamin A supplementation as the major intervention to address risks associated with all-cause mortality among children of six to 59 months and in special case to reduce diarrhea incidence and consequently to reduce burden of mortality. Most minerals are not commonly used in supplementation. However, iron-folate supplements are routinely provided to pregnant women.

Mineral fortification and supplementation give immediate results to restore pronounced deficiencies and are sustainable as long as they are affordable (Nilson & Pizza, 1998).

2.5.4. Biofortification

Biofortified common beans toward iron and zinc are bred varieties with relatively higher concentrations of iron and zinc compared to traditional lines. Mineral biofortification received higher priority when breeders realized that breeding for high micronutrient contents is not computing with superior agronomic traits. Quaim *et al*. (2007) reported high yields associated with common bean varieties grown in low-

quality soils, if the uptake of iron and zinc is high. This is due to the fact that these minerals are necessary for plant growth and vigor.

Several programmes have been launched through the term "biofortification" toward increasing micronutrient in staple crops. Through conventional selection methods exploiting available genetic variability, HarvestPlus Challenge Programme initiated by the Consultative Group on International Agriculture (CGIAR) has concentrated efforts in six staple food crops including common bean, wheat, rice, cassava, maize and sweet potato on iron, zinc and β-carotene (Quaim *et al*., 2007).

However, conventional techniques were inefficient toward increasing high β-carotene in rice grains; hence, the use of modern biotechnology methods particularly transgenic techniques were successful. This programme was leading by the International Rice Research Institute (IRRI) and resulted in production of Golden Rice (Ye *et al*., 2000). While several biofortified crops are still at research and development stage, Low *et al*. (1997) reported that orange-fleshed sweet potatoes have been promoted in several countries.

Biofortification has largely depended on technology efficacy and coverage and on local dietary patterns determining the target crop what should be an important staple food and regularly and largely consumed. Technology efficacy and coverage refer to the level of the micronutrient in the target crop, retention after any processing technology, bioavailability as well as adoption and acceptance by farmers and consumers (Quaim *et al*., 2007).

Post-harvest and processing techniques reduce to a certain extent micronutrients available. Sunlight as well as extreme heat have negative impacts on bioavailable β-carotene, vitamins and carotenoids. Minerals are less sensitive to these conditions.

Some other factors have influences on micronutrient biovailability. For instance, in common bean, Quaim *et al*. (2007) reported that Vitamin C intake has a positive impact on bioavailable iron, while phytates and tannins have rather negative impacts.

Adoption and acceptance of high micronutrient density cultivars by farmers and consumers are exacerbated by other characters that should be superior but also the efficiency of available seed distribution systems. These characters include agronomic traits especially the yield and resistance or tolerance to common stresses and quality traits including cooking time, color and starchiness (Teshome & Emire, 2012). These are the reasons why the focus should be on common local varieties available in the target region.

Varieties of staple food contain variable amounts of micronutrients. Costa *et al*. (2006) stated that seeds of common bean contain 18.8 to 82.4 ppm and more and 14.6 to 44.2 ppm of iron and zinc, respectively. Variability that exists among cultivars makes selection toward high micronutrient density food possible. However, limitations arise in case of a very limited available genetic variation leading to transgenic techniques to increase micronutrient levels in the target crop.

2.6. Antinutritional factors in common bean

Several antinutritional factors have been reported in common bean seeds. These factors include lectins, polyphenolics, phytic acid, condensed-tannins, raffinosaccharides, sapogenols (Giuberti *et al*., 2019). According to Welch & Graham (2004), lectin proteins of the group of phytohaemagglutinins as well as phytates reduce micronutrient (including iron and zinc) bioavailability inducing negative impacts on human health. Phaseolin is another factor, poorly digested. It is a major seed storage protein in common beans that contributes to elevated protein content, primarily stored as a

glycoprotein. However, its digestibility is limited in monogastric animals due to its resistance to proteolysis and its deficiency in essential amino acids such as tryptophan, cysteine, and methionine (De la Fuente *et al*., 2012). Despite efforts to improve nutritional quality, phaseolin-free common bean lines remain scarce on a global scale (Giuberti *et al*., 2019).

Phytic acid is another important antinutritional factor in common bean seeds and other plant seeds. Its amount in seeds averages 8.09 ppm (Campion *et al*., 2013). It represents the major form of phosphorous in many seeds (60 to 90%) and plays important physiological roles in plants. Lolas & Markakis (1975) reported respectively that phytic acid is involved in initiation of dormancy and serves as a source of phosphorous and cations for germinating seed. However, in animal nutrition, it chelates various minerals including iron, zinc, calcium, magnesium and molybdenum, reducing therefore their absorption at intestinal level.

The development of either low phytic acid (lpa) or biofortified common bean lines is the important sustainable approach to increase absorption of micronutrients including iron and zinc in common bean seeds. Evidence showed that iron absorption is notably higher in lpa maize compared to wild type (Hambidge *et al*., 2005). Campion *et al*. (2009) after isolation and characterization of lpa common bean mutant lines (with 90% reduction of phytic acid) derived from combined lectin-free (lf) and white seed coat (wsc) (with reduced condensed tannins) produced lf+lpa+wsc common bean lines that were subjected to further analyses. They realized that reduction or removal of these three antinutritional factors induces reduction of other antinutrients such as lignin and saponins but rather increases nutrients such as crude proteins as well as zinc and free phosphorous.

Other important antinutritional factors in common bean are tannins and polyphenols, concentrated in seed coat. Yang & Gan (2018) reported the broad classification of total polyphenols. They are classified into phenolic acids, flavonoids, proanthocyanidins, stilbenes and coumarins. In common beans, phenolic acids and flavonoids are the most important polyphenolics reported exacerbating antioxidant activity (Yang & Gan, 2018). Antioxidant activity is important in dark beans than in lighter colored beans. In fact, Yang & Gan (2018) reported that dark but also pinto kidney beans have higher amounts of polyphenolics. Tako & Glahn (2011), also reported earlier that common beans with white seed coats contain low amounts of polyphenolics compared to common beans with red seed coats. Joram (2007) stated that the total tannins concentration (including polyphenols) is influenced by the colour of the dry bean seed coats. According to the later author, coloured bean seeds have 2.2 to 12.6% of total tannins, while white bean seeds have tannins in the form of trace. The total polyphenols in common bean are determined as the amount of gallic acid equivalent (GAE) per 100 g or 1 g of dry weight. Yang & Gan (2018) reported 3,000 to 4,871 mg GAE per 100g of dry weight in seed coat of purple kidney, red to pinto bean and 33 to 63 mg GAE per 100g of dry weight in Turkish white beans. A part from antioxidant activity, polyphenols are involved in anti-diabetic, anti-inflammatory, anti-tumor, anti-mutagenic, anti-atherosclerosis and neuroprotective activities (Yang & Gan, 2018; Wu *et al*., 2004).

Polyphenols are concentrated in the seed coat and found also in cotyledons. They reduce mineral bioabsorption at intestinal level for monogastric animals including humans (Carvalho e*t al*., 2012; Giuberti *et al*., 2019). According to De la Fuente *et al*. (2012) ; Giuberti *et al*. (2019) they can reduce protein digestibility to 7-10% and exacerbate reduced absorption of iron and zinc at intestinal level. Petry *et al.* (2010)

reported that when young women were fed meals with removed polyphenols, the amount of absorbed iron doubled. In fact, in their study on iron absorption in young women using porridge made up with common bean flour, they showed that dephytinization increases iron absorption only in the absence of polyphenols. Iron absorption increased 3 to 4-fold in 90% dephytinization. The removal of polyphenols from dephytinized porridge doubled iron absorption. Tako and Glahn (2011) reported higher amounts of bioavailable iron in white beans where polyphenols are low compared to red beans containing more polyphenols. Mangan (1988) reported that polyphenols form stable complex with macronutrients such as proteins, cellulose and starch. They also form complex with digestive enzymes interfering with protein digestion (Singleton *et al*., 1999). However, polyphenols play an important role in protecting a plant against pathogens and UV radiation as well as acting as antioxidants (Petry *et al*., 2013).

2.7.Inheritance of iron and zinc concentration in common bean

Mineral bioavailability in higher plants tends to be controlled by many genes, each contributing a minor effect to the overall expression of the character. In *Arabidopsis thaliana* for instance, Vreugdenhil *et al*. (2004) found a quantitative and oligogenic mode of inheritance of micronutrient accumulation associated with several putative genes. Wu et al. (2008), focusing on iron and zinc uptake in cabbage (*Brassica napus*) leaves, reported a number of minor QTL effects associated with accumulation of these nutrients.

Using different backgrounds, Izquierdo *et al*. (2018) ; Cichy *et al*. (2005) ; Guzman-Maldonado *et al*. (2004) ; Forster *et al*. (2002) suggested that the inheritance of seed

iron and zinc is quantitative. Izquierdo *et al*. (2018) identified 12 meta-QTLs, where two were specific to zinc, two to iron and eight specific to iron and zinc.

The significance of biofortification in common bean has increased following evidence that enhancing iron and zinc concentrations does not compromise grain yield. Hence, genotypes enriched in these micronutrients have demonstrated consistently high yield performance (Kataliko *et al*., 2024 ; Kimani & Warsame, 2019 ; Ribeiro *et al*., 2014 ; 2013 ; Gregorio, 2002).

However, Diapari *et al*. (2014), in their study on chickpea, observed contrasting results. They reported that zinc is negatively correlated with grain yield. Nevertheless, correlation between iron concentration and yields was negative in one study site and positive in another site suggesting replicated experiments. The positive correlation tends to be due to the functions these minerals play in the plant growth. Philipo *et al*. (2020) reported that iron is involved in plant respiration, photosynthesis, nitrogen assimilation as well as DNA synthesis, while zinc is involved in the structure of enzymes hydrolyzing carbohydrate, proteins and lipids. Zinc is also involved in synthesis of auxin, formation of pollen and gene regulation toward environmental stress tolerance such that its deficiency may lead in sterility, chlorosis and susceptibility to biotic and abiotic stresses.

From their study involving mating between wild and cultivated common beans, Forster *et al*. (2002) ; Cichy *et al*. (2005) suggested that seed iron concentration is quantitatively inherited, while the inheritance of zinc tends to be simple and oligogenic. Blair *et al*. (2009) reported in addition an important quantitative inheritance of seed iron and zinc concentration in common bean. Using a recombinant inbred line population of common

beans, they identified 26 QTL of which half were for iron concentration and half for zinc concentration.

2.8.Breeding biofortified bean varieties in Eastern Africa

2.8.1. Germplasm collection, screening and release of fast-truck common bean varieties

Collection and screening of germplasm is a starting point of any breeding programme. A regional programme led by Pan-African Bean Research Alliance (PABRA) led on breeding micronutrient dense bean lines in Eastern Africa was initiated in 2001 and based at the University of Nairobi and Malawi National Bean programme in partnership with HarvestPlus and CIAT. This programme aimed to characterize iron and zinc variability among common bean accessions in east, central and southern Africa, and identify suitable parental lines for any breeding programme and lines that can be used as fast-track toward high iron and zinc concentration (Kimani et al., 2008).

A total of 2,853 lines were collected during five years from nine countries that belong to Eastern and Central African Bean Research Network (ECABREN). These countries include Burundi, DRC, Ethiopia, Kenya, Madagascar, Tanzania, Sudan, Rwanda and Uganda. These materials included varieties, landraces, germplasm accessions, introductions as well as breeding lines held in gene banks and national bean programmes. Chemical screening consisting of determination of iron and zinc concentrations and phytates in seeds was conducted in different labs of the University of Nairobi, at CIAT Columbia, Cornell University, University of Copenhagen as well as Sokoine University. Further, cooking time, mineral retention and other organoleptic characters were assessed. Most micronutrient dense beans were from the Great Lakes region particularly Rwanda and DRC.

On the other hand, from some wide crosses involving some genotypes screened from the above materials, some high iron lines were developed and introduced to breeding programmes in Rwanda and DRC. In DRC, the programme worked at the Institut National pour l'Etude et la Recherche Agronomique – INERA Mulungu in South-Kivu, INERA Kipoko in Katanga, INERA Mvuazi in Bas Congo and INERA Gandajika in Oriental Kasai provinces (Mulambu *et al*., 2017). High iron common bean lines were fast-tracked for delivery and release from 2011 to 2013 and are in use. These lines include HM 21-7, RWR 2245, PVA 1438, Namulenga, COD MLV 059 and Cuarentino (Mulambu *et al*., 2017). Since, activities toward high iron-biofortification spread over in DRC and Uganda. By 2016, more than 800,000 Rwandan common bean growers and consumers adopted biofortified beans (Mulambu *et al*., 2017).

Among the above materials collected, three hundred common bean lines from Tanzania, Rwanda, Uganda, Kenya and DRC had 40 to 105 ppm iron. Four landraces collected from Tanzania showed low iron concentration in white beans (73 ppm) but high rather in yellow beans (120 ppm) and in grey (114 ppm) and intermediate in kidney beans (87 ppm). Overall, common bean seeds showed higher iron concentrations compared to other grains including maize (19 ppm), banana (5 ppm), cassava (5 ppm), sweet potato (5 ppm) and potato (3 ppm) suggesting that iron deficiency can be well addressed with common bean.

Thirty-eight genotypes were identified and disseminated as fast-track lines for regional evaluation including resistance to major diseases across different agroecological conditions in more than 15 countries in Central, Eastern and Southern Africa. In addition to high mineral concentration, they revealed major farmer and consumer preferences and would require less time for further testing and release.

Micronutrient dense bean lines lacking preferred agronomic characters and susceptible to important diseases were integrated in hybridization to develop segregating populations subjected to selection pressure. The abilities considered were the good combination between high micronutrient content and high yield, resistance/tolerance to biotic and abiotic stresses and marketable grain types.

The yield associated with climbing beans was typically three to four times high compared to bush beans. Climbing bean lines were from CIAT, advanced for high mineral concentration and recognized as ideal in urban and rural regions where the size of the population is important and were subjected for preliminary evaluations in eastern, central and southern Africa. Genotypes with at least 70 ppm and 30 ppm of iron and zinc were evaluated for agronomic and consumer preferences in a participatory approach into nine countries in Eastern, Central and Western Africa. In particular, four candidate climbing bean lines were released as double fast-track in DRC. These lines included G59/1-2, VCB 81013, LIB 1 and Kiangara and showed adaptation from 500 to 1,500 masl (Kimani *et al*., 2008).

Overall, considering independent and multilocation evaluations conducted in eastern, central and western Africa toward high micronutrient content and agronomic performance, in December 2008, three candidate lines were recommended for full release. These lines were MV 19, MV 17 and MV 14 and had more than 2,100 kg ha^{-1} of yield potential. It was also reported by CIAT (2007) that iron concentration is much higher in leaves than in seeds (from 236 to 2,498 ppm), while the concentration of zinc in leaves varied from 20 to 67 ppm. It was also reported a high iron and zinc bioavailability in green shelled beans compared to dry seeds.

2.8.2. Breeding second-generation common beans

In Eastern and Central Africa, a breeding programme to develop micronutrient dense bean genotypes was initiated by CIAT and HarvestPlus and led by the University of Nairobi and Malawi National Bean programme. This programme aimed to exploit the available genetic variability among more than 2,800 accessions. Dissemination of genotypes developed was purposed to nine countries members of ECABREN (Kimani *et al*. (2008). Most of the high micronutrient dense bean genotypes were less robust and particularly less resistant to major diseases and abiotic stresses in Eastern and Central Africa. These diseases involved angular leaf spot (ALS), anthracnose (ANTHR) and root rots (RR) (Kimani & Warsame, 2019). This is why they were referred to as the first generation of fast-track common bean lines.

Six among these lines selected for high iron and zinc concentrations were crossed to 11 commercial lines with resistance or tolerance to the three major diseases listed to develop 47 multiparent populations. The description of the six high iron and zinc lines is available in Table 2.1.

Table 2. 1 : Description of six fast-track common bean lines with high iron and zinc contents

Genotype	Origin	Growth habit and type	Seed color	Seed size	Iron (ppm)	Zn (ppm)
Nakaja	DRC	II, climbing	Brown	Small	76.6	43.0
Simama	DRC	II, bush	Red mottled	Large	82.7	34.5
MLB 49-89A	DRC	II, bush	Black	Medium	95.6	30.1
AND 620	CIAT	I, bush	Red mottled	Large	76.0	35.3
Gofta	Ethiopia	II, bush	Brown	Medium	74.4	40.1
HRS 545	Sudan	II, bush	Navy	Small	89.7	45.6

Growth habit : I – determinate ; II – indeterminate. Seed size : Small (<25 g/100seeds), Medium (25-39 g/100seeds), Large (>40 g/100seeds). Source : Kimani & Warsame, 2019.

Two of the commercial lines, G5685 54 and Mex were used for their resistance to ALS, while G685 was used for its resistance to fusarium. Lines AND1062, SCAM 80CM/15 and RWR 719 were used for their resistance to Pythium and fusarium root rots and tolerance to low soil fertility. Line G2333, highly susceptible to fusarium wilt, was used for its resistance to ANTHR. The other commercial lines included Rosecoco (GLP 2), a popular variety in Uganda and Kenya, Selian 97 and Lyamungu 85, popular in Tanzania and GLP 24 (Canadian Wonder), popular in Tanzania and Kenya. Despite their susceptibility to ALS, ANTHR and RR, they had farmer and consumer preferred traits (Musoni *et al*., 2010).

The six high iron and zinc parental lines were used as female parents, while the commercial lines were used as the sources of pollens. Through bulking during the 2008 and 2009 long and short rains, the F_1 progenies obtained into single, double and three-way crosses were advanced to F_4 generation at the field station of University of Nairobi (Kimani & Warsame, 2019).

Fourty-seven F_4 populations were advanced to $F_{4.5}$, $F_{4.6}$ and $F_{4.7}$ on individual plant selection. This selection was based on the plant vigor, reaction to the major diseases listed, growth habit, pod load, soil moisture stress, grain yield (at 14% seed moisture content) and seed iron and zinc concentrations.

From these selections, 84 $F_{4.7}$ promising lines were selected from eight outstanding populations. These lines had 66 to 136 ppm of iron and 10 to 60 ppm of zinc, tolerant to drought and were resistant to major diseases. Furthermore, they had 50 to 90% more yield increase compared to first generation, parental and commercial lines (Kimani & Warsame, 2019). These are the reasons why they were called second-generation biofortified common bean lines.

2.8.3. Yield potential and market classes of second-generation biofortified beans

High significant differences in grain yield have been reported among the 84 second-generation biofortified beans. The grain yields of second-generation varying from 803 to 3,323 kg ha^{-1} were 67% higher compared to those of the first generation that varied from 1,191 to 1,985 kg ha^{-1} (Kimani & Warsame, 2019). However, these authors reported that most of the second-generation fortified beans have climbing habit. Funes *et al*. (2019) reported an increase of at least 23% and 22% associated with the use of biofortified bush and climbing beans and particularly in Rwanda. Particularly, the biofortified line RWR2245 induced 49% of high yields compared to traditional bush bean.

Biofortified common beans are preferred when they have good agronomic qualities and particularly high yield. Iron and zinc accumulation alone do not represent an impediment to consumer acceptance (Mulambu *et al*., 2017). Unlike various developing countries, Murekezi *et al*. (2017) reported that in Rwanda, consumers and consequently the market prefer the iron and zinc biofortified bean varieties over the local varieties. This is why HarvestPlus and its partners such as CIAT and the Rwanda Agriculture Board disseminate these varieties throughout the country to alleviate micronutrient deficiencies and particularly iron deficiency, considered as the most important regarding its effects.

2.8.4. Resistance of second-generation biofortified beans to biotic and abiotic stresses

Biotic stresses scored included angular leaf spot (caused by *Pseudocercospora griseola*), anthracnose (caused by *Colletotrichum lindemuthianum*), common bacterial blight (caused by *Xanthomonas axonopodis pv phaseoli var. fuscans*) and finally root rots (caused by *Pythium* and *Physarium spp*.). Significant disease reactions were

observed among the 84 second-generation biofortified beans (Kimani & Warsame, 2019).

Anthracnose is reported as the most prevalent disease because more than 24 lines were affected, while other lines showed resistance to this disease. All the lines showed resistance or intermediate resistance to common bacterial blight and root rot. Nine second-generation biofortified bean lines showed horizontal resistance to all the diseases screened for, while the remaining lines showed combined resistance to either two or three diseases (Kimani & Warsame, 2019).

The abiotic stress considered was moisture-stressed condition. Significant differences were observed among the irrigated second-generation biofortified beans compared to moisture-stressed lines. High yields were reported associated with irrigated lines or on average 3138.9 kg ha^{-1} compared to 1657.8 kg ha^{-1} for stressed lines. The yield was reduced by 47% when drought-stressed conditions were imposed. However, the magnitude of reduction varied with lines and drought intensity index observed in a study site (Kimani & Warsame, 2019).

2.8.5. Molecular breeding of biofortified beans

Functional value of food crop varieties in human diets is a key characteristic determined through nutritional quality. In developing countries, micronutrient content in food crops, particularly in staple crops such as common bean is an important component of nutritional quality (Frossard *et al.*, 2000).

Common bean cultivars may be considerably variable regarding the accumulation of micronutrients in seeds (Islam *et al.*, 2002; Moraghan *et al.*, 2002). Additionally, wild or weedy common bean germplasm as well as related species have revealed important diversity for mineral concentration (Guzman-Maldonado *et al.*, 2004). Islam *et al.*

(2002) reported similar accumulation levels of minerals among the Mesoamerican and Andean gene pools even though common bean types that belong to the last gene pool have likely more iron concentration than the types that belong to Mesoamerican gene pool.

Little has been focused on QTL studies for iron (Fe) and zinc (Zn) accumulation in staple foods in developing countries. On cabbage (*Brassica napus*) leaves, Wu *et al*. (2008) found a number of minor QTL effects being associated with Fe and Zn uptake under various nutrient solutions. Working on chickpea, Diapari *et al*. (2014) identified eight SNPs associated with iron and/or zinc contents. One SNP located on the first linkage group was associated with both zinc and iron contents. Five SNPs were located on chromosome 4 ; three were associated with zinc content, while the two remaining were associated with iron content. In addition, two further SNPs were located on chromosomes 6 and 7 associated respectively with iron and zinc.

Few studies have focused on legumes especially on common bean. The study conducted by Blair *et al*. (2008) represented the first evaluation of QTLs for high mineral accumulation. Using a segregating population derived from a cross involving two cultivated common bean genotypes and using an anchored genetic map with complete coverage, they suggested a multigenically and oligogenically control of seed Fe and Zn accumulation in common bean. They also reported some common genes affecting accumulation of both Fe and Zn in the genetic background.

Previously, Gerlin *et al*. (2007) revealed at the end of their study analyzing seed Zn and other minerals in RIL populations of Navy bean, four microsatellite markers being associated with P, Zn and Ca. However, the Zn marker was not associated with Fe.

Furthermore, locus associated with seed Zn accumulation was found on the ninth linkage group.

Another study conducted by Blair *et al*. (2010) on QTL analyses for seed Fe and Zn contents in 100 Andean common bean RILs using composite interval mapping (CIM) and single-point analyses identified nine seed mineral QTLs (six for Fe concentration and three for Zn concentration) on five linkage groups. The common QTLs were on b02, while the others were on b06, b07 and b08.

During another study on meta-QTL analysis for seed Fe and Zn accumulation in seven populations of common beans including the Andean and the Mesoamerican gene pools, Izquierdo *et al*. (2018) identified 12 meta-QTLs. Two meta-QTLs were specific to Zn, two meta-QTLs specific to Fe and eight co-localized meta-QTLs for Fe and Zn across seven chromosomes giving rise to 12 individual meta-QTLs where candidate genes were found within five of the identified meta-QTL from six gene families associated with Fe and Zn plant transporter.

It is important to extend and elaborate the capacity of the regional breeding programme, addressing critical knowledge gaps to develop and disseminate high Fe and Zn bean varieties and also support the national agricultural system in DRC. Consequently, this could facilitate the development of basis on biofortified food crops and to address the double burden of malnutrition, by delivering tractable nutritional bean varieties that can be used in breeding programmes in Central Africa and particularly in Eastern DRC.

The QTL mapping is the first step of genome wide association studies (GWAS) designed to identify putative genes controlling quantitative traits. In fact, the GWAS approach involves a wide panel of diverse cultivars within a given species.

2.8.6. Enhancing bioavailability of iron and zinc of biofortified beans

Breeding to increase micronutrients bioavailability is recognized by La Frano *et al*. (2014) as one of top goals in common bean breeding programme and is achieved by increasing related promoters or decreasing related inhibitors through manipulation of some structures of the plant. Petry *et al*. (2013) reported a negative relationship between iron and zinc bioavailability and phytates and polyphenols. Previously, Petry *et al.* (2010) reported that iron is most bioavailable in dephytinized food products in the absence of polyphenols.

To increase mineral bioavailability in common bean, research has shifted from merely increasing total mineral concentration to reduction of polyphenol levels. Strategies toward reduction of polyphenol levels in common bean include breeding low-polyphenol genotypes, post-harvest processing techniques, molecular breeding and gene editing and integrated biofortification approaches.

Breeding low-polyphenol genotypes involves genetic variability among bean accessions. This strategy offers opportunities to select lines with naturally lower levels of seed coat polyphenols. For instance, white and yellow-seeded beans typically exhibit lower polyphenol content compared to darker-colored varieties, correlating with higher iron bioavailability (Glahn & Noh, 2021). Post-harvest processing techniques include techniques such as soaking, germination, fermentation, and thermal processing. These techniques can degrade or leach out polyphenols, thereby improving mineral bioavailability. However, they may also lead to nutrient losses and require careful optimization (La Frano *et al*., 2014). In addition, advances in genomics have enabled the identification of candidate genes involved in polyphenol biosynthesis. Targeted manipulation of these genes through CRISPR/Cas9 or marker-assisted selection could

allow the development of bean varieties with reduced antinutrient profiles without compromising agronomic performance (Glahn & Noh, 2021). Integrated biofortification approaches combining low-polyphenol traits with high mineral concentration and favorable agronomic characteristics can maximize the nutritional impact of common bean. This holistic breeding strategy is increasingly recognized as essential for achieving sustainable improvements in human nutrition (Cichy *et al*., 2022).

Despite the benefits, it is important to consider the dual role of polyphenols. While they inhibit mineral absorption, they also possess antioxidant, anti-inflammatory, and antimicrobial properties (Yang & Gan, 2018; Wu *et al*., 2004). Therefore, breeding efforts must strike a balance between reducing antinutritional effects and preserving health-promoting functions. Reducing polyphenol levels in common bean represents a viable pathway to enhance iron and zinc bioavailability. When integrated with biofortification and appropriate processing, reduction of polyphenols holds promise for improving the nutritional quality of diets in vulnerable populations.

2.8.7. Factors influencing mineral concentration in common bean

Genotype by environment interaction (GEI) is a critical factor that influences mineral concentration in common beans. It is important in plant breeding programmes for a good development, selection and recommendation of suitable cultivars. In fact, it assesses differences in performance that are observed among the study genotypes in a number of locations. Changes are often associated with genotypes grown in different environments. A good genotype should acceptably have high yield records compared to other varieties.

According to Tryphone & Nchimbi-Msolla (2010), iron and zinc concentrations are significantly influenced by the interaction between genotypes and environments in common bean. Similar observation was reported by Beebe *et al*. (2000). An environment refers to the result of three kinds of factors, each providing its effects on the development of a given organism: biological, physical and chemical factors (Mukamuhirwa *et al*., 2015). According to Murphy *et al*. (2007) cited by Mukamuhirwa *et al*. (2015), if interaction is particularly severe, selection of suitable genotypes may be hampered. Pereira *et al*. (2014) ; Mukamuhirwa and Rurangwa (2018) also reported that environments have significant influences in seed iron and zinc concentrations.

In addition, Mukamuhirwa *et al*. (2015) reported a strong significant interaction between common bean genotypes and environments toward high iron and zinc concentrations. Working in two different agroecological zones in Uganda (1,200 and 2,200 masl), they revealed that iron and zinc were highly accumulated in seeds under low altitudes. Philipo *et al*. (2020) reported similar findings working on 99 common bean landraces and cultivars that are grown in Tanzania. They indicated that the interaction effects between genotype and environment contribute 26.3% of the total sum of squares for seed iron, while for zinc 28.6% was noted.

Further, iron and zinc bioavailability is largely influenced by the presence of some compounds such as phytates. It is therefore important to reduce their concentration or increase concentrations of iron and zinc to improve the quality of absorption of these nutrients. On the other hand, phytates play important physiological functions in plant growth. According to Williams (1970) cited by Lolas & Markakis (1975), phytates represent an important source of phosphorous and cations for seeds to germinate. They are also involved in initiation of seed dormancy. Their significant reduction is associated with impairment of these physiological mechanisms. This is why it is more

efficient to breed toward important iron and zinc increase rather than significant phytates reduction.

Polyphenols play a given impact reducing iron bioavailability. However, according to La Frano *et al*. (2014), their impact is so minimal even though inconsistent results have been reported probably because of dilution of polyphenols to some extent. Nevertheless, polyphenols are involved in protection of common bean plants against pathogens and UV radiation such that their considerable reduction may lead to negative impacts on seed and plant performance.

2.9.Participatory breeding in common bean

Participatory plant breeding (PBB) integrates farmers into the breeding process to ensure that developed varieties align with local preferences, agro-ecological conditions, and socio-economic realities (Porch *et al*., 2019). It enhances varietal relevance through farmer input, accelerates dissemination and adoption and promotes genetic diversity and resilience. In common bean, it has gained traction due to its potential to improve adoption rates, address abiotic stresses, and enhance traits like iron and zinc accumulation.

In fact, PBB in common bean typically involves participatory variety selection (PVS), on-farm trials and trait prioritization workshops. During PVS, farmers evaluate advanced lines based on traits like yield, earliness, seed color, and cooking quality (Tigist *et al*., 2020). On-farm trials are conducted under farmer management to assess genotype × environment interactions or agronomic practices. During trait prioritization workshops, farmers rank traits such as drought tolerance, marketability and nutritional value (Asfaw *et al*., 2012).

National agricultural research systems and international partners, including CIAT, conducted PVS across multiple sites in East Africa. During sessions held, farmers ranked yield, seed size, and color as top criteria (Tigist *et al*., 2020). Their involvement led to rapid identification and dissemination of preferred common bean genotypes.

In addition, PBB can be strategically aligned with biofortification goals. By involving farmers in selecting iron- and zinc-rich genotypes, breeding programmes can ensure nutritional traits are not compromised by local preferences. This synergy is crucial for tackling hidden hunger.

However, balancing scientific rigor with farmer intuition and limited capacity for molecular validation in farmer settings can challenge this approach (Porch *et al*., 2019). The last constraint can be addressed through combination of PPB with genomic tools such as association mapping. Nevertheless, PPB can enhance gender inclusivity in trait prioritization and scale up community seed systems.

Participatory breeding in common bean is not merely a tool for varietal selection. It is a pathway to democratize breeding, enhance nutritional outcomes, and build climate resilience. Its integration with modern genetic tools and nutritional goals holds promise for sustainable agricultural development.

CHAPTER 3

3. FARMERS' VARIETAL PREFERENCES, PRODUCTION CONSTRAINTS AND PERCEPTIONS OF BIOFORTIFIED COMMON BEAN IN EASTERN DRC

3.1.Introduction

Common bean is consumed by the majority world's poor in Africa and worldwide (Pfeiffer & McClafferty, 2007). The crop is an important mineral supplier, particularly for iron and zinc which have been reported to be involved, with vitamin A, in hidden hunger. Hidden hunger remains a public health challenge affecting more than two billion people worldwide (HarvestPlus, 2015). Children under five years and women of childbearing age are the most at-risk groups.

Iron deficiency is a global burden of anaemia, characterized by a reduction of the volume of red blood cell and a decrease in the concentration of hemoglobin in blood (Mulambu *et al*., 2017). It is caused by a low iron intake particularly during rapid human growth periods and/or increased blood loss. Iron deficiency is also associated with many other health problems including chronic heart failure, kidney disease, cancer and inflammatory bowel disease (Rocha *et al*., 2018). Zinc is another mineral important in physiological functions and involved in immunity and regulation of growth (Caproni et al., 2020).

In Africa, common bean is the most consumed legume in Eastern and Central parts. In Democratic Republic of Congo (DRC), it ranks the first among the food legumes (Mbikayi *et al*., 2018). Other legumes include soybeans and groundnuts.

Apart from its accompaniment with other diets such as cereals, tubers, it is transformed into flour to make porridge or dough and it is used to make soup (Ugen *et al*. 2012).

North and South-Kivu provinces are the most important regions of Eastern DRC where most of common beans are produced and consumed (Civava, 2013). Dense iron and zinc common beans are the best option toward reduction of mineral deficiency in these provinces.

While the yield reported by FAOSTAT (2023) is 0.54 t ha^{-1}, through HarvestPlus programme, high yielding (up to 4.6 t ha^{-1}) iron and zinc biofortified common beans have been introduced in DRC, particularly in South-Kivu (Mbikayi *et al*., 2018 ; Mulambu *et al*., 2017). These genotypes are not widespread all over the country, particularly in North-Kivu province. Hence, farmers are still relying on low yielding varieties that have been in use since decades due to limited access to improved varieties (Kimani & Warsame, 2019) but also due to low involvement of common bean growers and consumers in varietal development processes. Mbikayi *et al*., (2018) have reported 8% of adoption rate of new varieties.

The success rate in adoption and utilization of new lines depends on whether they are accepted and consumed by target population (Chirimubwe *et al*., 2024). Therefore, there is a need to have an understanding of the practices of communities of common bean growers regarding production and utilization of new lines, preferences and attitudes toward adoption of these lines.

The objectives of this study were to:

i) identify farmers' production constraints and their preferred traits in common bean varieties, and

ii) determine farmers' perception on iron and zinc biofortified common bean.

3.2.Materials and methods

3.2.1. Study area

This study was conducted in Eastern Democratic Republic of Congo (DRC), North-Kivu province, particularly in Lubero and Beni territories. Figures reported a population density of 1,703,102 individuals on 17,095 km^2 of area and 1,427,608 individuals on 7,484 km^2 in these territories, in the respective order (2020 Annual Report of the Administration of the territory). They are located on the land that rises into a plateau ranging from 1,100 (western part of Beni territory) to more than 2,500 masl (eastern part of Lubero territory).

Common bean is one of the staple food crops grown in both territories. The choice of North-Kivu province relies on the fact that North-Kivu and South-Kivu provinces are the main provinces where common bean is produced and consumed. The consumption can reach 300 g per individual per day (Civava, 2013). For instance, in South-Kivu the demand per year exceeds production leading to import of beans from Rwanda (Vwima, 2014). Apart from the grains, the plant leaves are also consumed as vegetables.

The climate in Beni and Lubero territories belongs to the temperate tropical type influenced by the relief with slopes and mountains. Temperatures vary between 16.4° and 24°C and sometimes 26°C in the lowest altitudes varying between 870 and 1,250 masl in North and South of "Kyavinyonge" in the Graben Rift Valley (Table 3.1). The highest altitudes vary between 1,900 and 2,400 masl and reach 5,000 masl at the top of Rwenzori Mountain where permanent snow is seen.

Table 3. 1 : Climatic characteristics of North-Kivu province, Eastern DRC in 2023

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Av. temperature (°C)	20.1	20.5	20.1	19.7	19.9	20	20.3	20.3	19.9	19.4	19.2	19.6
Min. mean température (°C)	17.1	17.4	17	16.7	16.9	17	17.1	17.2	17	16.7	16.4	16.7
Max. mean température (°C)	23.4	24	23.6	23.1	23.2	23.3	23.8	23.9	23.5	22.9	22.6	22.8
Rainfall (mm)	196	196	317	292	182	96	54	108	233	384	399	259
Humidity (%)	78	78	84	85	82	76	69	71	79	85	86	82
Days of rains	14	13	19	19	17	11	8	13	18	20	20	17
Hours of sun light	7.3	7.5	6.4	6.5	7.3	7.8	8.6	8.3	7.1	5.8	5.5	6.6

Source : https://fr.climate-data.org/afrique/congo-kinshasa/nord-kivu-1565/r/janvier-1/

The rainfall pattern is bimodal. The short rain season occurs from late February to May, while the long rain season occurs from early September to late November or early December. June and January are the hottest months of the year.

Soils of alluvial plains and ferrosols are the important types found in North-Kivu province. In the valleys and alluvial plains, soil is black with an important fraction of sand supplied with water and moisture, while on the slopes there are shallow soils (Sahani, 2011).

The geographical location of the study region is presented in Figure 3.1, showing the map of North-Kivu province.

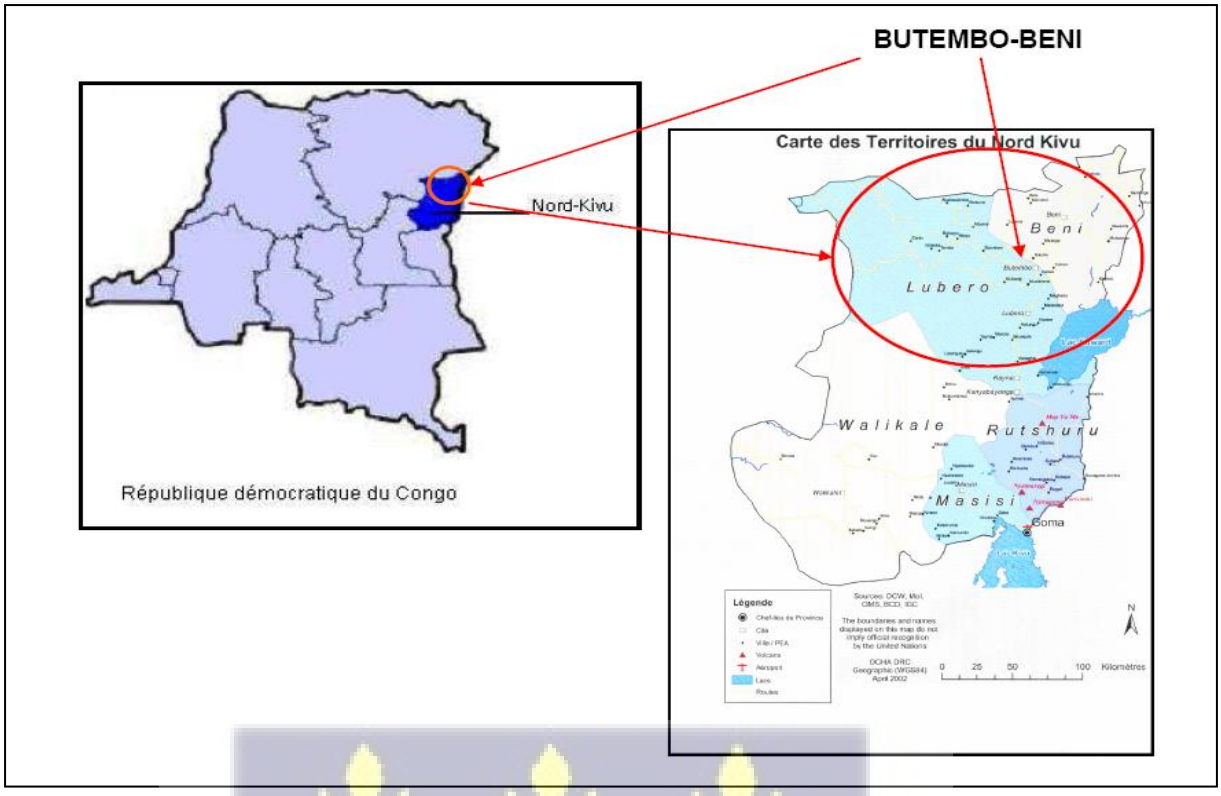


Figure 3. 1 : Map of Lubero and Beni territories in North-Kivu, Eastern DRC

Source: Annual Report of the Administration of the territory, 2020

Vegetation in the lower elevations is dense forest that predominates followed by the savanna shrubs and bamboo, while in the medium and high altitude (over 4,000 masl) mountains have low vegetation density.

3.2.2. Sampling

Surveys were conducted between May and September 2022 by a team comprising a breeder (the principal investigator) and two enumerators. The targeted population was constituted by the population of Beni and Lubero territories of North-Kivu province. These territories comprise more than 200 villages. A purposive sampling procedure was employed to identify villages in important axes where surveys were conducted. Axes considered are those where dense agricultural activities on common bean are concentrated and where there is no insecurity. The non-random selection of villages is

due to the dense insecurity affecting some regions in the study territories and the degraded status of roads. The sample interviewed was selected from a population of farmers who have grown or are growing common bean as a sole or a mixed crop in the study area. Selected farmers were those who have grown common bean for at least two years in the community.

Considering the percentage of common bean growers being high in Lubero territory (≥90%) and ≥80% in Beni territory (Vyakuno, 2006), the minimum sample size could have been constituted by 246 individuals (with p being 0.8) using Anderson's formula such that $n = \frac{z^2pq}{d^2}$ where n is the minimum sample size, z is 1.96 at 95% confidence interval, q the weighting variable computed as 1-p and d the acceptable error of 0.05 (Waluse, 2012). To reduce the likelihood of bias, sample size should be larger (Taherdoost, 2020). In this study, 320 individuals were interviewed, 180 in Lubero territory and 140 in Beni territory.

In Lubero territory, apart from the region surrounding Butembo town, two important axes were considered. These axes comprised Ngeleza-Luotu-Kitsuku-Magheria-Masereka-Kipese and Musienene-Lukanga-Lubero. In Beni territory, five axes were considered: Bunyuka-Isale, Vuhovi-Kyondo, Bingo-Kyanzaba-Mangina, Mabalako-Cantine and Bulongo-Mutwanga (Table 3.2).

Table 3. 2 : Numbers of respondents in each axis of Lubero and Beni territories

Territory	Axis	Number of respondents
Lubero	Ngeleza-Luotu-Kitsuku-Magheria-Masereka-Kipese	90
	Musienene-Lukanga-Lubero	60
	Region surrounding Butembo town	30
Beni	Bunyuka-Isale	25
	Vuhovi-Kyondo	30
	Bingo-Kyanzaba-Mangina	30
	Mabalako-Cantine	15
	Bulongo-Mutwanga	40

Information on geographic position was gathered using a global positioning system (GPS), while information on current rainfall and temperature patterns and types of soil was provided by the office of the Ministry of Agriculture.

3.2.3. Data collection

Information was collected on production practices, varieties grown and their attributes, productivity and utilization. Farmers were also asked about their production constraints, as well as perceptions on iron and zinc biofortification of common bean.

A mixed questionnaire was used where some questions had fixed responses and for others, respondents had to give their own opinions (Appendix 1). Questions were translated into the local languages ("Swahili" or "Kinande") to facilitate communication. This was done either by one of the research team members or a participant in the group of farmers.

3.2.4. Data analysis

Qualitative data was analyzed using Statistical Package for Social Science (SPSS), 15th version.

To get an insight toward analysis of data and interpretation of results, the study parameters including varietal attributes, production constraints, perceptions about iron and zinc in common beans and other relevant information were grouped under appropriate headings. To derive any other information that was not necessarily highlighted within the contents of the questionnaire, a flexible approach was used.

Factorial correspondence analysis was constructed to map how constraints to common bean production relate to each axis in Beni and Lubero territories. A one-sample t-test was performed using Statistix, 8th version, to picture how the yield recorded across various axes in both territories deviated from the minimum expected threshold (1,000 kg ha^{-1}).

3.3.Results

3.3.1. Respondents' characteristics, cropping system and purpose of common bean growing

Married female farmers were more important within the surveyed regions. These farmers did either primary or secondary school. The age for most respondents varied from 36 to more than 55 years (Table 3.3).

The sizes of farms for most farmers in Lubero territory are less than 0.25 ha. However, in axes of Beni territory, sizes of farms varied, for more than 23% of respondents, from 0.25 to 0.5 ha. More than 6.5% of farmers, particularly in Beni territory, use between 0.5 to 1 ha of land and few, particularly in Bingo-Kyanzaba-Mangina and Bulongo-Mutwanga axes use more than 1 ha. A land size of less than 0.25 ha is allocated for common bean in Lubero territory, while in Beni territory, except in Mabalako-Cantine axis, 5% of farmers and more allocated 0.25 to 0.5 ha of land to common bean. In

Bingo-Kyanzaba-Mangina axis, 33.3% of farmers allocated 0.25 to 0.5 ha to common bean cultivation.

For most of farmers surveyed, common bean is grown intercropped with other crops. These crops included maize, cassava, sweet potato and banana. Farmers do not use any fixed spacing or mineral fertilizer for common bean production. Common bean is grown as cash and a food crop.

Table 3. 3 : Respondents' characteristics, farm size, cropping system and purpose of common bean cultivation (%)

Variable	Position	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7	Axis 8
Gender	Male	9.3	10.4	5.9	11.4	12.1	22.5	17.8	24.6
	Female	90.7	89.6	94.1	88.6	87.9	77.5	82.2	75.4
Marital status	Single	3.3	5.0	10.0	12.0	6.7	13.3	13.3	10.0
	Married	91.2	90.0	80.0	86.0	80.0	73.4	73.3	77.5
	Divorced	0	0	3.3	0	3.3	3.3	6.7	2.5
	Widowed	5.5	5.0	6.7	4.0	10.0	10.0	6.7	10.0
Education level	No formal	11.1	11.6	13.4	12.0	10.0	13.3	20.0	12.5
	Primary	70.0	51.7	43.3	48.0	46.7	40.1	46.7	47.5
	Secondary	16.7	35.0	40.0	40.0	43.3	43.3	33.3	37.5
	Tertiary	2.2	1.7	3.3	0	0	3.3	0	2.5
Age	Below 25	4.4	3.3	0	8.0	13.3	13.3	0	7.5
	25-35 years	10.0	6.7	10.0	20.0	20.0	20.0	13.3	12.5
	36-45 years	31.1	25.0	26.7	36.0	23.3	23.3	33.3	25.0
	46-55 years	21.2	30.0	26.6	20.0	26.7	26.7	33.3	22.5
	Beyond 55	33.3	35.0	36.7	16.0	16.7	16.7	20.0	32.5
Farm size	Below 0.0625 ha	28.9	32.2	66.6	36.0	16.7	10.0	13.3	20.0
	0.0625-0.25 ha	65.5	61.1	26.7	32.0	53.3	33.3	40.0	20.0
	0.25-0.5 ha	4.4	5.5	6.7	24.0	23.3	40.0	40.0	30.0
	0.5-1 ha	1.1	1.1	0	8	6.7	13.3	6.7	22.5
	Beyond 1ha	0	0	0	0	0	3.3	0	7.5
Size for common bean	Below 0.0625 ha	65.5	70.0	86.7	32.0	33.3	23.3	46.7	30.0
	0.0625-0.25 ha	34.5	30.0	13.3	52.0	60.0	43.4	53.3	65.0
	0.25-0.5 ha	0	0	0	16	6.7	33.3	0	5.0
Cropping system	Monocrop	33.3	28.3	40.0	32.0	33.3	30.0	13.3	27.5
	Intercrop	66.7	71.7	60.0	68.0	66.7	70.0	86.7	72.5
Purpose of bean cultivation	Cash	35.5	31.7	26.7	28.0	23.3	30.0	40.0	20.0
	Food	33.3	33.3	53.3	20.0	30.0	26.7	26.7	25.0
	Cash & food	31.1	35.0	20.0	52.0	46.7	43.3	33.3	55.0

Axis 1 – Ngeleza-Luotu-Kitsuku-Magheria-Masereka-Kipese; Axis 2 – Musienene-Lukanga-Lubero ; Axis 3 – region surrounding Butembo town in Lubero territory ; Axis 4 – Bunyuka-Isale ; Axis 5 – Vuhovi-Kyondo ; Axis 6 – Bingo-Kyanzaba-Mangina ; Axis 7 – Mabalako-Cantine ; Axis 8 – Bulongo-Mutwanga in Beni territory

3.3.2. Major common bean varieties, yield and constraints to production

Common bean growers in Lubero and Beni territories did not use pureline varieties but a mixture of varieties. Even though growers preferred bush varieties than climbing varieties because of fearing charges of climbers, in Lubero territory, bush and climbing bean varieties were grown as well because climbers are available. However, in Beni territory, only bush varieties were grown because of a low availability of climbers. Except Mafutala, all the varieties listed in Table 3.4, have been in use for quite a number of years. All the varieties were said to be low yielding particularly in Lubero territory.

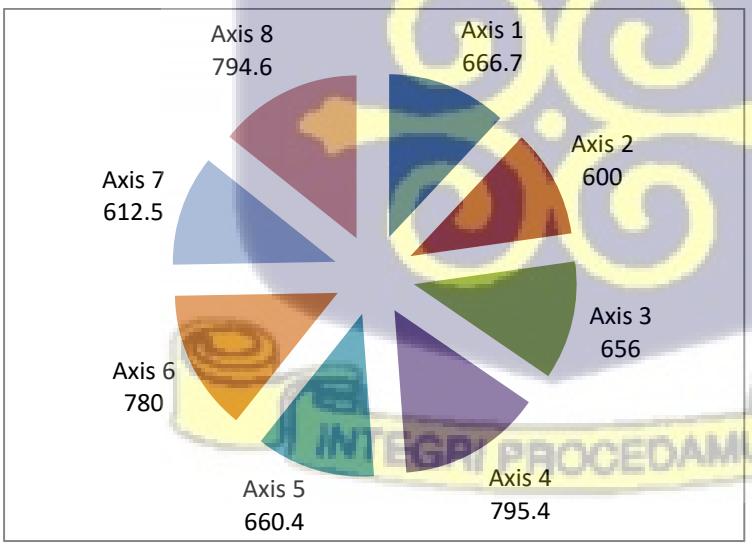
Table 3. 4 : Major common bean varieties grown in Lubero and Beni territories

Variety	Frequency (%) of use	Good trait	Bad trait
Lubero territory			
Kipumbafu	5.1	Large seeded	-
Kinimba	87.2	Good taste	Susceptible to pests
Museselo	12.8	Good adaptation	-
Kalangiti	15.4	Good taste	-
Demai	12.8	Good taste	-
Mafutala	2.6	Cook fast	-
Munyama	7.7	Early maturing	-
Musali	2.6	Large seeded	
Lodone	5.1	Cook fast	-
Kipisa	5.1	-	-
Kambine	5.1	Good taste	Black seed
Beni territory			
Maragi Kalanga	50	Good taste	-
Haricot blanc	41.7	Good taste, Cook fast	Break down at cooking
Kambine	16.7	Good taste	-
Kitenge	50	Good taste	-
Kyaregere	8.3	Cook fast	-
Malisawa	8.3	Good taste	-
Demai	8.3	Good taste	-
Kavundayiri	16.7	Good taste	-
Manzenene	8.3	Cook fast	-
Kinimba	8.3	Good taste	Susceptible to pests
Vijaune	33.3	Cook fast	Break down at cooking

Kalangiti was said to accelerate blindness of eyes. Kinimba was the most represented in mixtures of varieties commonly grown and used in Lubero territory, while Mafutala was the least represented.

In Beni territory, Maragi Kalanga, Kitenge and Haricot blanc were the most represented in mixtures of varieties used. The unique variety used solely is "Vijaune", a climbing variety with yellow seed coat, essentially cultivated as a cash crop because of its high price compared to others.

The seed yield of common bean is significantly lower ($p<0.0001$) than the minimum expected threshold (1,000 kg ha^{-1}). Among the recorded yields across all axes, high yields were observed in axes 4, 6 and 8 within Beni territory, whereas axis 2 in Lubero territory registered a low yield. At overall, yield of common bean in Lubero and Beni territories never reached 800 kg ha^{-1} in any of the axes surveyed (Figure 3.2). When analyzed by territory, Beni recorded a higher yield (728.6 kg ha^{-1}) compared to Lubero (640.9 kg ha^{-1}).



Sample mean	708.2
Expected yield	1000
Minimum	600
Maximum	795.4
Standard deviation	84.3
t value	-9.79
P-value	<0.00

Axis 1 – Ngeleza-Luotu-Kitsuku-Magheria-Masereka-Kipese ; Axis 2 – Musienene-Lukanga-Lubero ; Axis 3 – region surrounding Butembo town in Lubero territory ; Axis 4 – Bunyuka-Isale ; Axis 5 – Vuhovi-Kyondo ; Axis 6 – Bingo-Kyanzaba-Mangina ; Axis 7 – Mabalako-Cantine ; Axis 8 – Bulongo-Mutwanga in Beni territory

Figure 3. 2 : Yield (kg ha^{-1}) of common bean by axes averaged over Lubero and Beni territories

A number of constraints to common bean production in Lubero and Beni territories were reported. The most important constraints reported were low yield, pests and diseases, changes in climatic occurrences, poor soil fertility, animal wandering destroying the crop and small farm sizes.

Factorial correspondence analysis showed that poor soil fertility is an important constraint reported in Ngeleza-Kipese (Axis 1), Vuhovi-Kyondo (Axis 5) and Musienene-Lubero (Axis 2). Small farm size is most reported in Lubero territory and particularly in Ngeleza-Kipese axis. Low yield is most reported in Mabalako-Cantine axis (Axis 7), while changes in climatic occurrences represent an important constraint reported in Bunyuka-Isale and Bulongo-Mutwanga axes (Axes 4 and 8). Pests and diseases are factors constraining common bean production in Bulongo-Mutwanga axis, Bingo-Mangina axis and regions surrounding Butembo town. Wandering of animals such as goats decimates common bean crops and thereby represented a constraint to common bean production and occurred the most in regions surrounding Butembo town (Figure 3.3).

Others constraints reported included conflicts between different land owners, robbers, poor management practices, no improved seeds and charges associated with climbers.

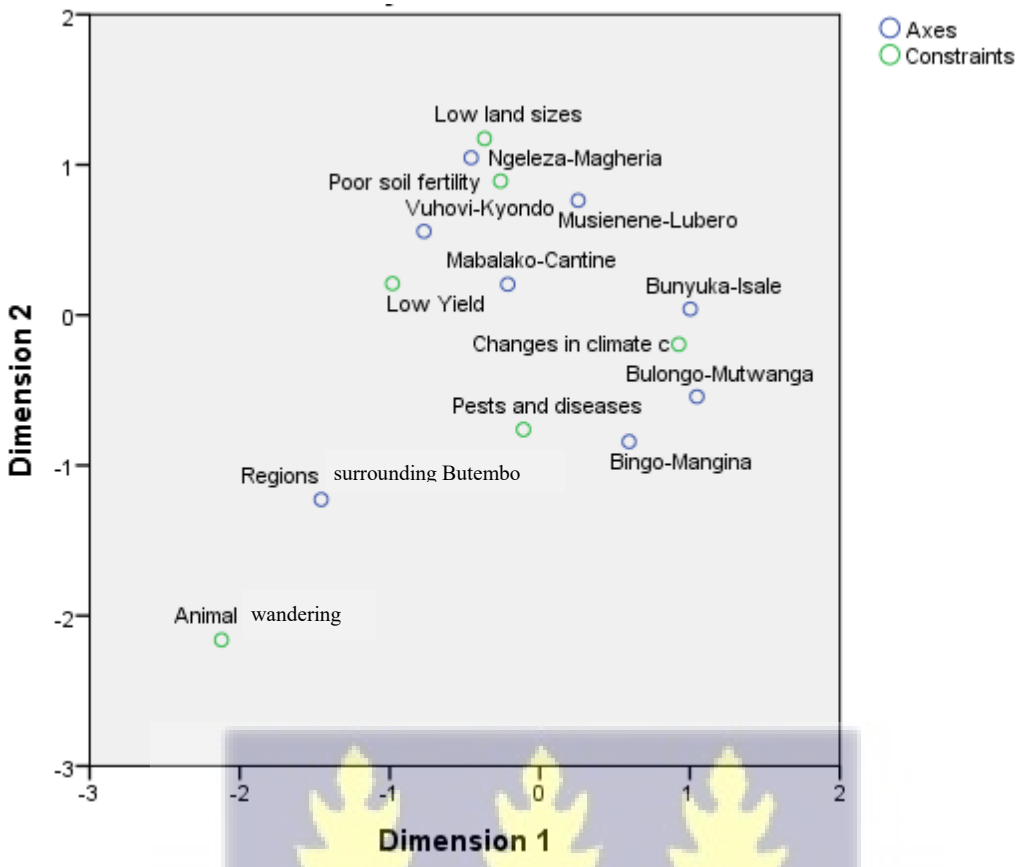


Figure 3. 3 : Factorial correspondences of common constraints in different axes of Lubero and Beni territories

3.3.3. Common bean productivity, utilization and marketing

Common bean growers in Lubero and Beni territories do not use neither fertilizers nor pesticides. A few farmers use only organic manure from livestock. The rate and nature depend upon the type and size of the livestock. But the rate never exceeded three plastic bags (60 kg at average) applied to the field occasionally, generally once a week, no matter the farm size is (Table 3.5). In general, the minimum land sizes farmers cultivated was 625 m^2.

The essential means by which producers get access to information on common bean farming was through parents. Producers reproduce what they saw or learnt from their parents or fellow farmers. The primary source of seeds was the market from

neighboring famers. From that point, farmers extracted seeds for the next seasons from subsequent productions (Table 3.5).

About one out of three farmers had heard about improved common bean varieties. However, it was from informal source, a neighbor or any group member. A few had planted improved common bean varieties. Nevertheless, farmers were willing to plant improved common bean varieties. The traits expected from improved varieties were in the decreasing order: high yield, resistance to abiotic stresses, early maturity and pest and disease resistance. In addition, farmers did not like common bean seeds that broke down when cooked. Few of farmers were just contented with the local varieties. Most of these farmers were the ones that have never used improved varieties or those who had bad perceptions on introductions.

Reduction in current yields compared to former yields was evident. For more than a half of respondents, the yield has been split to two.

Famers had to walk to get access to market. Few used motor bikes and bicycles. At harvesting, they all marketed dry common beans individually. When dry common beans were sold, money was mostly kept by parents to satisfy different charges that the family endorsed. Important common bean customers were represented by local traders and traders from far (towns) who met producers in local markets, the important mode of sale being by cash.

Table 3. 5 : Distribution of farmer responses on input use, variety awareness, and market engagement in Lubero and Beni territories

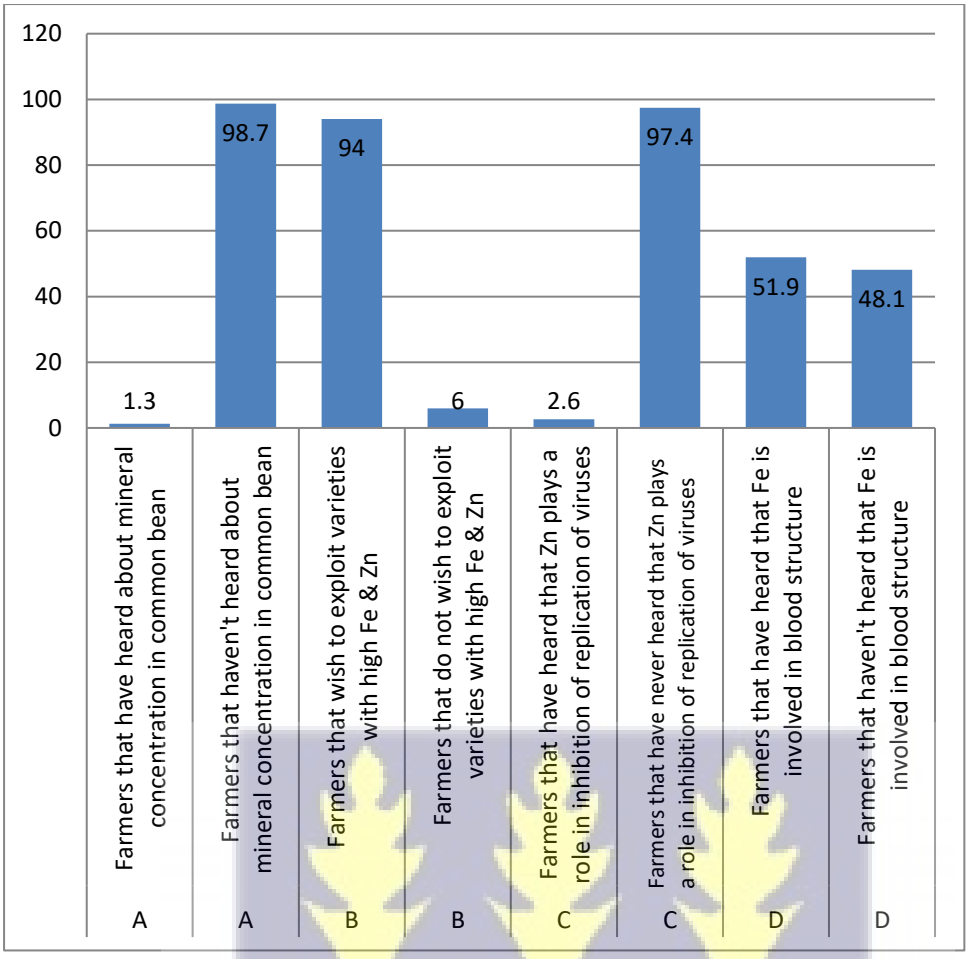
Variables	Farmer's position	Percentage
Use of inputs	No inputs used	93.6
	Use of manure	6.4
Means used to access information on common bean farming	Radio	1.9
	Fellow farmers	7.7
	Neighbor	1.3
	Group members	3.8
	Information from village	2.1
	Others : from parents	83.1
Source of planting material	Neighboring famers	45.4
	Agro vet	0.1
	Market	54.5
Information about improved varieties	Growers aware about improved varieties	30.1
	Growers not aware	69.9
Positions of farmers toward improved varieties	Farmers that have planted improved varieties	7.1
	Farmers that never planted improved varieties	92.9
Willingness to plant improved varieties	Farmers willing to plant improved varieties	83.7
	Farmers contented with local varieties	16.3
Reduction of yield experienced in last 1-2 years	Reduction of yield experienced	58.8
	Reduction of yield not experienced	41.2
Reduction of yield experienced in last 3-5 years	Reduction of yield experienced	65.9
	Reduction of yield not experienced	34.1
Means of travel to access to market	Walking	69.2
	Bicycle	12.8
	Motorbike	16.7
	Public vehicle	1.3
The money keeper after sale	Wife	64.7
	Husband	32.7
	Child-girl	1.3
	Child-boy	1.3
Important common bean customers	Local trader	55.1
	Trader from far	41.7
	Other farmers	3.2
The place where the sale is done	Urban market	1.9
	Local market	80.8
	Production site	7.1
	Others	10.2
Mode of sale	Credit only	4.5
	Cash only	89.7
	Both	5.8

3.3.4. Perceptions of iron and zinc biofortification in common bean

Most of farmers (98.7%) surveyed in Lubero and Beni territories had never heard about mineral levels in common bean but protein levels and did not know any known and reported iron and zinc biofortified common bean variety (Figure 3.4). However, most farmers wished to exploit varieties with high levels of iron and zinc. Only 6% of the farmers were not ready to exploit biofortified common beans.

Generally, farmers did not know about the role of zinc for humans. However, few farmers referring to the fact that they are frequently recommended to buy zinc in pharmacies for their children, assumed that zinc might be involved in increasing immunity.

In regard to the level of farmers' perceptions on the role of iron, about a half knew that iron is involved in blood structure in reference to the tablets women use during their pregnancies. Farmers who did not know the role of iron were mainly the male respondents.



A – Farmers' position about mineral concentration in common bean ; B – Farmers' position about cultivation of Fe and Zn biofortified common beans ; C – Farmers' position about the role of Zn in inhibition of replication of viruses ; D – Farmers' position about the role of Fe in blood structure

Figure 3. 4 : Level (%) of farmers' perceptions on iron and zinc concentration in common bean

3.4.Discussion

3.4.1. Farmers' constraints in common bean production

Several constraints were reported as the important drawbacks to common bean production in Lubero and Beni territories. Important constraints included low yields, pests and diseases, low farm sizes, poor soil fertility, changes in climatic occurrences, and animal damage.

The yield of common bean in Lubero and Beni territories were low and never reached 800 kg ha^{-1}. This is a very low yield compared to the yields reported. Kimani &

Warsame (2019) have reported 3,323 kg ha^{-1} associated with iron and zinc biofortified common bean in Kenya. Mondo *et al*. (2019) observed more than 3,323 kg ha^{-1} of yield in their study. Amongi *et al*. (2018) observed over 2,900 kg ha^{-1} of grain yield in their study. Mulambu *et al*. (2017) also observed more than 4,500 kg ha^{-1} for some of the common bean varieties considered in their study. The reason why yield is low in Lubero and Beni territories might be because farmers still use varieties that have been in use since decades as a result of lack of improved varieties. Prolonged use over multiple growing seasons compromises disease resistance, resulting in markedly reduced yields (Kimani & Warsame, 2019).

Reduction in current yields compared to former yields was found evident. Yields have considerably decreased, due to inbreeding depression as a result of using same varieties for decades (Acquaah, 2012), no available improved varieties, no use of fertilizers and pesticides (Table 3.5).

Yield of common bean in Lubero territory was lower than that in Beni territory. Regions of Beni territory should be ecologically suitable for common bean. This should be due to low acidic soils in these regions as a result of low rainfall amounts in low altitudinal positions and low mineral leaching. Soil pH in regions of Beni territory should range between 5.5 and 6.5. This is the limit under which common bean grows better (Salcedo, 2008). According to figures available on the NASA (2023) website, Lubero territory receives high amounts of rainfall annually (1,250 to 1,510 mm) than Beni territory (800 to 1,200 mm). Axes 4, 8 and 6 in Beni territories were associated with high yields. These axes should represent ecological zones suitable for common bean. In addition, Farrow & Muthoni-Andriatsitohaina (2020) reported that the upmost common bean productions are obtained at mid-altitudes (between 1,000 and 1,500 masl). Altitudes

above 1,500 masl are also suitable for high grain yields (Farrow & Muthoni-Andriatsitohaina, 2020).

Yields observed in Beni might also be a result of different fertility levels. Due to geographical position of these territories, soil of Beni territory should be more fertile than that of Lubero territory. This is due to important soil erosion in high altitudes. This is in line with Sahani (2011) who reported the importance of erosion in reduction of soil fertility, particularly in high altitudes of Lubero in Eastern DRC.

Low yields might also be a consequence of the no use of fertilizers or organic manure and pesticides. This is consistent to results of Figure 3.2 that reported low yields compared to the figures that have been observed (Kimani & Warsame, 2019 ; Mondo *et al*., 2019 ; Mulambu *et al*., 2017), particularly in fertilized farming systems. The quantity of organic manure (about 60 kg applied on 625 m^2 usually) that few farmers use is low. Per a hectare, Adamu *et al*. (2020) recommends a minimum rate of 10 tons and a maximum rate of 25 tons. The no application of fertilizers and pesticides is a behavior that growers copied from their progenitors or fellow farmers

Another reason that would constrain common bean yield in these territories is the source of seeds. While seeds would come from seed companies and research institutions, the primary source of seeds reported was the market from neighboring famers and then from subsequent productions. This is how inbreeding depression gets important from a season to another and induces low yields and breaks resistance of common bean varieties (Acquaah, 2012). Consequently, farmers use mixtures of seeds as they do not know which specific varieties will be suitable and resistant to ecological adversities and more productive. While in Beni territory, only bush varieties are used, in Lubero territory, climbing and bush beans are used. In Lubero terrirory, farmers prefer climbing

varieties. This is due to yield associated with climbing beans compared to bush beans. According to Nadeem *et al*. (2021), yield for bush common bean types rarely reaches 1,500 kg ha^{-1}, while high yielding climbing common beans can produce more than 3,500 kg ha^{-1} in suitable environments. In addition, Lubero territory is at altitudes higher than 1,700 masl where climbers are available on *Eucalyptus saligna*, the most represented tree species in this region.

Common bean production was also constrained by the unavailability of land sizes. Small farm sizes used might be linked to the context of insecurity that these territories face inducing important migrations of people and its concentration in particular geographic locations judged secure. For instance, farm sizes in Lubero territories were narrow than those in Beni territory. This might be explained by the intense insecurity that exists in Beni territory for more than two decades. Hence, people have to shift from regions where insecurity is severe to the places in Lubero judged secure. During the last decade, insecurity caused by Allied Democratic Forces, a rebel movement operating the most in Beni territory led to migration of people from insecure regions of Beni territory to Beni town and to some regions of Lubero judged more secure (Secretary-General, U. N., 2024). This might be the consequence of intercropping common bean with other crops such as cereals and tubers as well. Nevertheless, Mulugeta (2011) reported that cereal/common bean is the intercropping system widely used.

Poor soil fertility was highlighted as an important constraint reported in Ngeleza-Kipese, Vuhovi-Kyondo and Musienene-Lubero axes. This seems to be due to high altitudes of these locations. Using a GPS, altitudes increase from 2,088 masl at Ngeleza to 2,600 masl at Kipese. Altitudes in Vuhovi-Kyondo axis vary from 1,500 masl at

Vuhovi to 2,168 masl at Kyondo. In Musienene-Lubero axis, altitudes vary from 1,863 masl at Musienene to 1,871 masl at Lubero.

Small farm size is most reported in Lubero territory particularly in Ngeleza-Kipese axis. This might be attributed to a high population density in Lubero territory as a result of severe insecurity in Beni territory where populations have to migrate in Lubero territory. Figures reported, in 2020, a population density of 1,703,102 individuals in Lubero territory over 1,427,608 individuals in Beni territory (2020 Annual Report of the Administration of the territory). With intense insecurity in Beni territory, the density might have considerably increased in Lubero territory.

The study reported yields lower than the minimum threshold expected (1,000 kg ha^{-1}). Across axes in both territories, low yield was most reported in Mabalako-Cantine axis. This might be due to the fact that rice is the crop most common in this axis; hence, farmers, not interested in common bean, still use poor yielding common bean varieties. Changes in climatic occurrences were important in Bunyuka-Isale and Bulongo-Mutwanga axes. In these locations, sometimes rains with high magnitude destroy common bean, while on the other hand, drought spoils low drought tolerant crops, particularly common bean varieties in use. Pests and diseases were factors constraining common bean production in Bulongo-Mutwanga and Bingo-Mangina axes and regions surrounding Butembo town. Pest damages might be due to insects which attack common bean embryos in the soil. But damping off is another constraint that should be the utmost reason damaging common bean at germination. Diseases might be the leaf spots, root rots, anthracnose and common bacterial blight which are the most frequently reported in Eastern and Central Africa (Kimani & Warsame, 2019 ; Saettler, 1991). With observed spots and rots leading to drying of common bean, angular leaf spots and root rots might be the most important.

Wandering of animals such as goats was reported as the most constraint to common bean production in regions surrounding Butembo town. This is due to the fact that in towns, farms where to feed animals are not available. People, therefore, rely on leaving animals to wander and feed themselves at their own.

3.4.2. Farmers' preferred traits in improved common bean varieties

About one out of three farmers has heard about improved common bean varieties from either a neighbor or any group member. A few have planted improved common bean varieties. This is because there are no competitive means to get access to information on common bean farming and good management practices. This leads some farmers to fear whether improved varieties wouldn't impact soils negatively.

Farmers are willing to adopt common bean introductions, anticipating yields exceeding 1,000 kg ha^{-1}. These introductions are expected to demonstrate improved resistance to abiotic stresses, particularly drought. This trait is especially relevant in specific zones of the Beni territory, particularly in Bunyuka-Isale and Bulongo-Mutwanga axes where recent shifts in climatic patterns have been observed. These areas have experienced increasingly erratic weather conditions (Figure 3.3), including intense and unpredictable rainfall events as well as prolonged droughts. Heat and higher temperatures, frequent in lowlands represent serious drawbacks to common bean production (Farrow & Muthoni-Andriatsitohaina, 2020). Except Bunyuka which is at 1,848 masl, these regions are at altitudes varying from 1,100 to 1,200 masl.

In axes of Lubero territory, high yielding and early maturing varieties are the most expected improved varieties. In Musienene-Lubero axis, common bean takes four months to mature, while in Masereka and Kipese in Ngeleza-Kipese axis, it takes up to six months to mature. This is due to their high altitudinal positions and low

temperatures. Masereka and Kipese are at 2,500 and 2,600 masl, while Musienene and Lubero are at 1,863 and 1,871 masl. In addition, in these axes, temperatures vary from 13 to 24°C, while in most of axes of Beni territory, temperatures vary from 16.5 to 33.5°C ; hence, metabolic processes of plants are accelerated. This is in line with Mondo *et al*. (2019) and Singh *et al*. (2002) who observed accelerated common bean growth and early maturity in lowlands and delayed maturity in cooler conditions particularly in highlands.

Few of farmers are just contented with local varieties. This might be due to the lack of field day in these regions. A field day is one of the means of teaching and learning process of improved technologies. Mulugeta (2011) reported that farmers attending field day of improved varieties are more likely to use these varieties. In Lubero and Beni territories, farmers fear, in fact, introductions because they do not have information on attributes of introductions and have never participated in any activity where these genotypes are tested. In addition, improved varieties that can exist are not disseminated through formal means such as organizations or research institutions but they belong to some individuals who brought them from somewhere through unformal means.

3.4.3. Perceptions of iron and zinc biofortified common bean

Findings revealed that surveyed farmers in Lubero and Beni territories in North-Kivu province, Eastern DRC have never heard about mineral levels in common bean. But they have heard about protein availability in common bean such that this crop substitutes meat for poor people in these regions. This is because iron and zinc biofortification programme has never spread over the country. Iron and zinc biofortification programme in common bean operated during the period from 2009 to

2013 in four provinces (Mulambu *et al*., 2017). The North-Kivu was not part of them. It is therefore evident that farmers in these territories do not know specifically any iron and zinc biofortified common bean variety.

Few farmers, not willing to exploit iron and zinc biofortified common bean varieties, fear about negative impacts introductions might cause due to bad perceptions heard from their ancestors or progenitors. According to them, introductions may lead to soil unproductivity.

Farmers did not know that zinc is involved in inhibition of replication of viruses. This might be due to low education level in these territories (Table 3.3). However, they know that iron is involved in blood structure. The reason might be the importance that iron has gained particularly during pregnancy whereby pregnant women received pieces of advices and are required to take iron tablets. This is evident because farmers who did not know the role of iron in the organism were mostly among the male respondents.

3.5.Conclusion

Six constraints were identified in Lubero and Beni territories in North-Kivu province, Eastern DRC as the important drawbacks to common bean production. These constraints are low yields, pests and diseases, low farm sizes, poor soil fertility, changes in climatic occurrences, and wandering of animals decimating common bean crops. Causes of constraints were diverse including the absence of fertilizer and pesticide use, reliance on informal and outdated seed sources, insecurity and land disputes, inadequate infrastructure, and weak institutional support.

From the study, four major traits were desired by farmers in improved varieties. The foremost trait expected was the yield that should be high compared to that for the existing varieties. Other traits included the resistance to abiotic stresses, early maturity

and pest and disease resistance. In addition, seeds of improved varieties expected by farmers should not break down when they are cooked.

Farmers in Lubero and Beni territories had never heard about mineral levels in common bean but a half of them knew that iron is involved in blood structure in reference to the tablets women use during their pregnancies. But they did not know that zinc is involved in inhibition of replication of viruses. In addition, they did not know and had never used any known and reported iron and zinc biofortified common bean variety but wish to exploit dense iron and zinc varieties to reduce deficiencies in these minerals.

CHAPTER 4

4. COOKING TIME AND QUALITY OF HIGH PERFORMING SECOND-GENERATION BIOFORTIFIED COMMON BEAN IN DEMOCRATIC REPUBLIC OF CONGO (DRC)

4.1.Introduction

Common bean (*Phaseolus vulgaris* L.) is an important legume crop, source of daily proteins for almost half a billion people (Farrow & Muthoni-Andriatsitohaina, 2020). It represents the species of choice for the study of grain legume nutrition as half of the grain legumes consumed worldwide are common beans (Broughton *et al*., 2003). Common bean is consumed by 83% of the population of the Democratic Republic of Congo (Mbikayi *et al*., 2018).

A good analysis of factors influencing the acceptance of novel varieties should be done where farmers and consumers are involved in testing processes. Mulugeta (2011) listed high yield potential, duration to maturity, resistance to pests and diseases, grain color, grain size and storability as the main factors influencing adoption of novel varieties of common bean at farmers' level.

Consumer preferences are also determinant factors influencing adoption of novel varieties. These preferences include visual characteristics such as seed color and shape and culinary properties such as cooking time and texture of cooked beans (Bassett *et al*., 2017). Asiimwe *et al*. (2024) stated that the cooking time, taste, grain color and bean swelling on cooking are important attributes for consumers. Short cooking time is widely emerging as a consumer preferred character for variety acceptability and adoption (Yadji Haman *et al*., 2020). In Eastern Africa, due to scarcity of firewood and reduction in resources, fast cooking bean varieties would be a means to reach food

security (Petry *et al*., 2010). In addition, prolonged cooking time leads to important losses of proteins, starch and iron (Bassett *et al*., 2017).

Even when farmers are well involved in variety development, high yielding and disease resistant varieties newly developed are not necessary adopted at farmers' level when they lack consumer preferred traits such as short cooking time and good taste (Bassett *et al*., 2017). In addition, consumers do not prefer varieties that break at cooking (Mulugeta, 2011).

The second-generation biofortified common bean genotypes are a result of iron and zinc biofortification programme that was initiated in 2001 at the University of Nairobi (Kimani *et al.,* 2008). These varieties are reported to cook fast and give high yields of more than 4 t ha^{-1} (Kimani & Warsame, 2019 ; Mondo *et al*., 2019). Université Catholique du Graben of Butembo (DRC) requested for and received 285 lines, which were evaluated in destination site and assessed for cooking time together with other quality characteristics.

The objectives of this study were to:

i) assess agronomic performance of second-generation biofortified common bean genotypes, and
ii) determine the cooking time and quality of cooked beans of high performing second-generation biofortified common bean genotypes.

4.2.Materials and methods

4.2.1. Experimental sites

Field experiments were carried out in Butembo and Lukanga during the 2022 long rain season between October 2022 and January 2023. In Butembo, experiments were conducted at the field station of the Université Catholique du Graben (UCG-Butembo)

located at 0.1238815° latitude N, 29.268894° longitude E at 1,762 m above the sea level (masl). At Lukanga, studies were carried out in the field station of the Université Adventiste de Lukanga (UNILUK) at 0°3' latitude S, 29°1.8' longitude E at 1,952 masl in Lukanga city. Determination of cooking time and hydration coefficient were done in the laboratory of UCG-Butembo located at the University campus in Butembo in March 2023.

4.2.2. Experimental materials

Two hundred and eighty-five (285) second-generation biofortified common bean genotypes were used for field trial together with five local varieties. Biofortified genotypes were obtained from the University of Nairobi and their agronomic description has been studied by Kimani & Warsame (2019) and Mondo *et al*. (2019).

According to these authors, the average yield performance ranged from 0.8 to more than 4.0 t ha^{-1} across different test sites of Kenya. These sites include Kabete, Nakuru, Thika, Mwea and Tigoni. Using an Automated Mattson Bean cooker, Kimani & Warsame (2019) reported that these genotypes take less than an hour to cook.

4.2.3. Experimental design and crop management

Test genotypes were evaluated in a rectangular augmented design where only local varieties were replicated. Five blocks were used with block containing 57 biofortified genotypes and the five local varieties. Checks were involved to allow farmers make comparison between the later and biofortified genotypes.

An experimental unit was represented by a single row of 2.70 m containing 10 hills 30 cm apart each from another. The inter-row distance was 60 cm. A hill received two seeds. Thinning out to one plant per hill was carried out two weeks after emergence. Diammonium phosphate (18% N and 46% P_2O_5) was applied at planting at 150 kg ha^{-}

[1] as recommended by Hundie *et al*. (2000) cited by Kataliko *et al*. (2018). Plots were maintained weed free by manual weeding. Manual weeding was done at 25-days interval. Experimental conditions were what farmers used such that neither insecticide nor fungicide was applied. Harvesting was done when pods dried for each genotype.

4.2.4. Seed preparation for cooking and quality assessment

After harvesting, seeds were sun dried for three days and stored for one month. Thereafter, seeds for each genotype were sorted to remove broken, wrinkled and rotten seeds. Using an electronic computerized balance (WT-N Serries, WANT Balance Instrument Co., Ltd, Jiangsu, China), a sample weighing of 250g each genotype was soaked in water. As recommended by Munthali *et al*. (2022), equal volume of water is needed and should be high enough to cover the amounts absorbed during hydration of beans. In this study, equal volume of 1,000 ml of water was used in each soaking plastic container.

4.2.5. Data collection

Vigorous genotypes selected by farmers were used to determine hydration coefficient, cooking time (minutes) of dry beans, integrity of beans at cooking (%), particularly on selected genotypes. In addition, data collection was extended to visual evaluation and sensory parameters of cooked beans of selected genotypes.

Two trips were organized in each location where 25 farmers selected genotypes that they judged vigorous. The first trip was undertaken at flowering and the last at harvesting. During flowering, farmers selected genotypes whose plants were judged healthy and vigorous. Meanwhile, the color of flowers was mentioned during this activity. At maturity, selected genotypes at flowering that did not have high pod numbers were discarded. Farmers were interested in large numbers of pods. They

selected genotypes whose numbers of pods were large enough. Once selection was done, the number of pods was collected on selected genotypes. A sample of five plants per genotype was used to measure the number of pods per plant which was collected as the average from the plants sampled.

Cooking started one month after harvesting and took 11 days considering the capacity of the cook. Only four treatments were cooked at a time, while for a whole day, three rounds of cooking were realized. In total, 12 genotypes were cooked daily following a modified method proposed by Castillo *et al*. (2012). In fact, no Mattson cooker was available.

The common cooking protocol has been proposed by the "*Una Norma Española*" (UNE) 87028-1 (UNE, 1997). This protocol is general for legumes but differs in key ways from the methods consumers used. In addition, it leads to a high proportion of broken beans (Sanz, 1997). For this study, a revised protocol originally proposed by Castillo *et al*. (2012) was adopted, as it is better suited to the available resources.

According to the protocol, 250 g of dry beans were soaked in 1,000 ml of natural and cleaned water for 14 h and drained. Hydration coefficient (HC) was then determined by Van Der Merwe *et al*. (2006) formula as the ratio between the weight of soaked and drained beans (g) and the weight of dry beans (250g). These beans were then placed in a 1 l thick-bottomed stainless steel pot with water to cover the beans by 1 cm. This way increases the proportion of whole beans in cooked samples. When water overlaps the level of beans in a pot brought to a boil during cooking, and particularly when a smaller quantity of beans is cooked, important thermal differences result in streams in water and cause beans to move, collide one with others and break.

During cooking, the level of water was controlled and cold water was periodically added due to losses of water resulting from evaporation that occurs. To reduce the importance of water evaporation and maintain constant temperature in the pot, beans were cooked with a lid on. The protocol recommended that 2.5 g NaCl should be added shorty before the beans are cooked unless nutritional properties are to be performed. Beans were considered cooked when they were soft enough to be eaten. Cooking was realized at 135°C. The cooking time was recorded in minutes as the period from when beans are brought to a cook up to when they were soft enough to be eaten (Castillo *et al*., 2012).

From there, 100 g of cooled beans were drained and the whole beans were separated from the broken. Integrity of beans (%) was recorded as $\frac{quantity\ of\ whole\ beans}{quantity\ of\ whole\ beans + quantity\ of\ the\ broken\ beans} \times 100$ (Castillo *et al*., 2012).

A panel of 15 tasters was used in this study to appreciate visual characteristics and sensory parameters. Visual evaluation included the size, shape and the uniformity of beans, while sensory parameters included the taste, flavour and the starchiness of beans. For visual evaluation, a seven-point hedonic scale was used for size (1 indicates very small and 7 very large), shape (1 indicates very elongated and 7 very round) and uniformity (1 indicates very variable and 7 vey uniform) (Hosfield & Uebersax, 1980). However, a five-point hedonic scale was used to appreciate the starchiness (1 indicating that beans are very clear to 5 indicating that beans are extremely cloudy), the taste and flavour (1 indicates that parameter is judged unpleasant, while 5 indicates that the character is excellent) (Teshome & Emire, 2012).

4.2.6. Data analysis

Descriptive statistics and correlation analysis between cooking time, hydration coefficient and integrity of beans after cooking were performed using Statistix, 8th edition. Charts and a table were constructed for a view of sensory and visual characteristics.

4.3.Results

4.3.1. Farmers' selection of vigorous biofortified common beans

Performing second-generation biofortified genotypes were selected on the basis of healthy and vigorous leaves at flowering. At maturity, biofortified genotypes were selected on the basis of the number of pods per plant of a given genotype. Performing candidates selected were genotypes with large pod numbers.

A total of 124 biofortified genotypes were selected. The color of flowers for most genotypes was white. Number of pods per plant varied from 14 for genotypes BCB11-509, BF08-13-170 and BF08-1-30 to 27 for genotype BF08-13-44B (Table 4.1). The number of pods for local varieties varied from 9 for IKINIMBA to 13 for MAFUTALA.

Table 4. 1 : Candidate second-generation biofortified common bean genotypes selected at Butembo and Lukanga

N°	Genotype	Color of the flower	No. of pods/plant	N°	Genotype	Color of the flower	No. of pods/plant
1	BF08-26-69	White	15	63	KMA13-22-19	White	15
2	BF08-01-45A	Purple	15	64	BF08-03-05	White	15
3	BF08-13-102	White	15	65	KMA13-05-21	White	15
4	KMA13-28-5	White	15	66	BF08-14-153B	White	24
5	BF08-14-51A	White	18	67	BF08-7-19B	White	19
6	BF08-01-21	White	16	68	BF08-13-18D	White	15
7	KMA13-22-27	Purple	18	69	BF08-16-82B	White	22
8	BF08-3-23B	White	20	70	BF08-01-18B	White	23
9	MV-14	White	17	71	KMA13-23-22	White	15
10	BCB11-509	White	14	72	BF08-3-23A	White	20
11	BF08-07-74A	White	22	73	KMA13-10-18	White	21
12	G4-585	Purple	17	74	BF08-7-19A	White	21
13	BF08-14-20	White	15	75	BF08-1-51	White	17

14	BF08-13-38	White	18	76	RK12B	White	15
15	KMA13-10-05	White	17	77	BF08-07-112D	White	18
16	BF08-16-21	White	19	78	MC20	Purple	15
17	MBC23	White	15	79	BF08-16-14	White	16
18	BF08-7-112D	White	16	80	BF08-07-116	White	16
19	BF08-26-68B	Purple	20	81	KMA13-23-21	White	20
20	BF08-16-36A	White	19	82	BF08-16-52	White	17
21	NAIN DE KYONDO	White	16	83	G4-24A	White	15
22	BF08-7-114	White	15	84	BF08-01-90	White	15
23	BF08-16-76	White	21	85	BF08-03-20	White	15
24	BF08-13-44A	White	24	86	BF08-1-60B	White	15
25	G2333(B)	White	15	87	BF08-1-27	White	15
26	BF08-1-77	White	19	88	KENYA UMOJA	Pink	15
27	BF08-14-24	Purple	15	89	BF08-01-47B	White	23
28	BF08-03-13	White	19	90	BCB11-433	White	15
29	BF08-16-67B	Pink	15	91	BF08-01-92	White	15
30	BF08-1-18C	White	15	92	BCB11-372	White	15
31	BF08-14-116	Purple	15	93	BF08-07-74B	White	21
32	BF08-07-74C	White	21	94	BF08-16-18E	White	15
33	BF08-03-22	White	15	95	BF08-03-12A	White	19
34	BF08-13-47	White	16	96	SR9	White	17
35	BF08-13-44B	White	27	97	BF08-01-62B	White	16
36	RK14	White	15	98	BF08-01-01	White	15
37	BF08-16-67A	White	15	99	SER12	White	15
38	GLP585	White	16	100	KMA13-13-70	White	16
39	BF08-1-60	White	15	101	MEX54	Purple	15
40	BF08-1-47A	White	15	102	BCB11-303	Purple	15
41	BF08-1-29	White	16	103	BF08-01-62A	White	16
42	SR6	White	15	104	BF08-16-92	White	15
43	BF08-01-50	White	16	105	BCB11-138	Pink	15
44	BF08-36-53	White	15	106	BF08-7-80	White	15
45	BF08-14-153A	White	23	107	RK6	White	15
46	BCB11-492	Pink	16	108	BF08-26-162	White	15
47	BF08-13-43A	White	15	109	RK12A	White	14
48	KMA-21-19	White	15	110	BF08-16-31	White	19
49	BF08-14-96A	White	16	111	KMA13-03-35	White	14
50	BF08-13-170	White	14	112	MV1	White	14
51	BF08-14-102	White	15	113	BF08-16-68	White	15
52	BF08-14-51B	White	17	114	BF08-01-49A	White	15
53	BF08-01-18A	White	21	115	BF08-07-22	White	21
54	BF08-14-96B	White	16	116	MC29A	Purple	15
55	BF08-14-51C	White	18	117	BCB11-342	White	15
56	BF08-01-45B	White	15	118	BF08-7-75	White	15
57	KMA13-28-21	Purple	15	119	KMA13-32-28	White	15
58	BF08-03-12B	White	20	120	BF08-01-54	White	15
59	BF08-1-80	White	15	121	BF08-1-30	White	14
60	RK11	Purple	15	122	KMA13-19-33	White	15
61	BF08-03-13B	White	22	123	BF08-16-36B	White	20
62	BF08-1-49B	White	16	124	BF08-13-43B	White	16

4.3.2. Hydration coefficient, cooking time and integrity of beans at cooking

Hydration coefficient varied from 1.4 to 3.4 among all genotypes and biofortified types. Among checks, it varied from 1.7 to 2.0 (Appendix 2). Overall, biofortified genotypes had higher hydration coefficient compared to local varieties (Table 4.2).

Cooking time varied from 73 to 170 minutes among biofortified genotypes and from 120 to 144 minutes among checks. Among biofortified genotypes, BF08-01-21 cooked faster, while KMA13-28-21 cooked the last (Appendix 2). In general, biofortified genotypes cooked faster than checks (Table 4.2).

Percentage of beans remaining whole after cooking was high for checks than for biofortified common beans (Table 4.2). It varied from 21.3 to 100% and from 93.0 to 99.8% respectively for biofortified and local genotypes (Appendix 2).

A negative significant correlation ($p<0.001$) was observed between cooking time and hydration coefficient. Other correlations were not significant (Table 4.3).

Table 4. 2 : Hydration coefficient, cooking time and integrity of beans at cooking of biofortified genotypes and local varieties

Variable	Hydration coefficient	Cooking time (minutes)	Integrity of beans (%)
Lower 95% CI	1.9	112.05	89.3
	1.7	122.60	94.5
Mean	2.0	117.85	92.2
	1.8	134.40	97.9
Upper 95% CI	2.1	123.66	95.1
	2.0	146.20	101.4
Variance	0.13	513.69	127.9
	0.01	90.30	7.8
Coefficient of variation	18.2	19.23	12.3
	6.5	7.07	2.9
Minimum	1.4	73.00	21.3
	1.7	120.00	93.0
Maximum	3.4	170.00	100.0
	2.0	144.00	99.8
T-value	42.9	40.61	63.7
	34.3	31.63	78.2
P-value	0.00	0.00	0.00
	0.00	0.00	0.00

Table 4. 3 : Correlation between hydration coefficient, cooking time and integrity of beans at cooking

	Hydration coefficient	Cooking time	Integrity of beans
Hydration coefficient	1.00	-	-
Cooking time	-0.36***	1.00	-
Integrity of beans	0.006 ns	-0.05 ns	1.00

** p<0.001 ; ns – no significant

4.3.3. Visual characteristics of cooked biofortified common beans

Visual characteristics of cooked biofortified genotypes varied (Table 4.4). The size of cooked beans for most biofortified common bean genotypes varied from slightly small to indifferent. Some genotypes produced beans that were a bit smaller, while other genotypes produced beans whose size was neither notably small nor large. These sizes accounted for more than 70% of all biofortified genotypes. Checks were slightly small except KALANGITI whose size was judged moderately small.

Moderately elongated shape was the most represented among biofortified common beans followed by slightly elongated shape. These shapes accounted for more than 68% of biofortified genotypes. The shape for checks varied from slightly elongated to slightly round.

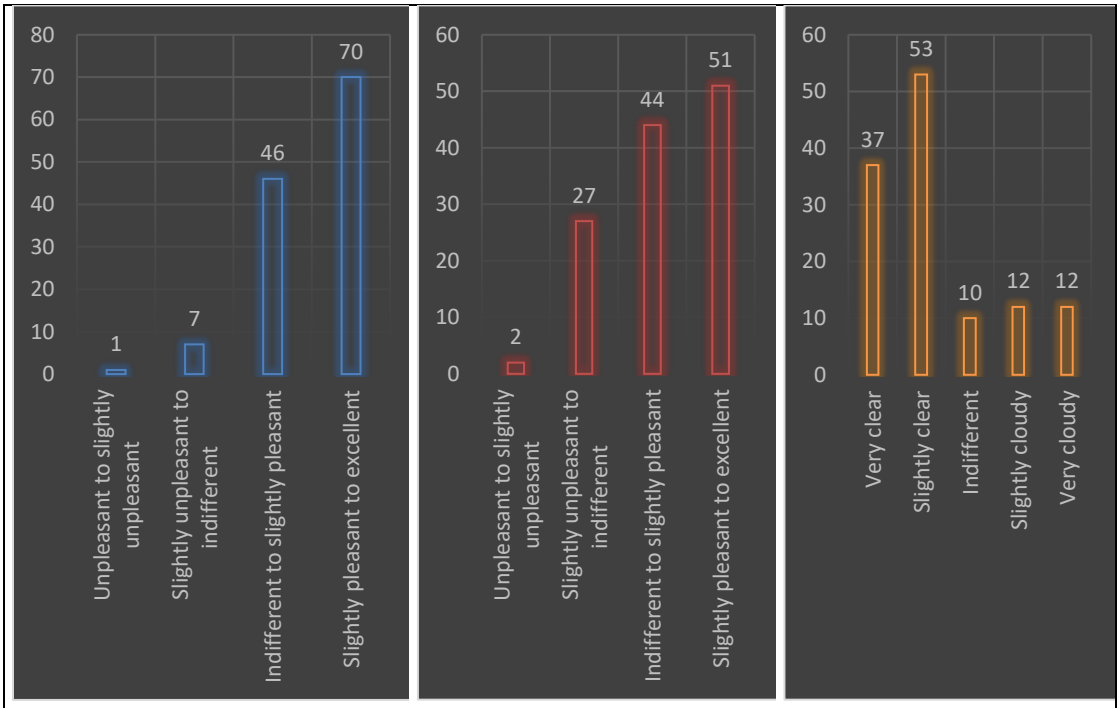
Most biofortified genotypes were moderately and very uniform. These genotypes accounted for more than 58% of biofortified genotypes. Very few (about 6%) biofortified genotypes were slightly variable. Among checks, IKINIMBA and MAFUTALA were very uniform, while others had indifferent to slightly variable cooked seeds.

Table 4. 4 : Visual characteristics of cooked biofortified and local common beans

	Size of common beans					
Category	Moderately small	Slightly small	Indifferent	Slightly large	Moderately large	Very large
Biofortified	5	34	54	18	10	3
Local	1	4	-	-	-	-
	Shape of common beans					
Category	Very elongated	Moderately elongated	Slightly elongated	Indifferent	Slightly round	Moderately round
Biofortified	13	46	39	13	3	10
Local	-	-	1	3	1	-
	Uniformity of common beans					
Category	Slightly variable	Indifferent	Slightly uniform	Moderately uniform	Very uniform	-
Biofortified	8	23	21	41	31	-
Local	2	1	-	-	2	-

4.3.4. Sensory characteristics of cooked biofortified common beans

The taste was unpleasant for BF08-26-68B and slightly pleasant to excellent for 70 biofortified genotypes. Flavour was unpleasant and slightly unpleasant for BF08-26-68B and RK13 and varied from indifferent to excellent for 95 biofortified common beans. Most cooked beans (about 90 biofortified genotypes) were slightly and very clear. They were slightly to very cloudy for 24 biofortified genotypes (Figure 4.1). For local varieties, cooked beans had indifferent starchiness and slightly pleasant taste and flavour.



A : Taste of cooked biofortified beans

B : Flavour of cooked biofortified beans

C : Starchiness of cooked biofortified beans

Figure 4. 1 : Sensory characteristics of cooked biofortified common beans

4.4. Discussion

4.4.1. Farmer assessment of agronomic performance of second-generation common bean genotypes

High yield potential is the foremost character that influences variety selection at farmer level (Mulugeta, 2011). The reason why farmers prefer genotypes with vigorous leaves in North-Kivu, particularly on climbing common beans, is because in this region, leaves are consumed as vegetable.

Farmers preferred genotypes with large pod numbers. This trait is a key determinant of grain yield. Its positive significant correlation with grain yield has been previously reported in several studies (Kataliko *et al*., 2024; Mondo *et al*., 2019; Batumike, 2018).

For all the biofortified genotypes, the number of pods per plant was larger than that for all the checks. This meets the breeding objective of these genotypes. In fact, these genotypes were bred to increase the yield and the mineral levels in seeds (Kimani & Warsame, 2019). In addition, these authors reported the short cooking time (about 45 minutes) associated with these genotypes.

4.4.2. Cooking time of the second-generation biofortified common bean genotypes

Cooking time is currently a priority for common bean improvement due to its implications for nutritional value, energy utilization and gender equity. Among biofortified genotypes, cooking time varied from 73 to 170 minutes with a mean of 117.85 minutes and from 120 to 144 minutes among checks with a mean of 134.40 minutes. Mukai (2017) found cooking time varying from 35 to 122 minutes with an average of 66 minutes. Yadji Haman *et al*. (2020) observed ranges from 49.28 to 89.28 minutes for freshly harvested beans and from 128.38 to 270.52 minutes for beans that were stored first for 10 days suggesting that storage period after harvest influences cooking time. Coelho *et al*. (2007) reported that freshly harvested beans cook 2-4 times faster than beans stored for six months.

Differences in cooking times observed from other studies arise from varietal differences, water absorption, cooking method used and storage period. While Yadji Haman *et al*. (2020) ; Mukai (2017) used 12 hours for soaking in distillated water and used an Automated Mattson Bean cooker developed by Canadian Grain Commission (Winnipeg, Canada), in this study, due to an unavailability of a Mattson cooker, a method proposed by Castillo et al. (2012) which recommended 14 hours for soaking was used.

Hydration coefficient varied from 1.4 to 3.4 among all genotypes and biofortified types. Among the checks, it varied from 1.7 to 2.0. Common beans whose water absorption is high tend to cook fast. In terms of water absorption expressed into percentages, it ranged from 41.4 to 235.3% among biofortified genotypes with an average of 102.7% and from 72 to 100% among the local varieties with an average of 84.8%. Mukai (2017) found water absorption varying from 63 to 137% in 152 common bean lines with an average of 94%. Variations might be due to varietal differences. Local varieties might have compact seed coat texture. This might be the reason why hydration coefficient was low and cooking time high for this category. This is in line with Shellie-Dessert & Hosfield (1990) who reported that water absorption capacity of beans influences their cooking time and is controlled by the texture of seed coat. Similar findings have been reported by Mukai (2017). Other factors affecting water absorption include differences in orifice dimensions in bean seed microphyle, presence and number of seed coat pores and the microstructural differences (Agbo *et al*., 1987).

Water absorption seems to be another important factor influencing the cooking time. Shorter cooking time might be associated with common beans which absorb enough water. Findings revealed a negative significant correlation ($r=-0.36$; $p<0.001$) between cooking time and hydration coefficients indicating that short cooking time occurred in bean with high water absorption. This might be attributed to the degradation properties of water, particularly its hydrolysis ability (IFC, 2016). When hydration coefficient is high, the cooking time is short. This is evident considering that biofortified genotypes, the most hydrated beans, cooked faster than the local varieties with low hydration coefficients. Similar findings were reported by Munthali *et al*. (2022). Shellie-Dessert & Hosfield (1990) also reported that a high state of cellular hydration allows cells to soften and separate. Even during her study, Mukai (2017) revealed a narrow range of

cooking time during the rainy season (from 35 to 100 minutes) and a delayed cooking time when the amount of rains decrease (from 43 to 122 minutes). This might be due to the fact that beans retained more water during the rainy season as the treatment processes were identical over the two seasons.

The temperature is another key factor of difference. For the study carried by Mukai (2017), the Mattson cooker operated at 350°C, while in this study, the cooker was at 135°C. This is a temperature that can be achieved by any farmer or consumer, and particularly under poor energy resources in Eastern and Central Africa.

Differences in cooking time might also have been attributed to the storage period before cooking. When storage period is too long, common beans take longer to cook. Similar findings have been reported by Yadji Haman *et al*. (2020). Freshly harvested beans cooked 3-4 times faster than beans that have been stored for 10 days. In fact, in seeds, as reported by Perera *et a*l. (2023) ; Mubaiwa *et al*. (2017), phytate storing calcium during storage, is broken down and free calcium is released to crosslink pectin in the cell wall leading to strengthening the last and increasing cooking time.

4.4.3. Quality characteristics of cooked beans of selected second-generation common bean genotypes

It was revealed that percentage of beans remaining whole after cooking was high for checks than for biofortified common beans. It varied from 21.3 to 100% among biofortified genotypes and from 93.0 to 99.8% among local varieties. This might be due to the texture of seed coat that should be more compact for local varieties than for biofortified genotypes. This is evident considering that hydration coefficient was low for local varieties indicating the compact structure and reduced orifice dimensions and few pores to allow important water penetration in cells as reported by Shellie-Dessert

& Hosfield (1990) ; Agbo *et al*. (1987). Barros & Helena (2016) observed percentages of whole beans after cooking ranging from 30.0 to 94.91%. Munthali *et al*. (2022) found percentages of split beans varying from 4 to 60%.

Another factor affecting integrity of beans might be the heating temperature and the amount of water used. When smaller quantities of beans are cooked in overlapping water, due to streams produced in the jar, beans move, collide and break (Van Der Merwe et al., 2006). High heating temperature may result in increased number of broken beans.

The size for most biofortified common bean genotypes varied from slightly small to indifferent. Biofortified common beans had moderately and slightly elongated shape and were moderately and very uniform. These attributes are linked to raw beans. For instance, when raw seeds are too small, after cooking, the size slightly increases due to the amount of absorbed water. In their study, Barros & Helena (2016), working on seven common bean genotypes with very small size, realized, after cooking, small size for six genotypes and very small size for the remaining genotype.

Color and lightness or clear aspects of beans influence their attractiveness. This attribute is the primary signal for consumer choice (Asiimwe *et al*., 2024). According to Barros & Helena (2016), consumers prefer lighter colored beans because they relate lighter and clear aspect to starchiness and darker color to old and hard beans that require more cooking time. Most of biofortified genotypes had yellow and lighter seed coats; hence, most of cooked beans were preferred and appeared clear and slightly clear.

Sensory characteristics might have been affected by genotypes. Similar observations have been reported by Barros & Helena (2016) ; Bassett *et al*. (2021). In addition to genotypic influences, the seed type might be another factor affecting sensory

characteristics. Bassett *et al*. (2021) reported that mottled/red speckled beans vary the most, while brown genotypes tend to vary the least. This might be due to the high level of polyphenolics, an aromatic compound, in red and purple seed coats as reported by Wu *et al*. (2004).

4.5.Conclusion

From the study, a total of 124 biofortified genotypes were selected from a panel of 285 genotypes by farmers on the basis of healthy and vigorous leaves at flowering and large numbers of pods per plant at maturity. The color of flowers for most genotypes was white with numbers of pods per plant varying from 14.2 to 26.8, while local varieties had numbers of pods per plant varying from 8.7 to 13.0.

Selected biofortified common beans cooked faster compared to local genotypes. Biofortified genotypes took 117.85 minutes to cook, while local genotypes took 134.40 minutes. Cooking time varied from 73 to 170 minutes among biofortified genotypes and from 120 to 144 minutes among local genotypes.

The percentage of beans remaining whole after cooking was high for local genotypes than for biofortified common beans. Among biofortified genotypes, it varied from 21.3 to 100% while among checks, it varied from 93.0 to 99.8%. The size for most cooked biofortified beans varied from slightly small to indifferent, while the shape was moderately elongated and slightly elongated for panelists. Cooked beans were moderately and very uniform. Characteristics such as taste and flavour were slightly pleasant to excellent for most cooked biofortified genotypes. Cooked beans were clear and slightly clear for most selected biofortified lines.

CHAPTER 5

5. GENETIC VARIABILITY AND YIELD STABILITY ANALYSIS OF IRON AND ZINC BIOFORTIFIED COMMON BEAN GENOTYPES IN EASTERN DRC

5.1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important source of proteins and minerals for many vegetarians. It is considered as the meat for poor people, particularly in Africa (Mbikayi *et al*., 2018).

The crop is cultivated from 500 to 2,500 masl (Farrow & Muthoni-Andriatsitohaina, 2020). The upmost common bean productions are obtained at higher altitudes (>1,500 masl) and at mid-altitudes (between 1,000 and 1,500 masl) (Farrow & Muthoni-Andriatsitohaina, 2020).

An adequate supply of iron and zinc is essential to prevent iron deficiency anaemia (IDA) and zinc deficiency associated with health problems in developing countries (Kataliko *et al*., 2024). Through iron and zinc biofortification programmes of HarvestPlus, some common bean varieties were released and adopted in DRC. These lines included G59/1-2, VCB 81013, LIB 1 and Kiangara (Kimani *et al*., 2008).

Following some wide crosses, six mineral dense common bean varieties, with more than 100 ppm of iron were developed and adopted in DRC. These varieties are HM 21-7, RWR 2245, PVA 1438, Namulenga, COD MLV 059 and Cuarentino (Mulambu *et al*. , 2017).

From another iron and zinc biofortification programme carried out by the University of Nairobi and the Malawi National Bean Programme, 84 F_4 - derived F_7 ($F_{4:7}$) lines with 66 to 136 ppm of iron and 10 to 60 ppm of zinc were developed. All these improved

genotypes gave higher seed yield than the traditional genotypes (Kimani & Warsame, 2019).

The performance of these iron and zinc biofortified common bean genotypes across the variable environments of DRC is lacking. The presence of interaction between genotypes and environments implies that the behavior of genotypes depends upon the particular environment in which they are tested. Inconsistent genotypic performances in different environments relative each to another result in changes to the ordering of genotypes from one environment to another (Falconer & Mackay, 1996). Interaction between genotypes and environments is a critical information to assign genotypes in their suitable environments.

The objectives of this study were to:

i. determine the performance of some selected iron and zinc biofortified common bean genotypes across contrasting environments in DRC, and
ii. assess the stability of these genotypes across environments.

5.2. Materials and methods

5.2.1. Experimental sites

Field experiments were carried out in Butembo, Lukanga and Beni. In Butembo and Lukanga, experiments were carried out during the 2023 short and long rain seasons from March to July 2023 and from October 2023 to January 2024. In Beni, due to an extreme and severe drought experienced during the 2023 short rain season, data was collected for only the 2023 long rainy season. In Butembo, experiments were conducted at the field station of UCG-Butembo, while at Lukanga, experiments were conducted at the field station of UNILUK. Geographical positions have been mentioned earlier in section 4.2.1. In Beni, the experiment was conducted at the field station of the

‹‹Association des Producteurs de Manioc (APROMA-Beni)›› located at 0.410355° latitude N, 29.585348° longitude E and 1,118 masl.

Soil analysis conducted at the laboratory of UCG in February 2023, revealed considerable differences in soil organic matter, pH and mineral content (Table 5.1). According to Salcedo (2008), suitable soils for common bean production should have pH (H_2O) between 5.5 and 6.5. Soil of UNILUK was a bit more acidic than the two other soils most likely due to the high rainfall (Table 5.2) in this site.

Climatic conditions are other important factors influencing common bean production. For Salcedo (2008), common bean grows well at temperatures ranging from 15 to 27°C and 29.5°C. But at temperatures close to or above 35°C and under moisture stress, particularly during flowering and pod setting and filling, abortion of blossoms and developed pods is important. This was what happened to the 2023 short rain experiment at Beni. A total of 350 to 500 mm of rains distributed during the growing season is required. Low relative humidity (<75%) is also required to minimize risk of bacterial and fungal diseases (Salcedo, 2008). Table 5.2 shows that Butembo and Lukanga were more suitable than Beni, considering the range of temperatures and amounts of rains. Moreover, temperatures at Beni were higher than at other locations particularly during the short rain season. Relative humidity was also high at Lukanga.

Table 5. 1 : Characteristics of soils of the study sites

Site	pH H_2O	pH KCL	Dry matter(%)	Org. matter (%)	P (%)	K (%)	N (%)	Fe (%)
UNILUK	5.24	4.54	83.73	6.03	0.543	0.296	0.126	0.000075
UCG	5.76	5.05	82.2	11.43	0.26	1.18	0.126	0.00265
Beni	6.57	5.28	76.8	8.4	1.3	0.395	0.1265	0.000011

Source : UCG lab, 2023

Table 5. 2 : Temperature, relative humidity and rainfall of the study sites during the growing seasons (2023-2024)

Climatic item	Site	Mar.	Apr.	May	Jun.	Jul.	Oct.	Nov.	Dec.	Jan.	Feb.
T_{max} (°C)	UNILUK	24.2	25.3	24.8	24.0	25.9	25.0	24.3	25.0	26.8	27.0
	UCG	28.7	27.3	25.3	28.2	27.0	27.9	27.1	27.3	29.4	30.7
	Beni	35.5	33.9	31.2	31.1	31.7	31.1	32.8	29.9	32.4	32.6
T_{min} (°C)	UNILUK	14.9	15,4	14.9	14.6	14.3	15.9	15.5	15.5	15.0	15.0
	UCG	16.5	15.8	15,7	15.4	15.2	16.4	16.7	17.0	15.5	16.0
	Beni	17.1	16.7	16.5	15.5	15.3	16.5	17.0	17.3	16.5	16.1
Relative humidity (%)	UNILUK	90.7	89.6	88.5	91.0	90.3	86.4	86.3	84.1	77.6	74.7
	UCG	74.6	79.7	83.5	77.0	73.4	82.0	85.5	82.1	76.8	74.7
	Beni	72.2	79.1	82.1	75.0	71.6	80.9	82.5	79.1	73.4	72.6
Rainfall (mm)	UNILUK	136.0	184.9	169.8	30.6	192.7	197.2	170.5	124.3	58.1	83.6
	UCG	119.9	162.1	147.0	29.6	141.4	138.4	156.5	102.7	52.4	67.3
	Beni	116.4	158.3	125.8	25.1	121.0	114.1	124.5	69.56	44.7	43.2

Source : NASA, 2024

5.2.2. Experimental materials

One hundred and sixty (160) iron and zinc biofortified common bean genotypes were used for this study together with five local commercial cultivars, namely IKINIMBA, MAFUTALA, KALANGITI, OBUSOSERA and DEMAI. The biofortified genotypes were of two origins, 126 genotypes were selected from the $F_{4:7}$ second-generation biofortified common beans developed at the University of Nairobi and 34 from CIAT and HarvestPlus programme. The second-generation biofortified lines were the candidate lines selected by farmers on the basis of their preferences (Chapter 4). Previously, they were described by Kimani & Warsame (2019) and Mondo *et al*. (2019). According to the later authors, their average yield performance ranged from 0.8 to more than 4.0 t ha^{-1}. In addition, according to Kimani & Warsame (2019) ; Amongi *et al*. (2018) and Mulambu (2017) mineral levels varied largely from 66 to 136 ppm and from 10 to 60 ppm for iron and zinc respectively.

5.2.3. Field evaluation and management

The 160 biofortified genotypes and five local genotypes were evaluated in an augmented design in 2023 short and long rainy seasons in three different ecological zones. Four blocks were used with each having 40 test genotypes and each of the five local varieties.

An experimental unit was represented by a single row containing 10 hills 30 cm apart each from another. A row was 60 cm apart from another, while the distance between blocks was 1 m. Two seeds were sown in a hill and thinned to one at two weeks after emergence. Diammonium phosphate (18% N and 46% P_2O_5) was applied at planting at 150 kg ha^{-1}. Plots were maintained weed free by manual weeding. A total of three manual weeding was done at 25-days interval.

5.2.4. Assessment of agronomic traits

Six plants in the middle of each plot were used to collect data. Data was collected on days to 50% flowering, 75% maturity and full maturity, number of pods per plant, the length of a pod, number of seeds per pod, grain yield and the 100-seed weight, following the standard procedure suggested by Schoonhoven & Pastor-Corrales (1987). The last four parameters were recorded at physiological maturity of plants.

Days to 50% flowering, 75% maturation and to fully maturity were recorded as the number of days from sowing to when 50% of plants within a plot initiate at least one opened flower or when 75% of individual plants or all the plants in a plot reach full maturity.

The number of pods per plant was collected as the average number of pods on the plants sampled. The length of pods was collected as the average length (cm) of pods on a sample of five pods, while the number of seeds per pod was recorded as the average

number of seeds from a sample of five pods. The 100-seed weight was assessed three times and recorded as the average weight of 100 random dry seeds using an electronic computerized balance (WT-N Serries, WANT Balance Instrument Co., Ltd, Jiangsu, China). Prior to the assessment of the 100-seed weight and grain yield, seeds were sundried for three consecutive days. Grain yield was assessed in grams on plot-level data by threshing all the plants, drying for three days, weighing seeds and scaling up the yield measured to the standard unit (kg ha^{-1}).

5.2.5. Determination of iron and zinc

Analyses to determine iron and zinc concentrations were conducted by Inductively Coupled Plasma (ICP) based-technique, Optical Emission Spectrometry. These analyses were carried out at the Department of Land Resource Management and Agricultural Technology (LARMAT), Faculty of Agriculture, University of Nairobi between September and October 2023.

Determination of these micronutrients was based on calibration curves using standard solutions of iron and zinc (1.5, 1.0, 0.5 and 0.25mg/kg) (Carvalho *et al*., 2012). A mixture of perchloric acid ($HClO_4$) and nitric acid (HNO_3) (in 1:2 ratio) was used to digest common bean samples as by Zarcinas *et al*. (1987) method. According to this method, 1.0 g of common bean seeds is grinded and transferred into a 100-ml Pyrex digestion tube. Thereafter 10 ml of $HClO_4$ and HNO_3 are added and the mixture stands overnight at room temperature until the reaction phase is over. These acids disappear when complete digestion is conducted in a Gerhardt Kjeldatherm, while temperature is raised to 235°C for 30 minutes or more (Zarcinas *et al*., 1987). The digest prepared was then cooled for a few minutes before a few drops of deionized water is carefully added to bring to the volume while washing down walls of tubes and funnels. The digest is

then undisturbed for a few hours. A blank (with no plant material) is prepared following the same protocol. The determination of iron and zinc was conducted using atomic absorption spectrophotometer (Varian Techtron Pty Ltd, Spectr AA-10). Prior to the measurement, a series of suitable standards with given iron and zinc concentrations were used to assure adequate analysis and to draw a calibration curve. The supernatant liquid is decanted and iron and zinc levels are determined in the aliquots according to the calibration curve. A given wavelength is used for adequate absorbance of iron and zinc. In the study, 248.33 and 213.86 nm were used as the respective wavelength for iron and zinc determination. These wavelengths have been successfully used previously by Kimani & Warsame (2019).

5.2.6. Data analysis

5.2.6.1. Genetic variability and interrelationships among genotypes

Data was subjected to a combined analysis of variance using R statistical software with locations, seasons and blocks as random factors and genotypes as a fixed factor. The factor genotypes was portioned into three components, the tested genotypes (160 biofortified genotypes), the checks (five local varieties) and the contrast between the tested genotypes and the checks (Gupta *et al*., 2015). Tukey test was used for mean separation.

The total variance was portioned into genotypic, environmental and phenotypic variance (VG, VE and VP) according to Ejigu *et al*. (2018) for three sites and two seasons. Broad-sense heritability (H^2) as well the genotypic and phenotypic coefficient of variation (GCV and PCV) were estimated based on the formula suggested by Falconer & Mackay (1996) as follow: $H^2(\%) = \frac{VG}{VP} \times 100$; $\mathrm{GCV}(\%) = \frac{\sqrt{\mathrm{VG}}}{\bar{\mathrm{X}}} \times 100$ and $\mathrm{PCV}(\%) = \frac{\sqrt{\mathrm{VP}}}{\bar{\mathrm{X}}} \times 100$ where $\bar{\mathrm{X}}$ is the grand mean of the study trait. The

heritability of a character was low when it is lower than 40% and high when it is above 70% (Falconer & Mackay, 1996). However, GCV and PCV were low when they were less than 10% and high when they were above 20%.

The expected genetic advance or genetic gain under selection (EGA) was estimated for selection intensity (k) at 5% following Tiwari *et al*. (2019) as follow $\mathrm{EGA} = \sqrt{\mathrm{VP}} \times \mathrm{k} \times \mathrm{H}^2$ (k=2.06 at 5% selection intensity). The genetic advance as percent of population mean (GA) was then estimated as $\mathrm{GA}\,(\%) = \frac{\mathrm{EGA}}{\bar{\mathrm{X}}} \times 100$. As suggested by Ghimire & Mandal (2019), if GA is low (<10%) for a trait, this trait is controlled by a non-additive gene action recommending heterosis as the appropriate selection method. However, if GA is high (>30%) the trait is controlled by an additive gene action and recurrent selection can be applied as the appropriate breeding method.

Pearson's correlation among characters was determined using Statistix-8, version 8.0. Distribution of genotypes under different clusters was conducted with R statistical software, version 4.4.0 following "Ward.D" method, while quantitative data distribution was realized following "Euclidean" method.

5.2.6.2. Yield stability analysis

To evaluate interactions between genotypes and environments, data was subjected to genotype main effect plus genotype x environment interaction (GGE) biplot analysis using the GGE biplot windows application (Yan *et al*., 2000) using the following model equation, $Y_{ij} - Y_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \sum_{ij}$; where Y_{ij} is the average performance of a genotype i for a character in environment j, Y_j the average performance across all genotypes in environment j, λ_1 and λ_2 the singular values for principal component (PC) 1 and 2, ξ_{i1} and ξ_{i2} the scores of PC1 and PC2 for genotype i, η_{j1} and η_{j2} the scores of

PC1 and PC2 for environment *j* and $\sum_{ij}$ the residual value of genotype *i* in environment *j*.

Selection of stable genotypes that interact less with the environments where they have to be grown is conducted using stability analysis. This analysis, helpful to describe the performance of a variety over a series of environments, was conducted following the linear model proposed by Eberhart & Russell (1966), $Y_{ij} = \mu_i + \beta_i I_j + \delta_{ij}$ where Y_{ij} is the mean of the i^{th} genotype at the j^{th} environment, μ_i the mean of the i^{th} genotype over all environments, β_i the regression coefficient that measures the response of the i^{th} genotype to varying environments, δ_{ij} the deviation from regression of the i^{th} genotype at the j^{th} environment and I_j the environmental index obtained as the mean of all genotypes at the j^{th} environment minus the overall mean.

The additive main effect and multiplication interaction (AMMI) analysis was used to determine the stability of the genotypes across locations using the AMMI stability value (Yan *et al*., 2000). AMMI stability value (ASV) was calculated using the following formula $\text{ASV} = \sqrt{\left[\frac{\text{SS IPCA1}}{\text{SS IPCA2}}(\text{IPCA1})\right]^2 + (\text{IPCA2})^2}$ where SS IPCA1 and SS IPCA2 are the sum of squares for interaction principal component analysis scores (IPCA1 and IPCA2) (Sudhagar *et al*., 2024).

The yield stability index (YSI) was calculated using the yielding ability and stability of a genotype as by the following formula YSI=rASV+rYSI where rASV is the AMMI-derived stability value rank of a genotype; rYSI the rank of yield stability index for a genotype. Low rASV and rYSI values earmark a stable and good-yielding genotype (Sudhagar *et al*., 2024).

5.3. Results

5.3.1. Genotypic variability and heritability estimates

Significant interactions ($p<0.05$) were observed between genotypes, locations and seasons for duration to flowering. Interactions between genotypes and locations were significant ($p<0.001$) for duration to flowering and duration to fully maturation. Significant interactions ($p<0.001$) were also observed between genotypes and seasons for duration to flowering (Table 5.3).

Iron and zinc biofortified genotypes revealed significant differences ($p<0.001$) over the checks for duration to flowering, 75% maturity and fully maturation. Differences among biofortified genotypes were significant ($p<0.001$) for these traits. Locational and seasonal effects were also significant ($p<0.001$) for these traits (Table 5.3).

Table 5. 3 : Mean squares for flowering and maturity of common bean genotypes at Lukanga, Butembo and Beni during the 2023 short and long rain seasons

Source of variation	df	DTF	DT75M	DTFM
Season (S)	1	7202.3***	1536.0***	2311.2***
Location (L)	2	47012.1***	140664.2***	167924.2***
Block	3	2.699	3.45	175.0***
Genotypes vs checks	1	1826.0***	7059.7***	7413.9***
Checks	4	61.7***	51.36*	26.9
Tested Genotypes	159	35.68***	72.7***	119.6***
Season x L	2	1828.6***	23552.2***	15684.0***
Genotypes x S	164	10.16***	11.9	13.0
Genotypes x L	228	12.8***	18.8	45.3***
Genotypes x L x S	228	8.12*	16.9	14.4
Pooled error	87	5.45	14.9	18.6

* $p<0.05$, ** $p<0.01$ and *** $p<0.001$; DTF – days to 50% flowering ; DT75M – days to 75% maturity ; DTFM – days to fully maturity ; df – degree of freedom

Among the biofortified common bean genotypes, RW 582 flowered earlier at 35.5 days from sowing. The rest of genotypes (including the checks) initiated flowers 5 to more days later than this genotype. However, CODMLV 059 was the late flowering biofortified genotype following by NGWINxCAB2 21311, RWV2359, BF08-03-13B and ICYANA and flowered after the period varying from 50.5 to 52.8 days. Fifty-seven iron and zinc biofortified genotypes and all the checks had duration to flowering that did not vary significantly from that of the earliest flowering genotype. Duration to flowering of these genotypes varied from 41.1 to 46.0 days (Appendix 3).

The test genotypes took an average of 44.7 days to reach 50% flowering. They flowered earlier at Beni and later at Lukanga, 33.2 and 55.6 days from sowing respectively. Furthermore, flowering was reached earlier for the short rain season compared to the long rain season. The local checks flowered four days earlier than biofortified genotypes (Table 5.5).

Iron and zinc biofortified common bean genotypes RW 582 and BF08-14-96A were the first to reach maturity. They matured respectively 88.7 and 79.2 days from sowing. Genotype BF08-14-96A matured earlier than all the checks whose duration to maturation varied from 92.4 days for KALANGITI to 95.0 days for MAFUTALA. Genotypes CODMLV 059, MAC 74 and BF08-7-19B were the late maturing iron and zinc biofortified genotypes and matured 109.7 to 118.3 days from sowing (Appendix 3).

The test genotypes took an average of 97.6 days to reach fully maturity. Maturation term was 2.5 months at Beni and averaged 4 months at Lukanga, while at Butembo it reached 3 months at overall. Iron and zinc biofortified genotypes matured eight days later compared to the checks. This period was longer for the long rain season compared to the short rain season (Table 5.5).

Interactions were significant between genotypes, locations and seasons, between genotypes and locations and between genotypes and seasons for number of pods per plant, grain yield and the 100-seed weight. Locational and seasonal effects were also significant for these traits. Significant contrasts were observed between biofortified genotypes and the checks as well as among biofortified genotypes for number of pods per plant, number of seeds per pod, grain yield and 100-seed weight (Table 5.4).

Number of pods per plant varied among genotypes (Appendix 4). Biofortified genotypes BF08-26-68B, BF08-01-47B, BF08-16-21, BF08-1-49B and BF08-03-05, with numbers of pods varying from 18.1 to 18.7 per plant, initiated significantly larger numbers of pods compared to the local varieties DEMAI and KALANGITI whose plants initiated respectively 8.1 and 7.4 pods at average. A total of 26 biofortified common bean genotypes initiated the least numbers of pods per plant varying from 4.8 to 7.4 and did not vary significantly from all the checks.

Table 5. 4 : Mean squares for pod and seed characters of common bean genotypes at Lukanga, Butembo and Beni during the 2023 short and long rain seasons

Source of variation	df	NPP	PL	NSP	GY	100-SW
Season (S)	1	2388.8***	0.0	2.6	43478168.1***	4239.8***
Location (L)	2	5408.3***	65.1***	1.2	94839055.9***	6595.3***
Block	3	292.7***	33.6***	2.45	2220916.5***	151.4***
Genotypes vs checks	1	217.9***	661.1***	4.5	42091736.3***	7576.9***
Checks	4	64.4*	1.50	12.46	2577025.0***	74.7***
Tested Genotypes	159	56.0***	36.15***	6.9	1676513.3***	232.1***
Season x L	2	7222.8***	0.00	1.5	108433634.4***	3325.1***
Genotypes x S	164	30.6*	0.00	0.59	951725.7***	41.2***
Genotypes x L	228	42.3***	23.9***	1.5	1595884.9***	61.7***
Genotypes x L x S	228	33.5**	0.00	0.6	1135010.0***	47.9***
Pooled error	87	21.1	0.64	5.2	276625.3	10.1

* p<0.05 , ** p<0.01 and *** p<0.001 ; NPP – number of pods per plant ; PL – pod length ; NSP – number of seeds per pod ; 100-SW – 100-seed mass ; GY – grain yield ; df – degree of freedom

Except at Beni, iron and zinc biofortified genotypes initiated large numbers of pods than the checks. Across locations and seasons, biofortified genotypes initiated more pods than checks. The long rain season was more suitable, except for Lukanga where large pod numbers were observed during the short rain season (Table 5.5).

The length of pods varied from 7.8 cm for genotype RW 805 to 13.3 cm for genotype BF08-1-60B (Appendix 4). In fact, seven iron and zinc biofortified common bean genotypes initiated pods whose the length was superior to 13 cm. Variation of pod length was not significant among checks. It varied from 8.9 to 9.5 cm.

The length of pods was 10.3 cm at average. This length was high at Butembo. Pods for biofortified genotypes were much longer compared to the local checks commonly used in study locations (Table 5.5).

The number of seeds per pod did not vary significantly neither among the study genotypes nor the environments. This number varied from 2.8 seeds for genotype BCB11-433 to 7.9 seeds for genotype KMA13-23-21. Among the checks, MAFUTALA initiated larger number of seeds per pod (5.4 seeds per pod). The four other checks initiated an average of 4.7 to 5.4 seeds per pod. One hundred iron and zinc biofortified common bean had numbers of seeds per pod numerically larger compared to MAFUTALA (Appendix 4).

Iron and zinc biofortified common bean genotypes initiated more seeds in pods compared to the checks except at Beni where the size was important for the checks. Large seeds per pod were initiated during the short rain season compared to the long rain season (Table 5.5).

There were significant variations observed in 100-seed weight (Appendix 4). Hundred seeds weight varied from 19.4 g for genotype RW 547 to 54.7 g for genotype BF08-16-67B among the study genotypes. Together with nine other biofortified genotypes, BF08-16-67B had the highest 100-seed weights significantly larger than that for the checks. The later genotypes had 100-seed weight varying from 22.3 to 26.6 g.

The average 100-seed weight was high for biofortified genotypes and the long rain season tended to be associated with high 100-seed weight particularly at Lukanga (Table 5.5).

Grain yield varied from 591.8 to 4,699.7 kg ha^{-1} among genotypes studied (Appendix 5). Except 16 iron and zinc biofortified genotypes, grain yield for the remaining biofortified genotypes was more than 1,000 kg ha^{-1}. Three checks namely OBUSOSERA, DEMAI and KALANGITI had low than 1,000 kg ha^{-1} of grain yield. Three iron and zinc biofortified common bean genotypes, G4-24A, BF08-01-47B and RWV 2359, had the grain yields (4699.7, 2818.3 and 2690.9 kg ha^{-1} respectively) significantly higher than that for the checks (Table 5.7).

Grain yield for biofortified common bean genotypes was high compared to that observed for checks. Grain yield for checks was 628.1 kg ha^{-1} lesser than that for iron and zinc biofortified genotypes. Grain yield was higher in Butembo, making it the most suitable location. However, at Beni, grain yield was the least important. While at Butembo, the long rain was the most suitable with greater grain yield, at Lukanga, the most suitable season was the short rain season (Table 5.5).

Table 5. 5 : Pooled performance of iron and zinc biofortified common beans and checks across locations and seasons in Eastern DRC

		Butembo			Lukanga			Beni			Combined locations		Across
		Short rain	Long rain	Combined seasons	Short rain	Long rain	Combined seasons	Short rain	Long rain	Combined seasons	Short rain	Long rain	locations & seasons
DTF	Genotypes	43.8	50.5	47.2	54.0	62.0	58.0	-	35.0	-	44.3	49.1	46.7
	Checks	37.3	49.1	43.2	47.5	58.8	53.1	-	31.4	-	38.7	46.4	42.6
	Mean	40.6	49.8	45.2	50.8	60.4	55.6	-	33.2	-	41.5	47.8	44.7
DT75M	Genotypes	85.3	105.4	95.3	118.9	105.5	112.2	-	72.3	-	92.1	94.4	93.3
	Checks	78.7	94.6	86.7	104.7	99.0	101.9	-	66.8	-	83.4	86.8	85.1
	Mean	82.0	100.0	91.0	111.8	102.3	107.0	-	69.6	-	87.8	90.6	89.2
DTFM	Genotypes	96.1	113.6	104.9	126.6	117.5	122.1	-	78.4	-	100.4	103.2	101.8
	Checks	88.9	104.8	96.9	112.4	108.4	110.4	-	73.0	-	91.4	95.4	93.4
	Mean	92.5	109.2	100.9	119.5	112.9	116.2	-	75.7	-	95.9	99.3	97.6
NPP	Genotypes	6.7	20.6	13.7	14.5	10.8	12.7	-	6.0	-	9.1	12.5	10.8
	Checks	5.4	12.1	8.7	15.7	8.0	11.8	-	7.5	-	9.5	9.2	9.3
	Mean	6.0	16.4	11.2	15.1	9.4	12.2	-	6.7	-	9.3	10.8	10.1
PL	Genotypes	-	11.9	-	-	11.1	-	-	11.1	-	-	11.4	-
	Checks	-	9.7	-	-	8.7	-	-	9.1	-	-	9.2	-
	Mean	-	10.8	-	-	9.9	-	-	10.1	-	-	10.3	-
NSP	Genotypes	5.6	5.4	5.5	5.6	5.6	5.6	-	5.4	-	5.5	5.4	5.5
	Checks	5.1	4.9	5.0	5.4	5.0	5.2	-	5.7	-	5.4	5.2	5.3
	Mean	5.3	5.1	5.2	5.5	5.3	5.4	-	5.6	-	5.5	5.3	5.4
GY	Genotypes	1128.2	2978.4	2053.3	2091.5	1656.7	1874.1	-	1003.8	-	1407.8	1879.6	1643.7
	Checks	1155.4	1141.7	1148.5	1257.8	782.8	1020.3	-	877.8	-	1097.0	934.1	1015.6
	Mean	1141.8	2060.1	1600.9	1674.6	1219.8	1447.2	-	940.8	-	1252.4	1406.9	1329.6
100-SW	Genotypes	31.0	33.0	32.0	33.2	44.2	38.7	-	30.0	-	31.4	35.8	33.6
	Checks	28.0	20.4	24.2	22.8	32.7	27.7	-	23.6	-	24.8	25.58	25.2
	Mean	29.5	26.7	28.1	28.0	38.5	33.2	-	26.8	-	28.1	30.7	29.4

DTF – days to flowering ; DT75M & DTFM – days to 75% and fully maturity ; NPP – number of pods per plant ; PL – pod length ; NSP – number of seeds per pod ; 100-SW – 100-seed mass ; GY – grain yield

Significant interactions between genotypes and locations were observed for iron and zinc. Variations among biofortified genotypes were also significant ($p<0.001$) for both quality characters. While variations among checks were significant ($p<0.001$) for iron, possible numerical variations observed for zinc were due by chance. The contrast between biofortified genotypes and checks was also significant for both quality characters (Table 5.6).

Iron and zinc varied among genotypes. Iron amounts varied from 43.3 ppm for genotype KMA13-10-05 to 172.0 ppm for genotype BF08-13-170, while zinc amounts varied from 19.1 ppm for genotype BF08-01-50 to 63.4 ppm for genotype RK 11 (Appendix 5). Thirty-three biofortified genotypes had numerically higher levels of iron compared to checks. Six biofortified genotypes, with more than 144.0 ppm, had the iron levels significantly higher than that for the high iron check, DEMAI. On the other hand, seven biofortified genotypes, with more than 57.2 ppm, had zinc amounts significantly higher than those for checks. About 100 biofortified genotypes had zinc amounts numerically higher compared to those for checks. Among checks, DEMAI and MAFUTALA had the highest iron and zinc amounts. At overall, biofortified genotypes RK 11, BF08-07-22 and BF08-26-162 combined iron and zinc amounts significantly higher than those for the high iron and zinc checks. Finally, a strong positive correlation ($r=0.52$, $p<0.001$) was observed between iron and zinc (Table 5.9).

Table 5. 6 : Mean squares of iron and zinc at Butembo and Lukanga

Source of variation	df	Iron	Zinc
Location (L)	1	377.35*	73.35
Block	3	2545.18***	193.08***
Genotypes vs checks	1	753.83**	1182.59***
Checks	4	2598.27***	51.40
Tested Genotypes	159	1167.73***	125.83***
Genotypes x L	164	196.78***	38.83*
Pooled error	25	56.38	20.60

* p<0.05 , ** p<0.01 and *** p<0.001 ; df – degrees of freedom

The partition of the total variance showed an importance of genotypic variance over the environmental component for pod length and a moderate importance for 100-seed weight and seed iron indicating high broad-sense heritability for pod length and moderate broad-sense heritability for 100-seed weight and seed iron (Table 5.8).

Table 5. 7 : Grain yield (GY), iron (Fe) and zinc (Zn) levels of top 10 percent of biofortified and five local common bean lines

Genotype	GY (kg ha^{-1})	Group	Genotype	Fe (ppm)	Group	Genotype	Zn (ppm)	Group
G4-24A	4699.7	a	BF08-13-170	172.0	a	RK11	63.4	a
BF08-01-47B	2818.3	b	RK11	148.4	ab	BF08-07-22	63.0	ab
RWV2359	2690.9	b	BF08-07-22	148.2	ab	BF08-16-31	60.1	abc
BF08-13-44	2620.7	bc	BF08-07-112D	146.7	ab	BF08-26-162	58.4	abc
BF08-03-13	2546.8	bcd	BF08-26-162	145.8	ab	BF08-13-47	57.6	abc
MAC49	2532.3	bcd	BF08-07-74A	144.1	ab	BF08-16-92	57.4	abc
RW298	2455.2	bcd	BF08-16-92	142.4	abc	RK12B	57.3	abc
BF08-16-21	2451.3	bcd	G4-585	141.4	abc	BF08-1-60	55.7	abcd
BF08-16-67B	2439.6	bcd	BF08-14-153B	140.1	abc	G4-585	55.2	abcd
BF08-1-77	2335.9	bcd	BF08-3-23B	134.3	abcd	BF08-13-170	55.9	abcd
RWV2887	2331.0	bcd	G4-24A	133.7	abcde	BF08-14-153B	54.5	abcd
BF08-7-80	2326.8	bcd	RWV2887	133.2	abcdef	CAB2	54.3	abcd
BF08-07-74B	2315.1	bcd	SR6	132.8	bcdefg	BF08-13-38	53.0	abcde
BCB11-342	2308.0	Bcd	RK12B	132.4	bcdefg	BF08-14-153A	52.8	abcde
KMA13-23-21	2304.4	bcd	BF08-01-45B	131.2	bcdefgh	G4-24A	52.3	abcde
BF08-13-38	2304.0	bcd	BF08-16-18E	128.3	bcdefghi	BCB11-433	52.1	abcde
MAFUTALA	1513.2	cdefgh	DEMAI	112.7	c-s	MAFUTALA	38.7	defghij
IKINIMBA	1147.4	efgh	MAFUTALA	105.7	d-u	IKINIMBA	37.9	defghij
OBUSOSERA	948.2	fgh	IKINIMBA	77.6	s-K	DEMAI	37.6	defghij
KALANGITI	703.6	h	OBUSOSERA	77.3	u-K	KALANGITI	35.7	efghij
DEMAI	765.4	gh	KALANGITI	71.2	v-K	OBUSOSERA	32.3	fghij

For other traits measured, the environmental component of variance was more important than the genotypic component. This led to low broad-sense heritability for these characters.

Genotypic coefficient of variation was important for pod length and moderate for grain yield, number of seeds per pod, number of pods per plant, 100-seed weight and seed iron suggesting important and moderate genotypic influence in the expression of these characters.

Genetic gain under selection was important only for pod length. This indicated that this trait might be controlled by an additive gene action and gain under selection can be achieved through selection in early generation of iron and zinc biofortified genotypes. Low genetic gain was observed for all other traits, suggesting the importance of dominance gene action under control of these traits (Table 5.8).

Table 5. 8 : Variability, heritability and genetic advance of studied traits in iron and zinc biofortified common bean genotypes

Trait	Mean	VG	VE	VP	H^2 (%)	GCV(%)	PCV (%)	GA (%)
DTF	46.3	0.766	5.454	6.22	12.3	1.89	5.39	1.36
DT75M	92.4	4.18	14.978	19.162	21.8	2.21	4.74	2.13
DTFM	100.9	7.797	18.61	26.407	29.5	2.76	5.09	3.09
NPP	10.6	2.275	21.132	23.408	9.7	14.2	45.54	9.10
PL	11.4	12.9	0.644	13.6	95.3	31.53	32.38	63.57
NSP	5.59	0.696	5.192	5.888	11.8	15.23	44.29	10.76
100-SW	32.7	13.549	10.111	23.661	57.2	11.27	14.89	17.55
GY	1573.9	90250.55	890733.7	980984.2	9.2	19.08	62.93	11.93
Iron	93.0	121.37	56.38	177.75	68.3	11.84	14.33	20.16
Zinc	41.6	10.87	20.6	31.47	34.5	7.94	13.50	9.60

DTF – days to 50% flowering ; DT75M – days to 75% maturity ; DTFM – days to fully maturity ; NPP – number of pods per plant ; PL – pod length ; NSP – number of seeds per pod ; 100-SW – 100-seed mass ; GY – grain yield ; VG, VE and VP – genotypic, environmental and phenotypic component of variance ; H^2 – broad sense heritability ; GCV and PCV – genotypic and phenotypic coefficient of variation ; GA – genetic advance of mean at 5% selection intensity.

5.3.2. Association among agronomic traits and seed iron and zinc levels

Positive significant correlations were found among all agronomic traits, except between seeds per pod and 100-seed weight, which showed a negative relationship. However, the correlation between number of seeds per pod and duration to flowering was not significant. An increase in seed size within pods results in a decrease in 100-seed weight. Positive significant correlations were observed between grain yield and each of the agronomic traits measured indicating that high yielding ability is observed in late flowering and maturing common bean genotypes for which numbers of pods per plant and seeds per pod, pod length and 100-seed mass are large (Table 5.9).

Iron levels correlated positively only with the pod length among the agronomic traits. Positive correlations were observed among zinc levels and pod length and 100-seed weight.

Table 5. 9 : Correlation among agronomic traits and seed iron and zinc levels

	DT75M	DTFM	DTF	Iron	NPP	Pod length	100-SW	Seeds per pod	Grain yield
DTFM	0.97***								
DTF	0.85***	0.86***							
Iron	0.03ns	0.04ns	0.05ns						
NPP	0.53***	0.50***	0.37***	0.02ns					
Pod length	0.13***	0.14***	0.09**	0.08**	0.20***				
100-SW	0.28***	0.30***	0.38***	0.05ns	0.06*	0.15***			
Seeds per pod	0.07*	0.07*	0.05ns	0.02ns	0.20***	0.40***	-0.14***		
Grain yield	0.45***	0.42***	0.29***	0.05ns	0.69***	0.26***	0.17***	0.13***	
Zinc	0.03ns	0.04ns	0.05ns	0.52***	-0.03ns	0.13***	0.12***	-0.00ns	0.02ns

* p<0.05 , ** p<0.01 and *** p<0.001 ; DTF – days to 50% flowering ; DT75M and DTFM – days to 75% and fully maturity ; NPP – number of pods per plant ; 100-SW – 100-seed weight

5.3.3 Interrelationships among genotypes of common bean

Silhouette plot analysis (Figure 5.1) showed that the maximum and minimum clusters under which the study genotypes can be classified are 10 and 1. An optimum of four clusters can be skewed.

Genotypes were clustered into four groups (Figure 5.2). Cluster I (black) comprised three genotypes, BF08-1-49B, BF08-01-54 and KAB06F2-8-27. Cluster II (green) had 14 genotypes. High yielding genotypes were represented under these clusters (Table 5.10). Cluster III (blue) was made up of 41 genotypes and 107 genotypes under Cluster IV (red) (Figure 5.2). The least yielding genotypes were represented under Cluster III (Table 5.10).

Except the check MAFUTALA which belonged to the Cluster IV, all the other checks were represented in Cluster III. Two of the top four most yielding iron and zinc biofortified genotypes (with more than 2,600 kg ha^{-1}), G4-24A and RWV2359 belonged to the Cluster II, while the other two BF08-01-47B and BF08-13-44 belonged to Cluster IV. The least productive genotypes, KALANGITI, MIB465, RW267 and BCB11-509 were represented in Cluster III.

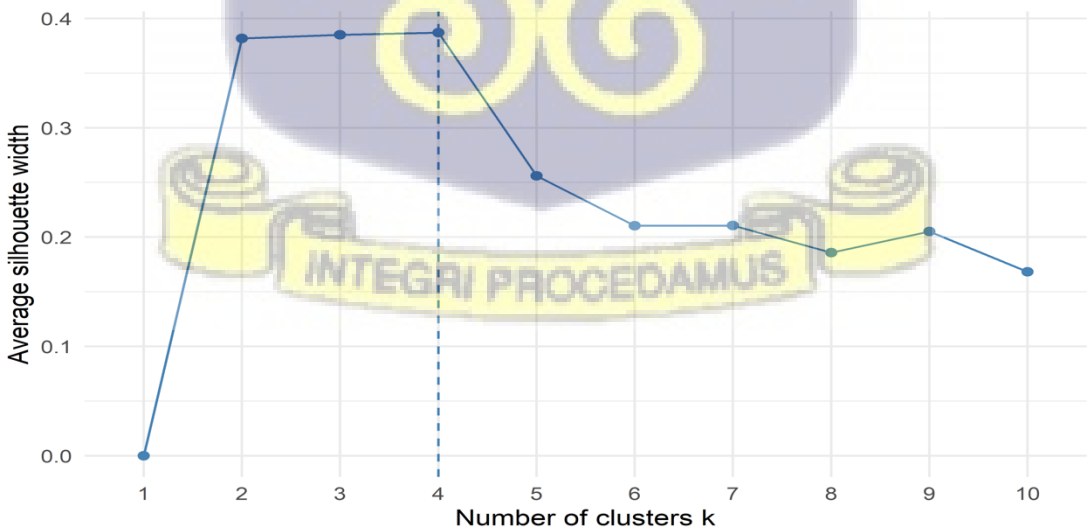


Figure 5. 1 : Optimum number of clusters to classify the study genotypes

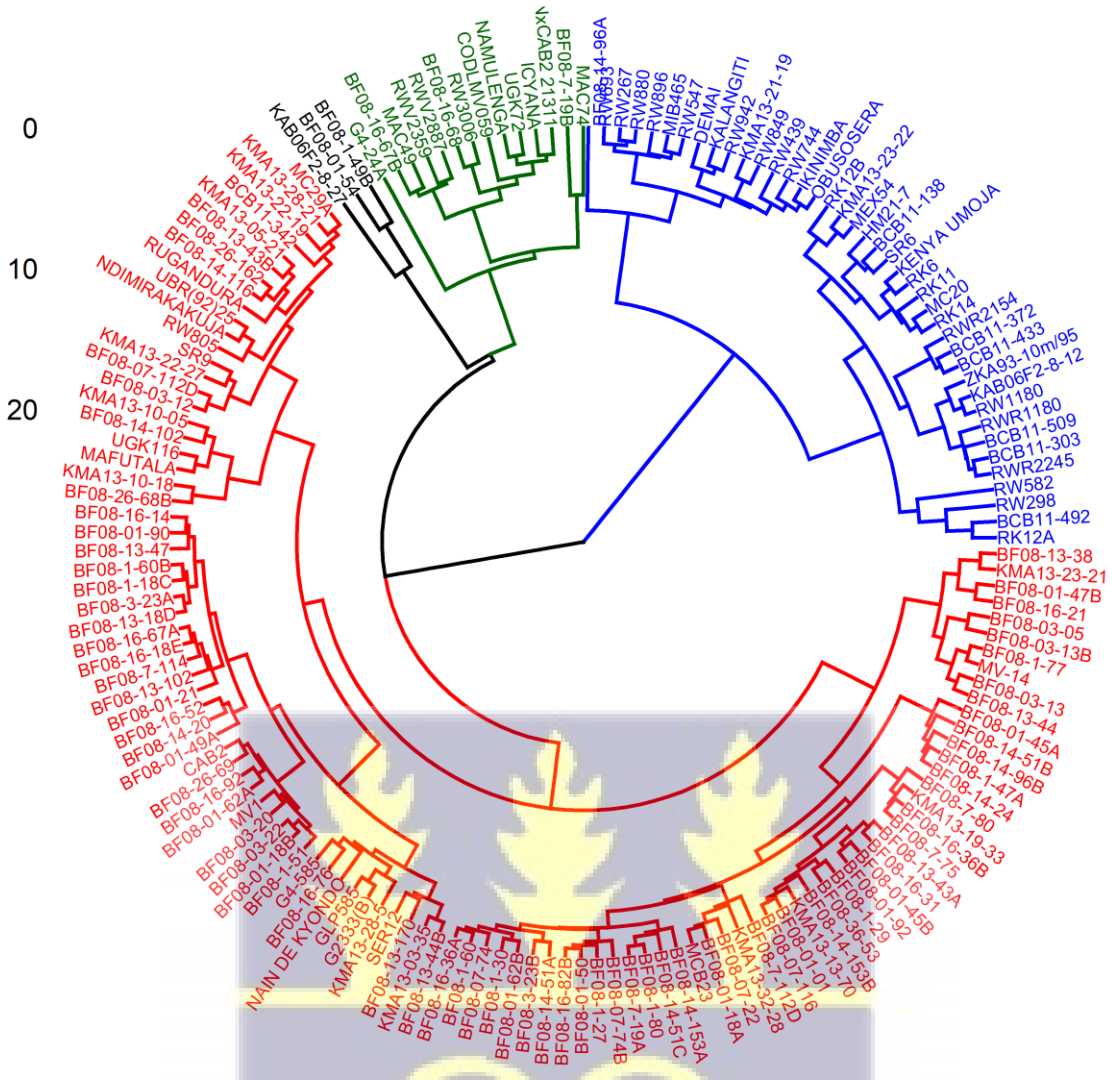


Figure 5. 2 : Dendrogram for different clusters for 165 common bean genotypes

Table 5. 10 : Cluster means for the agronomic traits

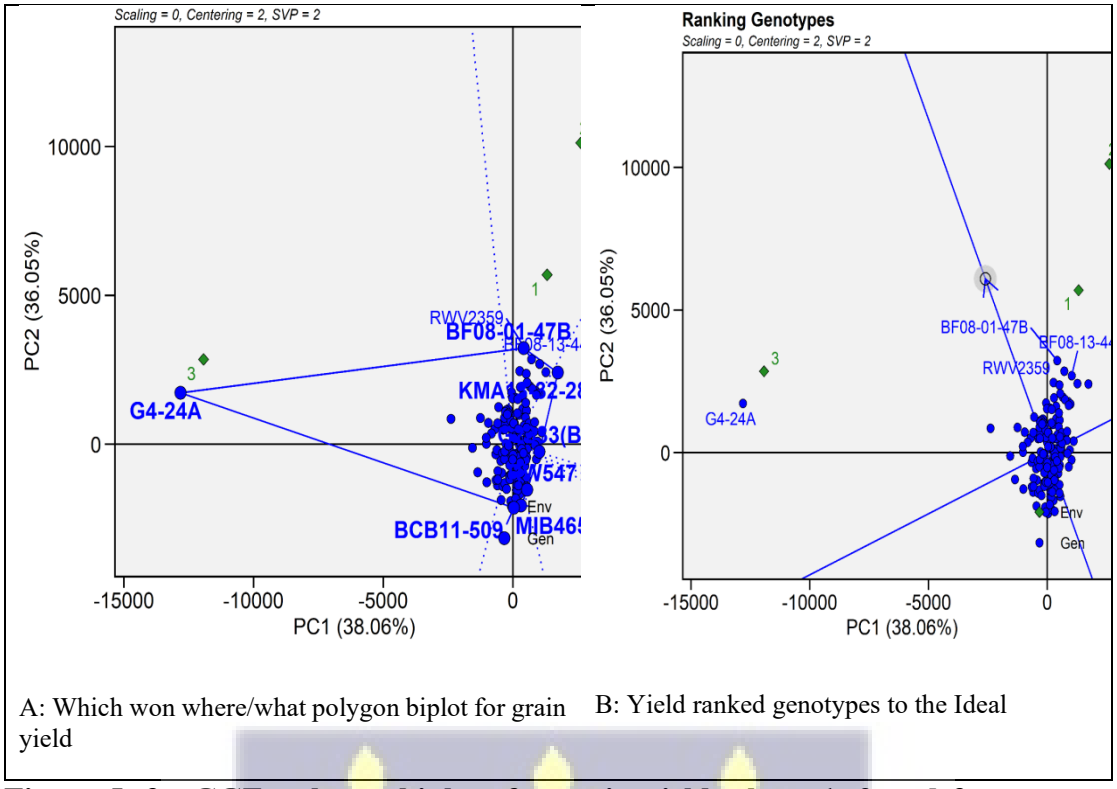
Trait	Cluster			
	I	II	III	IV
Days to flowering	46.9	48.8	43.4	47.3
Days to 75% maturity	94.8	96.5	87.2	94.3
Days to fully maturity	103.2	107.5	94.7	103.2
No. of pods per plant	17.5	9.5	7.7	12.0
Pod length (cm)	12.1	11.3	9.8	11.8
Seeds per pod	5.6	4.6	4.4	6.0
Grain yield (kg ha^{-1})	2110.7	2112.0	1076.4	1730.5
100-seed weight (g)	39.7	44.4	31.6	31.5

5.3.4. Yield stability

There was significant genotype by environment interaction. The first two principal components explained 74.1% of observed variability. The first component, PC1, contributed 38.06%, while the PC2 contributed 36.05% of the variability observed in interaction between genotype and environment (Figure 5.3).

Environments of Butembo and Lukanga were clustered together. They influenced genotypes in similar ways and were positively correlated, while no correlation was observed between Beni and each of these two environments. Butembo, an environment near the AMMI biplot origin, elicited only weak interactive forces, while Lukanga and Beni elicited strong environmental forces.

Many genotypes were scattered far from the AMMI biplot origin indicating important environmental instability for grain yield. Iron and zinc biofortified common bean genotype G4-24A, with the highest yield across sites, was most sensitive to environmental interaction and specifically adapted to environmental conditions of Beni (Figure 5.3A). None was ideal across all environments (Figure 5.3B).



A: Which won where/what polygon biplot for grain yield

B: Yield ranked genotypes to the Ideal

Figure 5. 3 : GGE polygon biplots for grain yield where 1, 2 and 3 represent environments Butembo, Lukanga and Beni

AMMI Stability Values (ASV) varied from 0.25 for genotype BF08-16-52 to 122.69 for genotype G4-24A. Despite being the high-yielding biofortified genotype, the later genotype was the most unstable. The 10 most broadly adapted iron and zinc biofortified common bean genotypes were presented in Table 5.11. Among these genotypes, BF08-14-96B, BF08-7-19B and BF08-14-51C can be advanced and used as the high yielding and stable iron and zinc biofortified common bean genotypes. At average, they yielded more than 1,800 kg ha^{-1}. Regardless to the rank of ASV (rASV) and rank of yield stability index (rYSI) that should be low for the good-yielding genotype, BF08-14-96B was ranked as the best. AMMI stability values varied from 4.23 for MAFUTALA to 11.81 among checks. IKINIMBA was the unstable check.

Table 5. 11 : AMMI stability values, ranking and grain yields of the 10 most stable common bean genotypes

Genotype	ASV	YSI	rASV	rYSI	Mean
BF08-16-52	0.25	100	1	99	1438.41
BF08-7-19B	1.18	51	2	49	1916.39
BF08-01-62A	1.46	104	3	101	1424.69
ZKA93-10m/95	1.60	160	4	156	874.28
BF08-13-44B	1.96	149	5	144	1020.25
BF08-14-51C	2.01	58	6	52	1864.68
BF08-14-96B	2.07	28	7	21	2190.92
KMA13-05-21	2.15	108	8	100	1432.73
BF08-03-12	2.96	128	9	119	1253.53
BF08-01-90	3.10	158	10	148	971.40

YSI – yield stability index, rYSI – rank of YSI, ASV – AMMI Stability Value and rASV – rank of ASV

5.4. Discussion

5.4.1. Performance of selected iron and zinc biofortified common bean genotypes

Findings showed significant interactions between genotypes, locations and seasons for grain yield, 100-seed weight, number of pods per plant and number of days to flowering. Findings also showed significant interactions between genotypes and locations for all the traits except for duration to 75% maturation and for number of seeds per pods but also significant interactions between genotypes and seasons for days to flowering, number of pods per plant, grain yield and 100-seed weight. Environmental factors played an important influence in phenotypic observations and genotypes responded differently across sites. Other studies (Mondo *et al*., 2019 ; Kataliko *et al*., 2024 and Kimani & Warsame, 2019) reported similar findings. No significant phenotypic contrasts were found for number of seeds per pod indicating that neither genotypic nor environmental factors were responsible for numerical variations observed for this character and that all genotypes behaved the same.

Mondo *et al*. (2019), during their study carried out on genotype x environment interactions on seed yield of 73 $F_{1.7}$ inter-racial common bean lines and five checks in

Kenya, found highly significant interactions between genotypes and environments for yield. Kataliko *et al*. (2024), during their study on genetic variability of iron and zinc biofortified common beans in eastern DRC, found significant interactions for the traits studied, except number of days to 90% maturation. They all suggested significant genotypic responses in different environments. Kimani & Warsame (2019) found significant interactions between genotypes and locations for yield. Furthermore, working on 110 $F_{4.7}$ common bean lines in four locations (Kabete, Nakuru, Thika and Tigoni), they found significant locational interactions with genotypes.

Genotypic differences were significant for all the traits, except for number of seeds per pod. Days to flowering and maturation among genotypes differed with the sites. Study genotypes flowered and matured earlier at Beni than at Butembo and Lukanga. This is due to the temperature and rainfall distributed during the growing seasons. While the optimum temperature for growth ranged between 27°C and 29.5°C and critical when it is close to or above 35°C (Salcedo, 2008), at Beni, during some months (Table 5.2), temperatures went beyond 35°C. This condition should be the cause why flowering and maturation periods were shortened in this particular location. The reason why flowering and maturation were late at Butembo and most particularly at Lukanga could be due to the relative cooler conditions (Table 5.2) considering their altitudinal positions (1,762 and 1,952 masl respectively). These cooler conditions led to slower plant growth delaying flowering and maturation. Similar results have been reported by Mondo *et al*. (2019) and Singh *et al*. (2002).

Genotypic performance in terms of number of pods per plant and grain yield was more important at Butembo and Lukanga compared to Beni. This could be a result of cooler conditions in these locations inducing slower plant growth, delaying maturity and important pod setting period resulting in higher grain yields. Mondo *et al*. (2019) and

Singh *et al*. (2002) have reported similar findings in independent studies. Lukanga tended to be more suitable for number of pods per plants and Butembo for grain yield. Furthermore, while the grain yield was estimated on the plot basis, the stand count of genotypes was high at Butembo than Lukanga. At Lukanga, all the plants did not stand for the entire period of cultivation. This could be due to the higher relative humidity observed at Lukanga (Table 5.2) leading to an important occurrence of bacterial and fungal diseases affecting the plants at late maturity. Soil acidity is another factor exacerbating the contrasting yields observed at these two locations. Soil at Lukanga was a bit more acidic than that of Butembo and Beni (Table 5.1). Similar findings were reported by Kataliko *et al*. (2024) but also Legesse *et al*. (2013) who evaluated 25 common bean genotypes under lime treated and untreated acidic soils in Ethiopia. The pH H_2O for soil at the experimental site at Lukanga was 5.24 (Table 5.1) which is less than the recommended limit lying between 5.5 and 6.5 (Salcedo, 2008).

The lower yield recorded at Beni in low altitude (1,118 masl) could be due to dry spells and erratic rainfall frequent in that location. In fact, the monthly maximum temperatures during the growing season were high than 29.5°C and for some months the temperature reached 35°C, while according to Salcedo (2008), for the optimum growth, the temperature should not go beyond 29.5°C and particularly under moisture stress. Among all the study locations, Beni was one with low values of relative humidity. In addition, maximum temperatures of 30°C at average were prevailing during the critical phases (flowering and pod setting and filling) that, according to Salcedo (2008), may be the cause of abortion of blossoms and developed pods.

The 100-seed weight for the genotypes was higher at Lukanga followed by Butembo and then Beni. This could be due to the rainfall amounts recorded but also the cooler conditions, most important at Lukanga. Lukanga recorded higher rainfall followed by

Butembo during the growing seasons. The monthly rainfall recorded at average was 142.8 mm at Lukanga, 119.92 mm at Butembo at 109.32 mm at Beni (Table 5.2).

Genotypes performed better at Lukanga during the short rainy season. This could be due to moderate rainfall during this season as high rainfall leads to mineral leaching and an increase of soil acidity particularly in higher altitudes such as Lukanga located at 1,952 masl (Table 5.2). According to Vyakuno (2016), nitrogen, very critical for plant growth, is the first essential mineral that is lost in increased rainfed conditions particularly in high altitudes. At Butembo, genotypes performed better during the long rainy season. Temperatures at Butembo were lying in between the limit recommended by Salcedo (2008). In addition, rainfall could be well distributed during the growing season (Table 5.2). This is in line with Singh *et al*. (2002) who reported that increased grain yields are observed in locations where temperatures do not go beyond 30°C and where rainfall is well distributed throughout the growing season.

Iron and zinc biofortified common bean genotypes performed better than the checks for yield and yield related traits. However, checks were the early flowering and maturing genotypes. This is evident and attributed to the breeding goal of biofortified genotypes. According to Kimani & Warsame (2019) ; Mbikayi *et al*. (2018); Kimani *et al*. (2008), iron and zinc biofortified common bean genotypes were bred to concentrate iron and zinc in seeds as the fast-track lines to address iron deficiency anaemia and to increase the income of farmers by increasing the grain yield. This breeding programme resulted in development of late maturing and yield performing biofortified common bean lines. This might also be explained by the type of correlation existing between the grain yields and other agronomic characters. Findings revealed strong positive correlations ($p<0.001$) between grain yield and days to flowering ($r=0.29$), grain yield and days to 75% maturity ($r=0.45$) and grain yield and days to fully maturity ($r=0.42$) suggesting

that large grain yields are observed in late flowering and maturing genotypes and that genes involved in control of these characters might co-segregate together. Similar findings have been reported by Mondo e*t al*. (2019) ; Kimani & Warsame (2019) ; Ghimire & Mandal (2019) ; Singh *et al*. (2002). In addition, findings revealed other strong positive correlations ($p<0.001$) between grain yield and all the yield components, number of pods per plant ($r=0.69$), pod length ($r=0.13$), number of seeds per pod ($r=0.13$) and the weight of 100 seeds ($r=0.17$) indicating that increases in these components result in increases of grain yield. This suggests that genes controlling these characters should co-segregate together. Similar findings have been reported by Mondo *et al*. (2019) ; Ghimire & Mandal (2019) ; Kataliko *et al*. (2023).

Another reason why grain yield was high for biofortified genotypes compared to the local lines could be the growth type. Most of biofortified genotypes were climbing lines, while only MAFUTALA was the climbing type among the local lines. Kimani & Warsame (2019) ; Mondo et al. (2019) stated that yield of climbing beans is four to 10 times more than that associated with bush beans.

Significant interactions were found between genotypes and locations for iron and zinc. This suggests that genotypes responded differently across sites. Biofortified common bean genotypes tested revealed significant variations for both quality traits suggesting that selection toward dense mineral concentration is possible among these genotypes. Similar findings have been reported by Kimani & Warsame (2019) ; Possobom *et al*. (2018) ; Amongi *et al*. (2018) ; Tryphone & Nchimbi-msolla (2010).

Large mineral variability observed would be a result of a large soil variability for mineral levels (Table 5.1) but also the genotypic differences considering different common bean lines used. Iron and zinc levels varied significantly among the germplasm

from 43.3 to 172.0 ppm, while zinc levels varied from 19.1 to 63.4 ppm in combined sites. Gelaw *et al*. (2023), in their work on 289 common bean landraces collected from Ethiopia, found iron and zinc amounts varying respectively from 63.6 to 88.8 ppm and 27.3 to 32.0 ppm. Using existing methods of mineral quantification, large iron and zinc variations have also been reported by many researchers; from 38 to 93 ppm and 19 to 43 ppm (Caproni *et al*., 2020), from 43 to 88 ppm and 28 to 41 ppm (Amongi *et al*., 2018), from 66.8 to 136.0 ppm and 22.2 to 41.3 ppm (Kimani & Warsame, 2019), from 28.0 to 98.0 ppm and 16.9 to 56.8 ppm (Blair *et al.*, 2011), from 34.3 to 117.8 ppm and 25.3 to 50.8 ppm (Delfini *et al*., 2021), 49.9 to 93.3 ppm and 19.3 to 35.6 ppm (Gunjača *et al.*, 2021), from 47.0 to 112.7 and 21.7 to 42.5 ppm (Binagwa *et al*., 2021).

Another reason explaining large variability observed might be the method used to determine mineral levels. In this study, inductively coupled plasma method, optical emission spectroscopy (ICP OES) was used. Other methods include atomic absorption spectrophotometry (AAS), colorimetry and advanced nondestructive estimation methods like X-ray fluorescence (XRF) (Elango *et al*., 2021). However, among methods for iron and zinc determination, Blair *et al.* (2011) reported that AAS and ICP OES are reliable and highly correlated. In addition, Elango *et al*. (2021) reported that the later methods are used as standard analytical tools for iron and zinc due to their reproducibility, accuracy and high throughput nature.

About 33 and 100 biofortified genotypes had higher levels of iron and zinc respectively compared to checks. This meets the breeding objective as reported by Kimani & Warsame (2019) ; IFC (2016) ; Blair, *et al*. (2010) ; Amongi *et al*. (2018). Among checks, findings showed that DEMAI and MAFUTALA had the highest iron and zinc amounts. These seeds look similar and their seed coats had similar color, black. However, MAFUTALA is a climbing type, while DEMAI is a bush type.

Most genotypes with high seed iron levels tended to have high seed zinc levels. This might be attributed to the type of correlation between iron and zinc. Findings revealed a strong positive correlation between iron and zinc suggesting that selection for superiority in one mineral would result in a high concentration in the other. The reason could be attributed to the fact that genes controlling these traits co-segregate together. Similar findings have been reported by Kimani & Warsame (2019) ; Amongi *et al*. (2018) ; Tryphone & Nchimbi-msolla (2010) ; Blair *et al*. (2009) and Beebe *et al*. (2000).

Large and diverse genotypic variability and performance might also be due to the importance of genotypic and phenotypic components of variation. This led to different heritability levels of characters. Findings revealed the importance of genotypic component of variance over the environmental component for pod length. Genotypic component of variance was moderate for 100-seed weight and least important for all other traits suggesting high and moderate heritability for pod length and 100-seed weight. Important and moderate genotypic coefficients of variation were observed respectively for pod length and grain yield, while genetic gain under selection in early generation was important for pod length and least important for all other traits. These findings could be attributed to additive and dominance gene effects governing these characters and suggest the importance of recurrent and heterosis breeding strategies to develop high performing biofortified common bean genotypes toward agronomic characters. Similar observations have previously been reported by Kataliko *et al*. (2024) ; Ejigu *et al*. (2018) ; Tiwari *et al*. (2019).

Low and moderate heritability governing zinc and iron levels respectively suggested low and moderate genotypic components of variation over the environmental factor. Genetic gain under selection as well as genotypic coefficient of variation were moderate

for iron and low for zinc indicating the importance of both dominance and additive gene effects controlling these characters and suggesting recurrent selection and heterosis breeding strategies to develop dense mineral common bean genotypes. During their study, Possobom *et al*. (2018), found broad-sense heritability for zinc varying from 67.21 to 90.03% and from 17.32 to 27.80% of genetic gain using a diverse population of F_1, F_1 reciprocal, F_2, F_2 reciprocal, backcross (BC_{11} and BC_{12}) generations of common bean lines developed through crosses involving two Andean and two Middle American lines. Lamptey *et al*. (2023) found broad-sense heritability varying from 62 to 82% for iron with 18.71 to 30.49 % of expected genetic gain and from 60 to 74% for zinc with 15.94 to 23.77% of genetic gain under selection suggesting the importance of both additive and non-additive gene effects. However, Mukai (2017) found higher broad-sense heritability estimates for iron (99%) and zinc (99%) suggesting that additive gene effects are more important that dominance effects.

Based on similarity of quantitative characters measured, findings revealed four important clusters. Cluster IV was the most important with 107 genotypes followed by cluster III with 41 genotypes. Clusters I and II comprised most of the yield performing genotypes. Cluster I was the least important with three genotypes. Genotypes assigned to a common cluster demonstrated closely aligned agronomic traits whereas those in separate clusters showed marked dissimilarity. Ghimire & Mandal (2019), based on quantitative data measured, also realized four clusters during their study carried on evaluation of 13 common bean genotypes in Nepal. Kataliko *et al*. (2024), characterizing 169 common bean genotypes, found four clusters, based on quantitative data assessed. Characterizing 57 common bean genotypes based on qualitative traits measured in Benin, Laura *et al*. (2018) realized nine different clusters. Kataliko *et al*.

(2023) found 12 clusters during their study on characterization of 65 common bean genotypes.

5.4.2. Yield stability

The scores for the first two interaction principal component axes accounted for up to 74.11% of the total genotype environment interaction suggesting that the use AMMI model fit the data well and justified its use.

Treatments (genotypes, environments and their interaction) contributed the most to the total variability. This indicated environmental diversity and the existence of significant genetic variations among genotypes for seed yield. Similar findings have been reported by Ashango & Alamerew (2016) ; Mondo *et al*. (2019) ; Gereziher *et al*. (2017). Although, the environment is a very broad factor including predictable and unpredictable factors (Falconer & Mackay, 1996), observations might have mainly been influenced by the temperature and the rainfall distribution. Lukanga and Butembo were locations with low temperatures and high rainfall, while Beni was the location with high temperatures and low rainfall distribution (Table 5.2). This could also be the reason why according to AMMI output, Butembo and Lukanga were clustered together and influenced genotypes in similar ways unlike Beni.

The other key environmental factor was the soil pH which was low at Lukanga (acidic soil) and high at Beni (least acidic to neutral soil) (Table 5.1). This factor was not important for yield increase as Beni, with the least acidic soil, was not the site with important grain yields.

Due to significant genotype – environment interactions, test genotypes were not stable and responded differently across locations and should be recommended to specific environments. Regarding their ASV, high yielding lines, particularly the most yielding

biofortified genotype, G4-24A, was the most unstable across locations. These findings are in line with what Mondo *et al*. (2019) ; Tadesse *et al*. (2018) ; Swegarden *et al*. (2016) reported. Stable lines are not necessarily the high yielding genotypes. Gepts & Debouck (1991) reported that the center of domestication is another factor affecting the yield stability among dry bean cultivars. Swegarden *et al*. (2016) reported that Andean (large-seeded) lines, in general, exhibited lower yield and stability than their Mesoamerican (small and medium-seeded) counterparts. This seems to be one among the factors of the stability of the most stable biofortified genotypes, BF08-14-96B, BF08-7-19B and BF08-14-51C. These genotypes are the medium-seeded lines. Regardless, genotype G4-24A, small to medium-seeded biofortified genotype was the most unstable line, sensitive to environmental interaction and specifically adapted to environmental conditions of Beni due probably to the environmental conditions of Beni (high temperatures and low rainfall) limiting the growth of large-seeded genotypes, particularly the high-yielding types. These types require enough period to mature as reported by Mondo *et al*. (2019) and Singh *et al*. (2002). In fact, hot conditions led to shortening maturation and impacted the yield of late maturing genotypes particularly.

5.5. Conclusion

Iron and zinc biofortified common bean genotypes flowered and matured later but performed better for yield and yield-related traits than the local varieties. Performance at Butembo and Lukanga was better than that at Beni. The long rain season allowed a good performance than the short rain season. Three biofortified genotypes, G4-24A, BF08-01-47B and RWV 2359, had grain yields (4699.7, 2818.3 and 2690.9 kg ha^{-1} respectively) significantly higher than that for checks. Iron and zinc levels varied respectively from 43.3 to 172.0 ppm and from 19.1 to 63.4 ppm. Three biofortified

genotypes, RK 11, BF08-07-22 and BF08-26-162, combined iron and zinc levels significantly higher than those for the high iron and zinc checks.

Among genotypes, none was ideal across locations but based on their AMMI stability values, biofortified genotypes BF08-14-96B, BF08-7-19B and BF08-14-51C were the most stable with more than 1,800 kg ha^{-1} of grain yield. Genotype G4-24A, the most high yielding, was the most unstable line and adapted to environmental conditions of Beni.

CHAPTER 6

6. INHERITANCE OF ACCUMULATION OF IRON AND ZINC AND REDUCTION OF POLYPHENOLS IN COMMON BEAN

6.1.Introduction

Common bean (*Phaseolus vulgaris* L.), also known as pinto, navy, red kidney or French bean is the third most important legume in the world after soybean and peanut in regard to the production. It has been ranked as the world's most important legume food crop (Amongi *et al*., 2018).

Beans are regarded as an important component of healthy diets not only because of their high nutritional values (20-28% of proteins, 56% of carbohydrates with the least amount of fat) (Amongi *et al*., 2018), but also owing to their functional components. These components include lectins, soluble fibers and total polyphenols (Yang & Gan, 2018).

Polyphenols represent antinutritional factors reducing mineral bioavailability in foods. They chelate minerals such as iron, zinc, calcium, magnesium and molybdenum and thereby reduce their bioabsorption (Campion *et al*., 2009). However, polyphenols are associated with anti-inflammatory, chemopreventive and antibacterial effects and can reduce risks associated with conditions such as obesity, diabetes, heart diseases and also certain types of cancers (Yang & Gan, 2018).

Petry et al. (2010) suggested that the level of polyphenols and other antinutrional factors should be reduced if the aim of a breeding programme is to improve bioabsorption of iron and zinc in a staple food. Welch & Graham (2004), reported a reduction of iron and zinc bioavailability in common beans with the high level of polyphenols. However, antioxidant activity decreased when common beans had low polyphenol contents (Campion *et al*., 2009).

There is a dearth of information on the genetic relationships between polyphenols and mineral contents, especially for iron and zinc. Very little information exits on the inheritance of iron, zinc and polyphenols in common bean. Such information is necessary for developing common bean varieties with high iron and zinc contents with minimal levels of polyphenols.

The objective of this study was to determine the performance and mode of inheritance of iron, zinc and total polyphenols in common bean.

6.2.Materials and Methods

6.2.1. Description of the study sites

Experiments were conducted in Butembo and Lukanga in Lubero territory, Nord-Kivu province, Eastern DRC between November 2022 and July 2023. Generation of hybrids was done between November 2022 and February 2023 in a greenhouse in the field station of the Université Catholique du Graben (UCG Butembo) in Lubero Territory. Evaluation of F_1 hybrids with their parents was conducted in the field stations of UCG Butembo and Université Adventiste de Lukanga (UNILUK) during the 2023 short rain season between March and July 2023. Their geographical positions have been described in section 4.2.1.

Prior to the field evaluation, soil analysis of the study sites was conducted at the laboratory of UCG in February 2023 (Table 6.1). The soil type belonged to the group of Nitosols. According to Van Diepen (1985), nitosols refer to soils that have an argillic horizon with about 80% and above of clay within 150 cm of the surface.

Considering that common bean grows better in a range of pH H_2O lying between 5.5 and 6.5 (Salcedo, 2008), soil of UCG was sweet than that of UNILUK. Soil of UNILUK was a bit more acidic.

Table 6. 1 : Characteristics of soils of the study sites

Site	pH H_2O	pH KCL	Dry matter(%)	Org. matter (%)	P (%)	K (%)	N (%)	Fe (%)
UCG	5.76	4.95	82.1	12.2	0.364	1.18	0.1264	0.000015
UNILUK	5.41	4.68	83.2	7.6	0.512	0.3958	0.1266	0.000015

According to Salcedo (2008) common bean grows well when temperatures range from 15 to 27°C and 29.5°C. Both sites were suitable considering the range of temperatures and amounts of rainfall recorded during the growing season. However, relative humidity was rather high at Lukanga (Table 6.2).

6.2.2. Plant Materials

The first phase of the study involved hybridization of six female with three male parents. The six females were MAFUTALA, CAB2, NDIMIRAKAKUJA, RW582, RW547 and RW1180. The three males were 042/5, 041/1 and 720/12. The female genotypes were selected because of their high levels of iron and zinc and adaptation (Amongi e*t al*., 2018). These female genotypes had high contents of polyphenols than the males. The male parents were phaseolin and seed lectins mutants with low polyphenol contents (Giuberti *et al*., 2019 ; UCG lab, 2023) (Tables 6.3 and 6.4).

Table 6. 2 : Temperature, relative humidity and rainfall of the study sites during the 2023 short rain season

Climatic item	Sites	Month				
		March	April	May	June	July
T_{max} (°C)	UCG	28.7	27.3	25.3	28.2	27.0
	UNILUK	24.2	25.3	24.8	24.0	25.9
T_{min} (°C)	UCG	16.5	15.8	15,7	15.4	15.2
	UNILUK	14.9	15,4	14.9	14.6	14.3
Relative humidity (%)	UCG	74.6	79.7	83.5	77.0	73.4
	UNILUK	90.7	89.6	88.5	91.0	90.3
Rainfall (mm)	UCG	119.9	162.1	147.0	29.6	141.4
	UNILUK	136.0	184.9	169.8	30.6	192.7

Source : NASA, 2023

Table 6. 3 : Concentration of iron, zinc and total polyphenols in female dry beans

Genotype	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Total pphenols (mg GAE g^{-1})
Males			
041/1	84.77	37.94	1.71 – 2.89
042/5	91.37	35.55	0.92 – 1.71
720/12	83.77	34.36	1.05 – 1.71
Females			
MAFUTALA	130.1	38.5	39.46 – 78.92
CAB2	123.3	63.9	34.2 – 55.24
NDIMIRAKAKUJA	95.5	37.5	40.78 – 76.29
RW582	76.2	44.1	59.19 – 98.65
RW547	101.3	39.1	55.90 – 92.08
RW1180	81.8	42..0	59.19 – 105.88

Sources: Giuberti *et al*., 2019 ; Amongi *et al*., 2018 ; Mulambu *et al*., 2017 ; UCG lab, 2023

Male parents do not carry any mutation of low phytic acid contents but rather of the absence of Phaseolin and the presence of α- amylase inhibitor (Table 6.4).

Additional information on other plant characteristics of parental lines is presented in Table 6.5. Male parents are early maturing compared to the female parents and have low yield potentials compared the later parents (Kataliko *et al*., 2023).

Table 6. 4 : Characteristics of the male parents

Genotype	Presence of the low phytic acid mutation	Absence of Phaseolin	Absence of Phaseolin + presence of α- amylase inhibitor	Genus of origin
041/1	0	1	1	*P. vulgaris* + *P. coccineus*
042/5	0	1	1	
720/12	0	1	1	

Value 1 is attributed to the genotype carrying the genetic trait or a combination of traits, while 0 means the absence of the trait (or combination of traits) identified

Source : Giuberti *et al*., 2019

Table 6. 5 : Additional characteristics of the parental lines

Genotype	Flower colour	DTF	DTM	100-seed mass (g)	Seed type	Growth Type	Yield (kg ha^{-1})
041/1	Purple	43	84	23.2	Purple mottled	Bush	40 – 105
042/5	Purple	42	86	16.86	Red mottled	Bush	250 – 580
720/12	Pink	43	82	17.8	Purple mottled	Bush	75 – 150
MAFUTALA	Purple	51	110	25.13	Black beans	Climbing	800 – 1500
CAB 2	White	61	110	28.53	Navy beans	Climbing	1300 – 2400
NDIMIRAKAKUJA	Pink	51	105	29.33	Pinto beans	Climbing	1100 – 2200
RW582	Pink	59	106	50.46	Red mottled	Bush	800 – 1500
RW547	Purple	49	114	21.53	Clay	Climbing	750 - 1450
RW1180	Purple	45	89	51.86	Purple beans	Bush	450 – 1200

DTF - Days to 50% flowering ; DTM - Days to maturity

6.2.3. Hybridization

6.2.3.1.Crop management and preparation of female parents

Hybridization of the parental lines was done in a greenhouse at UCG Butembo following a line x tester mating design where females were considered as the lines and the males as the testers.

Parental lines were sown four times at 7-days interval to synchronize flowering and to ensure sufficient hybridization (Vollmann *et al*., 1992). The first set of parental lines was sown on 1 November 2022 and the fourth one on 22 November 2022. Each line was planted in 10 plastic pots. In total, 40 pots were sown for each line. A pot was 37 cm long with 30 and 50 cm of diameter at the base and the top.

Before sowing, seeds were mixed with thiram 75WP at a recommended rate of 1 kg of seeds in 2 g of the product to prevent seed borne fungi (Singh *et al*., 2014). Growth media was prepared using a 3:1:1 ratio of soil, sand and organic manure according to Kataliko *et al*. (2018). Four seeds were sown in a pot and thinning to two seedlings was

done after emergency. The name of the genotype and the date of planting were the elements used to label pots.

Pots were maintained free from weed and hand irrigation was done twice a day, in the morning and the evening. From when hybridization starts, the floor was kept wet to increase relative humidity. This is because high relative humidity seems to improve the success of artificial hybridization (Fehr, 1991).

Female flowers likely to be pollinated were chosen on the basis of some criteria : they were likely to open the next day and the colour of the corolla (purple or white depending on the variety) appeared inside the sepals. These parents had swollen buds. Stamens were emasculated to avoid self-pollination. On each raceme, two to four buds were prepared, while immature buds and self-mated flowers were removed using forceps with curved tips. Calyx and corolla were removed on female flowers ready for crosses by taking each of them using the forefinger and the thumb. The corolla was taken out, grasping it above the scar of the calyx without injuring the stigma. After locating the stigma which occupies the opposite side of the flower, anthers were also removed. In fact, they form a certain ring surrounding the stigma (Walter, 1980).

6.2.3.2.Collection of pollen, pollination and labeling

Pollens were collected from male flowers. These flowers should be opened to allow pollen shedding. Collection of pollens was done by removing the entire flower after the removal of the calyx and corolla.

Hybridization started 32 days from sowing, from 3 December 2022 and took 42 days. The hooking coupled with the emasculation method of pollination as described by Fehr (1991) was followed to increase the success rate of hybridization. Pollination took place early in the morning and late in the afternoon as recommended (Fehr, 1991). This is

because shedding of pollens begins at 0700 hours when temperatures average 30°C (Walter, 1980). Female flowers were immediately hand-pollinated after being prepared. Hand pollination was carried out using forceps by removing the stamens and pistil of a recently opened flower used as the male parent and brushing gently the anthers against the stigma of the female flower (Walter, 1980). After being brushed, the male flower was hooked to the female flower. Forceps were treated with 95% alcohol to avoid unintended pollination.

Paper tags were used to identify pollinated flowers. Apart from information on the parents involved in the cross, the tag carried information on the person who made the cross, the number of female flowers mated on a raceme and the date of the cross.

6.2.3.3.Seed harvesting and processing

The success rate was recorded two weeks after the pollination for each cross. Rather than falling, flowers for successful crosses developed small pods.

At complete maturity, dry seeds were harvested from dried pods into paper bags after identifying the specific cross made. Seeds were thereafter sun-dried for three days and saved for field evaluation.

6.2.4. Field evaluation

6.2.4.1.Experimental design

The 18 F_1 progenies and their parental lines were evaluated from March to July 2023 in two different agroecological zones: at the field stations of UCG and UNILUK. The trial was laid out in a 9x3 alpha-lattice design. A plot consisted of three rows of 90 cm long, four plants per row and 12 plants per plot. The spacing was 40 cm between rows

and 30 cm between plants. Blocks were separated by 0.5m wide array, while a replicate was 1 m apart from the other.

6.2.4.2.Land preparation and crop management

Land at each trial site was prepared by ploughing and harrowing to a moderate tilth. Diammonium phosphate (18% N and 46% P_2O_5) was mixed with soil before sowing at a rate of 150 kg ha^{-1} as recommended. Field experiments were kept free from weeds throughout the cropping season. Weeding was carried out times on 21-days interval from sowing.

6.2.5. Assessment of agronomic traits

Data was collected on days to 50% flowering, 75% maturity and full maturity, number of pods per plant, pod length, number of seeds per pod and 100-seed weight. Five middle plants were used for data collection. Data on grain yield was collected on the plot basis.

6.2.6. Determination of iron (Fe) and zinc (Zn)

Analyses to determine Fe and Zn concentration were conducted by Inductively Coupled Plasma (ICP) based-technique, Optical Emission Spectrometry at the Department of Land Resource Management and Agricultural Technology (LARMAT), Faculty of Agriculture, University of Nairobi between September and October 2023 following the procedure described in section 5.2.5.

6.2.7. Determination of total polyphenols

Total polyphenols analysis was conducted at the Laboratory of UCG and was based on the Folin-Ciocalteau colorimetry method as described by Singleton *et al*. (1999) after crushing the samples of dry beans. This method has an advantage of a fairly equivalent

response to different phenols but slightly interferes with sugar and sulfur dioxide (Waterhouse, 2002).

According to the method followed, 1 g of grinded common bean seeds was diluted with deionized water (ten-fold) to fall into the range of the standards. The reason why dilution took place prior to the process and measurement is because the absorbance of the sample would be above that of the 500 mg/l standard. Thereafter, 1 ml sample, a gallic acid calibration standard or blank (deionized water) was placed in a 100-ml volumetric flask. From there, 70 ml of water and 5 ml of folin-Ciocalteau were added and the mixture was swirled and incubated for 4 to 8 minutes at room temperature. Thereafter, 15 ml of sodium carbonate solution was added. The volume was then brought to 100 ml line by adding deionized water and incubated two hours at room temperature. Finally, 2 ml of the preparation was transferred to a 1-cm, 2-ml plastic cuvette to measure its absorbance at 765 nm in a spectrophotometer. The absorbance of the blank from all readings should be substracted and using the standards, a calibration curve was created (Waterhouse, 2002).

The calibration curve was then used to determine the corresponding gallic acid concentration of the samples. This concentration was multiplied by the dilution factor (10) and values were reported in mg of gallic acid equivalent (GAE) per g of dry common bean seeds (Waterhouse, 2002).

6.2.8. Data analysis

6.2.8.1.Genotypic performance

All agronomic data was subjected to a combined analysis of variance using Genstat (21st edition) with replications and locations as random factors and all the genotypes

(crosses and their parents) as the unique fixed factor, while the traits measured were considered as the variables.

The separation of the total variance was done as suggested by Falconer and Mackay (1996) to estimate the importance of the genetic component of the variance controlling the traits measured. Fisher's Protected Least Significant Difference at 95% confidence interval was used for mean separation.

Data on iron, zinc and polyphenol contents were first subjected to a descriptive statistical analysis. Pearson's correlation between iron, zinc and total polyphenols was done using Statistix, version 8.0.

6.2.8.2.Genetic analysis

The total variance was partitioned to assess diversity between and within lines and testers using R statistical software, version 4.4.0. The line x tester linear model for the phenotype was expressed for any observed value from each experimental unit Y_{ijk} as by Nduwumuremyi *et al*. (2013) as $Y_{ijk} = \mu + a_i + b_{kl} + v_{ij} + (av)_{ijl} + e_{ijkl}$ where μ refers to population mean, a_i and b_{kl} to location and block or replication effects within each location ; v_{ij} the F_1 hybrid effect which refers to the sum of general combining ability (GCA) effect of the i^{th} parental line (g_i), GCA effect of the j^{th} tester (g_j) and the specific combining ability (SCA) effect of the ij^{th} hybrid (s_{ij}), $v_{ij} = g_i + g_j + s_{ij}$; $(av)_{ijl}$ refers to the interaction effect between ij^{th} hybrid and l^{th} location and e_{ijkl} the deviation from the observed mean or the residual effect.

Narrow sense heritability (h^2) was estimated as follow $h^2 = \frac{VA}{VA+VD+VE}$ while broad-sense heritability (H^2) was calculated as by this formula $H^2 = \frac{VA+VD}{VA+VD+VE}$ where VA, VD and VE are respectively the additive, dominance and environmental components of variance

(Acquaah, 2012). Best-parent heterosis (HPH) was calculated as follow $HPH(\%) = \frac{F_1 - HP}{HP} \times 100$ where F_1 and HP are respectively the average performance of the hybrid and the best parent for any given character (Acquaah, 2012). To validate the average performance of a genotype in a series of hybrid combinations or a deviation of a hybrid combination than would be expected on the basis of the average performance of the parents, parents-offspring (X-Y) regression estimate of heritability was conducted using Statistix, version 8.0, particularly on mineral composition of the study genotypes. The adjusted R^2 was used to explain how the observations fit the model (Y=f(X)).

To identify the best combiners toward a study trait, general and specific combining ability (GCA and SCA) effects were computed using AGD-R 5.1, an R statistical package developed by CIMMYT (https://prezi.com/m63mqfpwh-j1/agd-r/?fallback=1, Sept 2023).

6.3.Results

6.3.1. Pollination success rate

The number of matings realized daily varied from 10 to 15 for each cross during the period of pollination, pollination taking place in the morning and late in the afternoon. We made between 379 to 744 matings for each cross (Table 6.6). We did as much matings as we can for crosses where the success rate was low.

The number of seeds we obtained for each cross varied from 72 to 103. Crosses involving the tester 042/5 tended to result with a high success rate and high seed numbers (Table 6.6).

The success rate was less than 15% for each of the crosses made. Pods from successful crosses developed 1 to 2 seeds and rarely 3 or 4 seeds. These seeds were a bit small compared to those obtained from self-pollination.

Table 6. 6 : Pollination and success rate

Cross	Number of pollinations made	Number of successful crosses	Success rate (%)	Number of seeds harvested
Mafutala x 042/5	585	76	13	102
Mafutala x 041/1	500	55	11	79
Mafutala x 720/12	483	58	12	81
CAB 2 x 042/5	744	67	9	96
CAB 2 x 041/1	540	54	10	72
CAB 2 x 720/12	550	55	10	75
Ndimirakakuja x 042/5	555	61	11	87
Ndimirakakuja x 041/1	450	54	12	73
Ndimirakakuja x 720/12	536	59	11	77
RW 582 x 042/5	593	83	14	103
RW 582 x 041/1	617	74	12	96
RW 582 x 720/12	608	73	12	91
RW 547 x 042/5	392	51	13	77
RW 547 x 041/1	491	54	11	79
RW 547 x 720/12	483	58	12	82
RW1180 x 042/5	482	53	11	77
RW1180 x 041/1	423	55	13	72
RW1180 x 720/12	379	53	14	73

6.3.2. Genotypic performance and correlation between characters

Highly significant differences ($P<0.001$) were observed among genotypes for all the agronomic characters studied (Table 6.7). Location effects were significant for all the characters except the number of pods per plant (NPP) and 100-seed weight (100-SW). Interaction between genotypes and locations was significant for all the traits except NPP, number of seeds per pod (NSP) and grain yield (GY).

Table 6. 7 : Combined analysis of variance for hybrids and parental lines across Butembo and Lukanga

Source of variations	df	DTF	DT75M	DTFM	NPP	NSP	100-SW	GY
Replications	2	31.1	7.6	18.4	241.4	0.4	9.2	1977322
Genotypes (G)	26	86. 3***	130.8***	148.7***	282.9***	2.9***	167.7***	1358844***
Locations (L)	1	1963.6***	13384.3***	19734.2***	149.7	6.0**	1.0	4186096***
G x L	26	35.7***	60.4***	63.8***	55.3	0.4	17.9**	198835
Pooled error	161	4.97	5.7	7.5	44.7	0.8	9.1	225475

DTF – days to 50% flowering ; DT75M – days to 75% maturity ; DTFM – days to fully maturity ; NPP – number of pods per plant ; NSP – number of seeds per pod ; 100-SW – 100-seed mass ; GY – grain yield ; df – degree of freedom ; * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

The partition of the total genotypic variance showed significant interactions between the lines, testers and locations for the number of days to flowering (DTF) and to full maturation (DTFM). Interactions between lines and locations were significant for DTF, days to 75% maturation (DT75M) and DTFM and 100-seed weight (100-SW), while interactions between testers and locations were significant only for DTF. Interactions between the lines and testers were significant for DTF, DT75M and DTFM. Between the tested hybrids and locations interactions were significant for DTF, DT75, DTFM and 100-SW (Table 6.8).

Highly significant differences ($P<0.001$) were observed among lines for all the characters. Among testers, observed differences were significant only for 100-SW.

Table 6. 8 : Mean squares of the agronomic characters at UCG and UNILUK

Source of variation	df	DTF	DT75M	DTFM	NPP	NSP	GY	100-SW
Locations (S)	1	1247.12***	9259.26***	14352.1***	104.03	3.65*	2047332*	0.108
Replications	4	21.04***	15.47	22.52*	61.48	0.99	532503.3	27.58
Genotypes (G)	17	77.46***	126.34***	147.17***	191.13***	2.5***	960733***	132.3***
Line (L)	5	193.34***	400.33***	449.18***	548.58***	6.79***	2584201***	377.22***
Tester (T)	2	3.95	6.132	20.89	122.16	0.02	227463.1	93.63***
L x T	10	34.22***	13.38*	21.42*	26.20	0.87	285862.5	17.61
G x L	17	39.35***	53.24***	55.81***	59.12	0.49	173434.8	19.34*
L x S	5	40.23***	152.01***	149.13***	54.54	0.67	443657.5	24.51*
T x S	2	9.45	21.89*	13.36	42.03	0.65	86941.34	15.03
L x T x S	10	44.89***	10.13	17.64*	64.83	0.36	55622.06	17.63
Pooled error	56	3.80	6.22	8.32	49.69	0.53	243269.8	9.43

df- degrees of freedom ; DTF – days to 50% flowering ; DT75M and DTFM – days to 75% and fully maturity ; NPP – number of pods per plant ; NSP – number of seeds per pod ; 100-SW – 100-seed weight ; GY – grain yield (kg ha-1) ; * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

Flowering occurred six to eight days earlier at UCG-Butembo than at UNILUK (Table 6.9). Genotypes RW582 and CAB 2 were respectively the early and the late flowering lines. All the testers took similar period to initiate opened flowers, 42 days from sowing.

Among the F_1 progenies, hybrids RW1180 x 720/12 and RW1180 x 041/1 reached earlier 50% flowering, 41 days from sowing, while CAB2 x 041/1 flowered later, 53 days from sowing. In all combinations where the line RW1180 was involved, 50% of flowering was reached earlier, while when CAB2 was involved, F_1 hybrids flowered later (more than 46 days from sowing).

Table 6. 9 : Days to flowering of hybrids and parental lines at UCG Butembo and Lukanga

Testers	Butembo	Lukanga	Combined sites
042/5	41bcd	45cd	43
041/1	38cd	46cd	42
720/12	40bcd	45cd	43
Mean	39	46	42
$LSD_{0.05}$	-	-	3.4
CV (%)	-	-	6.3
Lines			
Mafutala	40bcd	56b	48b
CAB2	49a	63a	56a
Ndimirakakuja	43abcd	49c	46bc
RW582	40bcd	45cd	42d
RW547	42bcd	47cd	44cd
RW1180	43abcd	44cd	44cd
Mean	43	51	47
$LSD_{0.05}$	-	-	3.3
CV (%)	-	-	5.9
Hybrids			
Mafutalax042/5	40bcd	61a	50b
CAB2x042/5	46ab	46cd	46c
Ndimirakakujax042/5	41bcd	46cd	44defg
RW582x042/5	42bcd	45cd	43defgh
RW547x042/5	41bcd	45cd	43efgh
RW1180x042/5	37d	46cd	41ghi
Mafutalax041/1	41bcd	49cd	45cdef
CAB2x041/1	44abc	62a	53a
Ndimirakakujax041/1	44abcd	47cd	45cd
RW582x041/1	40bcd	45cd	43fghi
RW547x041/1	41bcd	47cd	44cdef
RW1180x041/1	37cd	44cd	41i
Mafutalax720/12	41bcd	46cd	44defg
CAB2x720/12	47ab	56b	51ab
Ndimirakakujax720/12	43abcd	47cd	45cde
RW582x720/12	37cd	45cd	41hi
RW547x720/12	44abcd	47cd	45cd
RW1180x720/12	38cd	43d	41i
Mean	41	48	45
$LSD_{0.05}$	-	-	2.3
CV (%)	-	-	4.4

LSD0.05 – least significant difference α =0.05 ; CV % – coefficient of variation

Genotypes matured after 84 days from sowing at UCG-Butembo and 22 days later at UNILUK. Testers matured earlier than the other two groups of genotypes. They

matured four days earlier than the lines. With 88 days to full maturity, genotype 720/12 was the early maturing tester, while RW1180 was the early maturing line (86 days to maturity). The line CAB 2 with 102 days to maturity was the late maturing genotype (Table 6.10).

Crosses RW1180x042/5 and RW1180x720/12 were the early maturing genotypes and CAB2x042/5, CAB2x041/1 and CAB2x720/12 the late maturing. In fact, crosses where CAB 2 was involved matured later.

A large variability was also observed among genotypes for pod and seed characters (Table 6.11). Deviation in the number of pods per plant from the mean varied significantly among lines. Lines CAB2, Mafutala and Ndimirakakuja initiated significantly large pod numbers (≥22.75) compared to RW582 and RW1180 (≤11.76 pods per plant).

Among crosses, numbers of pods per plant varied (42.1% of CV). Hybrid CAB2x042/5 had the large pod number. Many other crosses produced pod numbers statistically similar to this cross. Crosses RW1180x042/5, RW1180x041/1 and RW1180x720/12 initiated low pod numbers.

Table 6. 10 : Days to maturity of hybrids and parental lines at UCG Butembo and Lukanga

	Days to 75% maturity			Days to fully maturity		
Testers	Butembo	Lukanga	Combined sites	Butembo	Lukanga	Combined sites
042/5	79cde	90ghi	84b	83bcd	98ij	91b
041/1	79cde	100cdef	90a	84abcd	106defghi	95a
720/12	79abcde	87hi	83b	84abcd	92j	88c
Mean	79	92	86	83	99	91
$LSD_{0.05}$	-	-	2.7	-	-	1.8
CV (%)	-	-	2.4	-	-	1.6
Lines						
Mafutala	79cde	107abcd	93b	83bcd	113abcde	98b
CAB2	84a	107abc	96a	89a	116abc	102a
Ndimirakakuja	81abcde	100cdef	91c	85abcd	107cdefghi	96b
RW582	76e	94fg	85d	81d	104efghi	92c
RW547	78de	99cdef	89c	84abcd	107cdefghi	96b
RW1180	77de	83i	80e	81d	91j	86d
Mean	79	99	89	84	106	95
$LSD_{0.05}$	-	-	2.3	-	-	3.1
CV (%)	-	-	2.2	-	-	2.7
Hybrids						
Mafutalax042/5	80abcde	99ef	90ef	83abcd	111abcdefg	97bcd
CAB2x042/5	84ab	108ab	96a	88abc	117ab	106a
Ndimirakakujax042/5	82abcd	100cedf	91cde	86abcd	107cdefghi	96cd
RW582x042/5	79abcde	96fg	88fg	83cd	105efghi	94ef
RW547x042/5	78de	95fg	86gh	83bcd	99hij	91f
RW1180x042/5	77de	85hi	81j	81d	94j	87g
Mafutalax041/1	77de	101cdef	89efg	82cd	108bcdefgh	95cde
CAB2x041/1	79acde	108a	94abc	86abcd	118a	102a
Ndimirakakujax041/1	82abcd	105abcde	94abc	86abcd	111abcdefg	99bc
RW582x041/1	79abcde	96fg	88fg	83bcd	108cdefgh	95cde
RW547x041/1	80abcde	100acdef	90def	85abcd	109bcdefgh	97cd
RW1180x041/1	78de	86hi	82ij	82d	94j	88g
Mafutalax720/12	81abcde	99cef	90def	85abcd	106defghi	95cde
CAB2x720/12	84abc	106abcde	95ab	88ab	117ab	103a
Ndimirakakujax720/12	81abcde	104abcde	93ab	86abcd	114abcd	100ab
RW582x720/12	78cde	91gh	85hi	81d	103eghi	92ef
RW547x720/12	79abcde	102abcdef	90def	84abcd	113abcdef	98bc
RW1180x720/12	76e	85hi	801	81d	94j	87g
Mean	80	98	89	84.	107	96
$LSD_{0.05}$	-	-	2.9	-	-	3.4
CV (%)	-	-	2.9	-	-	3.1

$LSD_{0.05}$ – least significant difference α =0.05 ; CV % – coefficient of variation

Table 6. 11 : Number of pods per plant of hybrids and parental lines at UCG Butembo and Lukanga

Testers	Butembo	Lukanga	Combined sites
042/5	4.0d	5.2cd	4.6b
041/1	8.5bcd	6.4bcd	7.4a
720/12	5.1cd	2.5d	3.8b
Mean	5.8	4.7	5.3
$LSD_{0.05}$	-	-	2.669
CV (%)	-	-	39.4
Lines			
Mafutala	19.3abcd	28.7a	23.9a
CAB2	28.7a	22.7abcd	25.7a
Ndimirakakuja	21.2abcd	24.3abc	22.9a
RW582	10.3abcd	13.0abcd	11.7bc
RW547	13.2abcd	25.9abc	19.6ab
RW1180	7.5bcd	5.5cd	6.5c
Mean	16.7	20.02	18.4
$LSD_{0.05}$	-	-	8.0
CV (%)	-	-	36.4
Hybrids			
Mafutalax042/5	16.8abcd	27.2ab	22.0ab
CAB2x042/5	25.5ab	23.2abcd	24.3a
Ndimirakakujax042/5	24.3abc	20.3abcd	22.3ab
RW582x042/5	15.6abcd	11.6abcd	13.6cdef
RW547x042/5	16.9abcd	25.2abc	21.1abc
RW1180x042/5	8.3bcd	6.9bcd	7.6f
Mafutalax041/1	14.1abcd	18.0abcd	16.0bcde
CAB2x041/1	19.7abcd	23.8abc	21.8ab
Ndimirakakujax041/1	13.2abcd	26.3abc	21.8ab
RW582x041/1	15.0abcd	11.5abcd	16.8abcde
RW547x041/1	12.6abcd	21.1abcd	16.9abcde
RW1180x041/1	8.5bcd	5.9bcd	7.2f
Mafutalax720/12	15.3abcd	9.8abcd	12.6def
CAB2x720/12	13.1abcd	29.0a	21.1abc
Ndimirakakujax720/12	19.4abcd	20.4abcd	19.9abcd
RW582x720/12	12.6abcd	8. 7abcd	10.6ef
RW547x720/12	17.8abcd	16.6abcd	17.2f
RW1180x720/12	9.5abcd	5.3cd	7.4f
Mean	15.45	17.77	16.67
$LSD_{0.05}$	-	-	8.073
CV (%)	-	-	42.1

$LSD_{0.05}$ – least significant difference α =0.05 ; CV % – coefficient of variation

Number of seeds per pod did not vary significantly from the mean (3.6 seeds per pod) among testers (Table 6.12). Dispersion of seed numbers within a pod among testers from the mean was not important (13.2%).

Lines CAB2 and Mafutala initiated numbers of seeds per pod (5.4 seeds per pod) significantly higher. Line RW 582 initiated low seed numbers in pods.

Among hybrids, RW 547 x 041/1 initiated large number of seeds per pod (5.7 seeds per pod) followed by CAB2 x 720/12 (5.6 seeds per pod). Hybrid RW 1180 x 720/12 initiated the least seed numbers within pods (3.5 seeds per pod).

The 100-seed weight (100-SW) was high for lines and hybrids (26g) compared to the testers (20.3g). Among the testers, 042/5 had the highest 100-SW, while 720/12 had the lowest 100-SW. Among lines, RW 582 and RW 1180 had the highest 100-SW (≥34.4g), while RW547 and Mafutala had the lowest 100-SW (≤20.6g) (Table 6.13).

Among hybrids, RW582x042/5 was the best performer with 34.2 g of 100-SW. This cross was followed by CAB2x042/5, RW1180x042/5 and RW1180x041/1 for which the 100-SW was more than 30g. Mafutalax042/5 and Mafutalax720/12 were hybrids that had the lowest 100-SW (≤19.1g) (Table 6.13).

Table 6. 12 : Number of seeds per pod of hybrids and parental lines at UCG Butembo and Lukanga

Testers	Butembo	Lukanga	Combined sites
042/5	3.9	3.1ab	3.5
041/1	4.1	3.7ab	3.9
720/12	3.8	3.1ab	3.5
Mean	3.9	3.3	3.6
$LSD_{0.05}$	-	-	2.0
CV (%)	-	-	13.2
Lines			
Mafutala	5.5	5.3ab	5.4a
CAB2	5.6	5.3ab	5.4a
Ndimirakakuja	5.1	5.2ab	5.2ab
RW582	4.2	3.9ab	4.1c
RW547	5.0	5.2ab	5.1ab
RW1180	4.9	3.7ab	4.3bc
Mean	5.0	4.7	4.9
$LSD_{0.05}$	-	-	0.9
CV (%)	-	-	15.5
Hybrids			
Mafutalax042/5	5.8	4.6ab	5.2abcde
CAB2x042/5	5.7	4.9ab	5.3abcd
Ndimirakakujax042/5	5.4	5.0ab	5.2abcde
RW582x042/5	4.2	4.1ab	4.2fgh
RW547x042/5	5.3	5.1ab	5.2abcde
RW1180x042/5	4.4	3.7ab	3.7gh
Mafutalax041/1	5.0	4.9ab	5.0abcdef
CAB2x041/1	4.3	4.5ab	4.4efg
Ndimirakakujax041/1	5.4	4.6ab	5.0abcdef
RW582x041/1	4.5	4.6ab	4.5defg
RW547x041/1	6.0	5.5ab	5.7a
RW1180x041/1	3.9	3.8ab	3.9gh
Mafutalax720/12	4.9	4.6ab	4.8cdef
CAB2x720/12	5.5	5.8a	5.6ab
Ndimirakakujax720/12	5.5	5.2ab	5.4abc
RW582x720/12	4.3	4.3ab	4.3fgh
RW547x720/12	4.5	5.2ab	4.8bcdef
RW1180x720/12	4.3	2.8b	3.5h
Mean	4.9	4.6	4.766
$LSD_{0.05}$	-	-	0.849
CV (%)	-	-	15.5

$LSD_{0.05}$ – least significant difference α =0.05 ; CV % – coefficient of variation

Table 6. 13 : 100-seed weight (g) of hybrids and parental lines at UCG Butembo and Lukanga

Testers	Butembo	Lukanga	Combined sites
042/5	27.2bcdef	22.0efgh	24.6a
041/1	19.6fgh	19.9fgh	19.7b
720/12	14.2g	19.0fgh	16.6c
Mean	20.3	20.3	20.3
$LSD_{0.05}$	-	-	1.0
CV (%)	-	-	3.9
Lines			
Mafutala	20.7efg	20.1fgh	20.4c
CAB2	24.0bcdef	23.5defgh	23.8bc
Ndimirakakuja	25.0bcdef	26.5defg	25.7b
RW582	36.2a	34.9abc	35.6a
RW547	21.4defg	19.7fgh	20.5c
RW1180	31.4abc	37.2ab	34.4a
Mean	26.5	27.0	26.7
$LSD_{0.05}$	-	-	3.5
CV (%)	-	-	10.9
Hybrids			
Mafutalax042/5	19.8fg	18.3gh	19.1j
CAB2x042/5	30.0abcd	30.7bcd	30.4bcd
Ndimirakakujax042/5	27.6bcdef	23.6defgh	25.6efg
RW582x042/5	29.5abcd	38.8a	34.2a
RW547x042/5	25.1bcdef	22.0efgh	23.6ghi
RW1180x042/5	29.4abcd	36.9ab	33.2ab
Mafutalax041/1	19.9fg	20.2fgh	20.1ij
CAB2x041/1	26.0bcdef	27.7cdef	26.8defg
Ndimirakakujax041/1	27.0bcdef	27.7cdef	27.3def
RW582x041/1	29.5abcd	28.9cde	29.2cde
RW547x041/1	22.9cdef	19.9fgh	21.4hij
RW1180x041/1	31.8ab	31.9abcd	31.8abc
Mafutalax720/12	20.7efg	16.7h	18.7j
CAB2x720/12	25.1bcdef	23.8defgh	24.5fgh
Ndimirakakujax720/12	26.3bcdef	25.5defg	26.1efg
RW582x720/12	28.9abcde	26.2defg	27.5def
RW547x720/12	22.3def	21.1efgh	21.7hij
RW1180x720/12	26.6bcdef	29.6cde	28.1def
Mean	26.0	26.1	26.1
$LSD_{0.05}$	-	-	3.7
CV (%)	-	-	12.4

$LSD_{0.05}$ – least significant difference α =0.05 ; CV % – coefficient of variation

Significant variations were observed among all the genotypes studied, the 18 hybrids and particularly among the lines for grain yield. Testers had low grain yields compared

to the lines and hybrids. Testers had an average grain yield of 186.5 kg ha^{-1}; lines and hybrids had 1,120 and 890 kg ha^{-1} respectively (Table 6.14).

Line CAB2 had the highest grain yield followed by Ndimirakakuja. However, RW1180 had the least grain yield. Among hybrids, CAB2 x 042/5 had the highest grain yield followed by CAB2 x 041/1 and Ndimirakakuja x 720/12. In fact, in each cross where lines CAB 2 and Ndimirakakuja were involved, except the combination where Ndimirakakuja and 042/5 were involved, grain yield tended to be high. In contrast, hybrids RW1180x041/1, RW582x720/12 and RW1180x720/12 had the least grain yields.

Descriptive statistics for iron, zinc and total polyphenols showed that iron contents varied from 65.4 to 140.6 ppm among the 27 study genotypes. Zinc contents varied from 41.9 to 74.7 ppm, while total polyphenol contents varied from 0.9 to 45.2 mg of gallic acid equivalent per g of dry common bean seeds (Table 6.15).

Dispersion from the mean was important for total polyphenols and low for zinc contents.

Table 6. 14 : Grain yield (kg ha^{-1}) of hybrids and parental lines at UCG Butembo and Lukanga

Testers	Butembo	Lukanga	Combined sites
042/5	72c	45.5b	58.7b
041/1	557abc	262.6ab	409.7a
720/12	76c	106.0ab	90.9b
Mean	235	138	186
$LSD_{0.05}$	-	-	127.8
CV (%)	-	-	53.3
Lines			
Mafutala	1250abc	1098.5ab	1174bc
CAB2	2245a	1224.7a	1735a
Ndimirakakuja	2038ab	1050.5ab	1544ab
RW582	1073abc	848.5ab	961cd
RW547	972abc	820.7ab	896cd
RW1180	372bc	443.2ab	408d
Mean	1325	914.35	1120
$LSD_{0.05}$	-	-	556.5
CV (%)	-	-	41.5
Hybrids			
Mafutalax042/5	1056abc	1106.1ab	1080.8abcd
CAB2x042/5	1965ab	1048.0ab	1506.3a
Ndimirakakujax042/5	1045abc	515.2ab	780.3defg
RW582x042/5	854abc	813.1ab	833.3cdef
RW547x042/5	1000abc	1065.6ab	1032.8abcde
RW1180x042/5	503abc	416.7ab	459.6efg
Mafutalax041/1	952abc	732.3ab	842.2cdef
CAB2x041/1	2030ab	949.5ab	1489.9a
Ndimirakakujax041/1	1783abc	972.2ab	1377.5abc
RW582x041/1	881abc	818.2ab	849.7bcdef
RW547x041/1	886abc	601.0ab	743.7defg
RW1180x041/1	386bc	234.8ab	310.6fg
Mafutalax720/12	773abc	292.9ab	532.8defg
CAB2x720/12	1596abc	1154.2ab	1375.1abc
Ndimirakakujax720/12	1745abc	1090.9ab	1417.9ab
RW582x720/12	548abc	305.6ab	426.8fg
RW547x720/12	717abc	697.0ab	707.1defg
RW1180x720/12	265bc	246.2ab	255.7g
Mean	1054.7	725.5	890
$LSD_{0.05}$	-	-	574.8
CV (%)	-	-	56.1

$LSD_{0.05}$ – least significant difference α =0.05 ; CV % – coefficient of variation

Table 6. 15 : Descriptive statistics of iron, zinc and polyphenol of common bean

	Iron, Fe (ppm)	Zinc, Zn (ppm)	Polyphenol (mg of GAE g^{-1})
Low 95% Confidence interval	86.5	47.6	22.2
Mean	94.0	50.6	26.7
Up 95% Confidence interval	101.5	53.6	31.3
Standard deviation	19.1	7.6	11.5
Variance	363.3	57.8	131.4
Coefficient of variation (%)	20.3	15.0	42.9
Minimum	65.4	41.9	0.9
Median	89.6	48.9	27.7
Maximum	140.6	74.7	45.2

GAE g^{-1} – gallic acid equivalent per gram of common bean

No significant differences were observed among the 27 genotypes for iron, zinc and total polyphenols (Table 6.16). The observed individual variations from the average performance of 94 ppm, 50.58 ppm and 26.7 mg of GAE g^{-1} respectively for iron, zinc and total polyphenols, were not significant and thereby due by chance.

Analysis of variation of separated genotypes showed significant differences among testers for zinc and iron ($P<0.01$) (Table 6.17). The amounts of iron and zinc in tester 720/12 were significantly higher than in the other two testers (Table 6.18). However, there were no significant differences among parents and hybrids for total polyphenols.

Table 6. 16 : Mean squares of combined 18 common bean hybrids and nine parental lines for iron, zinc and total polyphenols

Source of variations	df	Iron	Zinc	Total polyphenols
Locations	1	6740.2	8.01	25979.0
Genotypes	26	726.5	115.55	262.8
Error	26	700.1	98.74	227.8

df – degree of freedom

Table 6. 17 : Mean squares of iron, zinc and total polyphenols in common bean genotypes

Source of variation	df	Polyphenols	Iron	Zinc
Lines	5	0.01	374.0	54.0
Error	6	0.24	470.9	59.3
Testers	2	6.66×10^{-5}	1959.6**	666.2**
Error	3	0.00	60.2	20.2
Hybrids	17	0.02	679	78.3
Error	18	0.16	1219	120.0

** $p<0.01$; df – degrees of freedom

Among lines, RW582 and RW1180 had the highest iron amounts, while CAB2 had the least polyphenol content (Table 6.18). In general, total polyphenol contents in lines were high than in testers. Hybrids RW582 x 041/1, CAB2 x 720/12, CAB2 x 042/5 and RW547 x 042/5 had the least total polyphenol amounts. Mineral bioavailability in these genotypes should be high compared to other hybrids.

The hybrid CAB2 x 720/12 had the highest zinc amount, while hybrids RW547 x 720/12 and RW1180 x 720/12 had the highest iron amounts. These genotypes can be advanced through selection for iron.

It was also observed, in separated data, high iron and less total polyphenol amounts for genotypes at Lukanga than at UCG Butembo. However, zinc amounts did not vary in these sites.

A significant positive correlation ($r=0.40$, $p<0.01$) was observed between iron and zinc suggesting that selection for high iron accumulation results in high zinc content in the study common bean genotypes (Table 6.19). Non-significant positive correlation ($r=0.17$, $p>0.05$) was observed between iron and total polyphenols, while the type of correlation between zinc and polyphenol was negative ($r=-0.14$, $p>0.05$). Total polyphenol levels positively correlated with number of pods per plant ($r=0.23$, $p<0.05$).

Grain yield positively correlated with each of the agronomic traits except the 100-seed weight.

Table 6. 18 : Iron, zinc and polyphenol contents in common bean parental lines and F_1 hybrids grown at two locations in Eastern DR Congo

Testers	Total polyphénols (GAE g^{-1})	Zn (ppm)	Fe (ppm)
042/5	0.85	42.6 b	70.3 b
041/1	1.71	43.6 b	65.4 b
720/12	0.85	74.7 a	121.9 a
Mean	1.14	56.6	85.9
Lines			
Mafutala	28.16	53.1	84.4
CAB2	23.04	57.1	77.7
Ndimirakakuja	29.87	41.9	74.6
RW582	37.97	52.9	107.2
RW547	38.40	48.5	76.4
RW1180	34.56	51.7	100.0
Mean	32.00	58.9	86.7
Hybrids			
Mafutalax042/5	34.99	46.4	109.1
Mafutalax041/1	43.52	46.8	110.4
Mafutalax720/12	28.16	55.4	105.3
CAB2x042/5	24.32	52.0	90.3
CAB2x041/1	33.28	53.2	98.4
CAB2x720/12	18.77	68.0	89.1
Ndimirakakujax042/5	26.45	46.2	83.5
Ndimirakakujax041/1	22.61	42.7	106.0
Ndimirakakujax720/12	37.97	53.1	87.5
RW582x042/5	27.31	43.4	87.0
RW582x041/1	17.49	43.1	89.6
RW582x720/12	45.23	52.3	88.2
RW547x042/5	21.76	43.7	94.6
RW547x041/1	31.57	48.4	71.1
RW547x720/12	30.72	53.4	140.6
RW1180x042/5	26.88	48.9	109.7
RW1180x041/1	27.73	45.9	69.0
RW1180x720/12	27.73	55.9	131.2
Mean	29.25	49.9	97.9

Table 6. 19 : Correlation among characters in common bean F_1 hybrids

	DT75M	DTFM	DTF	Iron	N.pods	Pphenols	100-SW	Seeds/pod	Yield
DTFM	0.98***								
DTF	0.74***	0.74***							
Iron	-0.10	-0.07	-0.05						
N.pods	0.38***	0.34***	0.35***	-0.11					
Pphenols	0.02	0.05	0.05	0.17	0.23*				
100-SW	-0.14	-0.10	-0.14	-0.03	-0.10	0.20			
Seeds/pod	0.10	0.06	0.09	-0.15	0.46***	0.24	-0.17*		
Yield	0.01*	0.04**	0.12**	0.11	0.67***	0.21	0.07	0.48***	
Zinc	0.01	0.03	0.16	0.40**	-0.08	-0.14	-0.21	-0.03	-0.03

DTF – days to 50% flowering ; DT75M and DTFM – days to 75% and fully maturity ; N.pods – number of pods per plant ; 100-SW – 100-seed weight ; *** p<0.001 ; ** p<0.01 ; * p<0.05

6.3.3. Heritability of characters

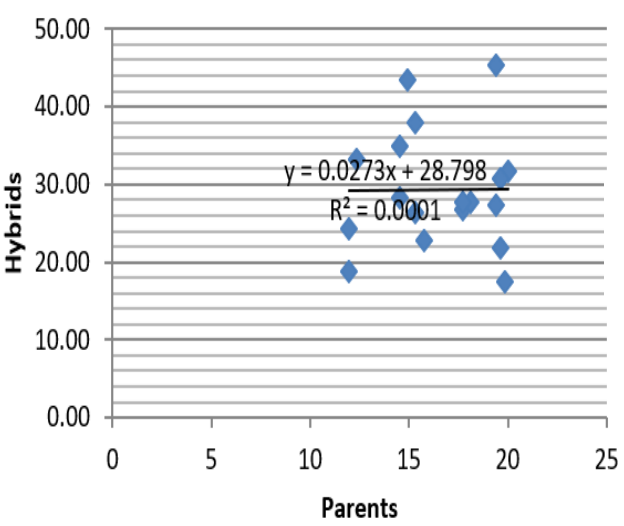
Broad-sense heritability was important for all the study characters (>0.70) indicating the importance of genetic effects controlling the inheritance of characters. The breakdown of the total genetic variance showed that additive genetic variance was more important for all agronomic characters and least important for all the quality traits (Table 6.20). This indicated that recurrent selection aimed at accumulating additive genes for improvement of the agronomic traits is the most viable breeding approach to develop the most promising advanced lines. However, to develop quality beans in regard to low total polyphenols, high iron and zinc in common beans, heterosis breeding was the most viable approach. For these characters, non-additive genetic component of variance was more important.

Table 6. 20 : Genetic components of variance and heritability estimates of agronomic and quality traits studied

	V_A	V_D	V_G	V_E	V_P	h^2	H^2
DDF	25.40	$8.84x10^{-14}$	25.40	6.559	31.959	0.79	0.795
DT75M	48.73	0.7427	49.473	8.874	58.347	0.83	0.848
DT90M	60.93	4.041	64.971	9.264	74.235	0.82	0.875
NPP	88.01	0	88.01	9.67	97.68	0.90	0.901
NSP	1.32	0.146	1.466	0.085	1.551	0.85	0.945
GY	518793.3	54635.87	573429.17	37894.63	611323.8	0.85	0.938
100-SW	75.318	0.2776	75.5956	3.2245	78.8201	0.95	0.958
Polyphenols	$5.4 x10^{-5}$	0.0162	0.0162	0.0038	0.02	0.0027	0.809
Zn	0	49.9839	49.9839	25.6224	75.6063	0	0.661
Fe	0.7015	460.877	461.578	189.5971	651.175	0.001	0.709

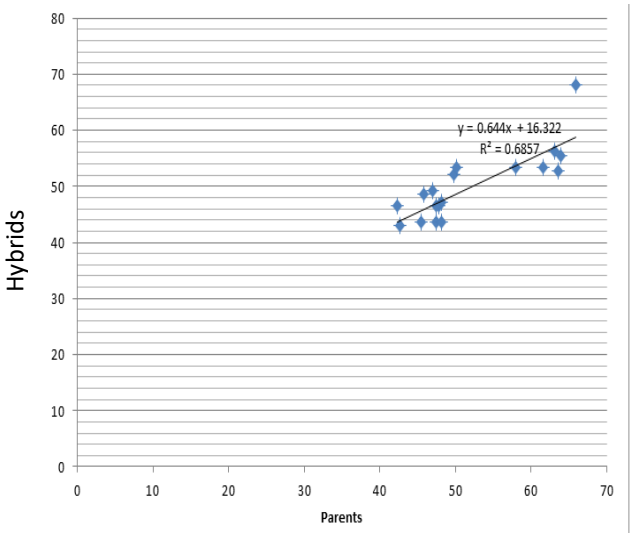
VA, VD, VG, VE, VP –additive, dominance, genotypic, environmental and phenotypic variances; h^2 and H^2 – narrow and broad-sense heritability ; DTF – days to 50% flowering ; DT75M and DTFM – days to 75% and fully maturity ; NPP – number of pods per plant ; NSP – number of seeds per pod ; 100-SW – 100-seed weight ; GY – grain yield ; Zn – zinc ; Fe – iron.

Parents-offsprings linear regression in regard to the quality traits (Figure 6.1) showed low heritability coefficients particularly with respect to the total polyphenol levels in dry common bean genotypes. This suggested low resemblance between hybrids and their parents particularly in regard to this quality character. However, the adjusted R^2 is very low to explain how the observations fit the model. Nevertheless, moderate to high heritability coefficients were found to be associated with iron and zinc respectively with 9.42 and 68.57% of adjusted R^2. This indicated a moderate or a high resemblance between hybrids and their parents in regard to these characters.



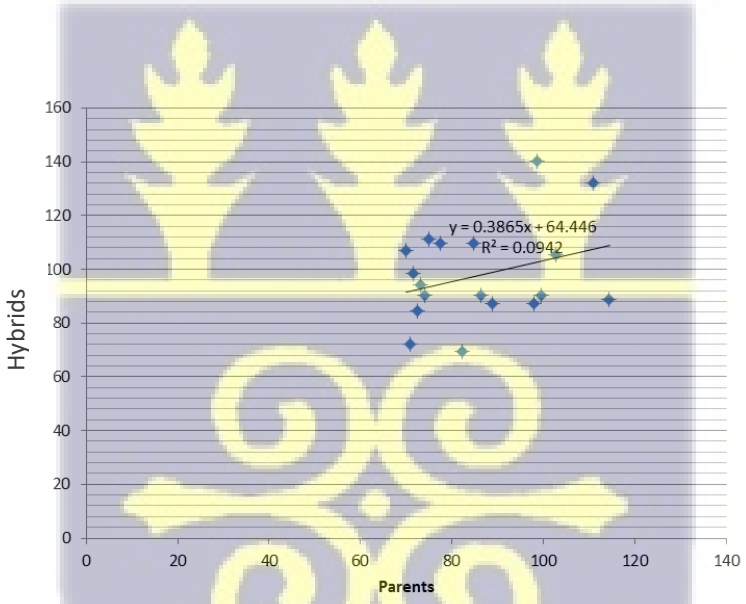
h^2 = 2.73%

A : Resemblance between hybrids and parents in regard to the total polyphenols (mg of GAE g^{-1})



h^2 = 64.4%

B : Resemblance between hybrids and parents in regard to the composition of beans in zinc (ppm)



h^2 = 38.65%

C : Resemblance between hybrids and parents in regard to the composition of beans in iron (ppm)

Figure 6. 1 : Regression coefficients of hybrids to their parents

6.3.4. Heterosis of characters

High-parent heterosis among hybrids varied depending on the character considered. A reduction of more than 3 % of the number of days to flowering was associated with

hybrids RW1180 x 042/5, RW1180 x 041/1 and RW1180 x 720/12. In addition, a reduction of 3.1% of days to flowering was associated with hybrid RW 582 x 720/12 (Table 6.21).

Only Mafutala x 041/1 hybrid allowed a reduction of 0.8% and an increase of 0.6 % of days to 75% and fully maturity than would be the case when parents are the ones grown. Hybrid RW 547 x 042/5 allowed an increase of 0.5% of days to fully maturity. Hybrid CAB2 x 720/12 was a late maturing genotype and allowed an increase of 16.9% of days than when parents are the ones grown.

Hybrid RW582 x 041/1 allowed an increase of 43.2% of pods per plant and 10.2% of seeds per pod. Two other hybrids, RW582 x 042/5 and RW1180 x 042/5, resulted in increases of 16.2 and 16.9% of pods per plant. Hybrid RW547 x 041/1 allowed 12.4% increase of number of seeds per pod. Two hybrids, RW547 x 042/5 and RW1180 x 042/5, allowed respectively 15.3 and 12.6% increases of grain yields.

The best combinations being the hybrids with the least or negative heterosis percentages in regard to the total polyphenol amounts, hybrid RW582 x 041/1 was the most promising such that its use would be associated with low rate of accumulated polyphenolics. However, in regard to iron and zinc, the most promising genotypes were the ones found with high heterosis percentages. Hybrid Ndimirakakuja x 042/5 was the most promising toward zinc increase, while about 10 hybrids were the most promising toward iron increase. Among these hybrids, Ndimirakakuja x 041/1 was the most promising with 42.1% increase followed by Mafutala x 041/1 and Mafutala x 042/5 respectively with 30.9 and 29.3% increases (Table 6.21). Hybrids RW547 x 042/5 and RW1180 x 042/5 were the ones combining increased heterosis for grain yields and iron accumulation.

Table 6. 21 : Heterosis (%) of agronomic and quality traits of common bean

Génotype	DTF	DT75M	DTFM	NPP	NSP	GY	100-SW	Pphenols	Zn	Fe
Mafutalax042/5	17.5	6.3	6.9	-7.9	-3.7	-7.9	-22.4	4000.0	-12.5	29.3
Mafutalax041/1	6.2	-0.8	0.6	-32.9	-7.4	-28.3	-1.6	2450.0	-12.0	30.9
Mafutalax720/12	2.4	8.2	8.6	-47.4	-11.7	-54.6	-8.2	3200.0	-25.8	-13.6
CAB2x042/5	8.1	14.2	13.0	-5.3	-2.5	-13.2	23.4	2750.0	-8.9	16.2
CAB2x041/1	25.6	4.4	7.8	-15.3	-18.2	-14.1	12.7	1850.0	-6.9	26.7
CAB2x720/12	20.8	14.0	16.9	-18.1	4.3	-20.7	2.9	2100.0	-8.9	-26.9
Ndimirakakujax042/5	1.9	8.1	6.2	-2.8	0.0	-49.5	-0.4	3000.0	8.5	11.9
Ndimirakakujax041/1	7.4	4.4	4.1	-4.9	-3.8	-10.8	6.4	1225.0	-2.1	42.1
Ndimirakakujax720/12	5.9	11.4	14.1	-13.0	3.2	-8.2	1.5	4350.0	-28.9	-28.3
RW582x042/5	2.0	3.9	3.3	16.2	1.6	-13.3	-4.0	3100.0	-17.9	-18.8
RW582x041/1	1.1	2.9	3.4	43.2	10.2	-11.6	-18.0	925.0	-17.3	-16.4
RW582x720/12	-3.1	1.8	5.0	-9.1	5.5	-55.6	-22.7	5200.0	-30.0	-27.7
RW547x042/5	0.3	2.6	0.5	7.6	2.0	15.3	-4.2	2450.0	-9.9	23.8
RW547x041/1	4.3	1.7	2.0	-14.0	12.4	-17.0	4.4	1750.0	-0.2	-6.9
RW547x720/12	6.7	8.8	12.0	-12.1	-4.9	-21.1	5.8	3500.0	-28.5	15.3
RW1180x042/5	-3.2	1.3	1.8	16.9	-13.2	12.6	-3.4	3050.0	-5.5	9.7
RW1180x041/1	-4.0	2.9	2.2	-2.8	-9.7	-24.2	-7.3	1525.0	-11.2	-31.1
RW1180x720/12	-4.7	0.6	1.8	13.8	-17.8	-37.3	-18.2	3150.0	-25.2	7.6

DTF – days to 50% flowering ; DT75M and DTFM – days to 75% and fully maturity ; NPP – number of pods per plant ; NSP – number of seeds per pod ; 100-SW – 100-seed weight ; GY – grain yield ; pphenols – total polyphenols ; Zn – zinc ; Fe – iron.

6.3.5. General and specific combining ability (GCA and SCA) of common bean genotypes

No significant GCA effects were observed among parents for iron, zinc and polyphenols (Table 6.22). This suggested insignificant breeding values toward the inheritance of these specific characters. Among the female parents, line RW1180 had significant negative GCA effects with respect to the number of seeds per pod, grain yield but positive GCA effects in regard to the 100-seed weight suggesting that, under specific limitations of this study, this parent is not a good combiner for the first two traits but a good combiner for the last trait. Line RW547 had significant positive GCA effects at UNILUK with respect to the number of seeds per pod but negative effects in regard to the 100-seed weight at both sites. Line RW582 had significant positive GCA

in regard to 100-seed weight at both sites suggesting that this parent is a good combiner and can be used successfully in a breeding programme toward increasing the 100-seed weight. Lines Ndimirakakuja and CAB2 can be used in a breeding programme aimed at improving the grain yield at UCG. They had significant positive GCA effects. Rather than increasing the weight of 100 seeds, the use of line MAFUTALA in a breeding programme can reduce weight of seeds. This is due to the significant negative GCA effects observed in regard to this trait.

Few significant dominance effects were observed controlling the inheritance of some traits. Significant positive SCA effects were observed in hybrid MAFUTALAx042/5 for number of days to flowering at UNILUK suggesting a significant increase of days to flowering than would be expected on the basis of the average performance of the parents involved. However, hybrids CAB2x042/5 and RW582x720/12 had significant negative SCA effects respectively at UNILUK and UCG-Butembo. Hybrids CAB2x041/1 and RW547x042/5 had significant negative SCA effects respectively for number of days to 75% maturity at Butembo and number of days to fully maturity at UNILUK suggesting significant decreases of days to maturity than would be the case considering the additive gene action of the parents. Hybrid CAB2x720/12 had significant negative SCA effects at UCG-Butembo for number of pods per plant, while RW582x042/5 had significant positive SCA effects for 100-seed weight at UNILUK. Significant positive SCA effects were observed for iron for hybrid RW547x720/12. Significant SCA effects were positive for hybrids MAFUTALAx041/1 and RW582x720/12 and negative for hybrid RW582x041/1 for total polyphenol contents suggesting that hybrids RW547x720/12 and RW582x041/1 can respectively be advanced and used toward increased iron amounts and reduced polyphenolics levels in common bean seeds (Table 6.23).

Table 6. 22 : General combining ability (GCA) of testers and lines for the study characters

	DTF		DT75M		DT90M		NPP		NSP		GY		100-SW		Phenols	Zn	Fe
Testers	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk			
042/5	-0.278	-0.018	0.315	-0.898	-0.296	-1.574	2.208	1.416	0.183	-0.127	15.778	102	0.512	1.56	-0.0269	$4.4x10^{-12}$	-0.296
041/1	-0.055	0.815	-0.407	1.352	0.037	0.870	-1.164	1.282	-0.077	0.062	98.278	-7.667	0.088	-0.04	0.00139	$2.0x10^{-12}$	-3.549
720/12	0.333	-0.796	0.092	-0.453	0.259	0.704	-1.04	-2.698	-0.105	0.065	-114.05	-94.33	-0.601	-1.52	0.0255	$1.0x10^{-11}$	4.796
Lines																	
Mafutala	-0.833	3.814	-0.296	1.407	-0.518	1.259	-0.277	0.674	0.234	0.112	-112.07	-9.438	-5.5**	-6.91*	0.0739	-0.291	0.088
CAB2	4.389	6.592	2.592	9.241	3.370	10.48	3.759	7.679	0.152	0.412	715.7**	203.3	0.97	1.157	-0.0444	7.320	-0.038
Ndimirakakuja	1.389	-1.518	1.815	5.185	1.704	3.704	4.624	4.672	0.365	0.306	415.9*	83.74	0.88	-0.36	-0.0027	-2.386	-0.039
RW582	-1.611	-3.185	-0.852	-3.926	-1.629	-1.963	-1.276	-4.742	-0.455	-0.212	-258.62	-49.97	3.05*	4.66*	0.0089	-3.172	-0.074
RW547	0.722	-1.93	-0.629	0.685	-0.074	-0.185	0.104	3.329	0.250	0.568*	-169.9	38.99	-2.43*	-4.59*	-0.0144	-1.201	0.040
RW1180	-4.055	-3.740	-2.629	-12.593	-2.852	-13.29	-6.93	-11.61	-0.55*	-1.18**	-591.0*	-266.6*	3.02*	6.04*	-0.0211	0.329	0.044

DTF – days to 50% flowering ; DT75M – days to 75% maturity ; DTFM – days to fully maturity ; NPP – number of pods per plant ; NSP – number of seeds per pod ; 100-SW – 100-seed mass ; GY – grain yield ; pphenols – total polyphenols ; Zn – zinc ; Fe – iron ; * $p<0.05$ and ** $p<0.01$

Table 6. 23 : Specific combining ability (SCA) of hybrids for the study characters

Hybrids	DTF		DT75M		DTFM		NPP		NSP		GY		100-SW		Phenols	Zn	Fe
	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk	Butembo	Uniluk			
Mafutalax042/5	0.5	8.79*	0.35	0.34	0.185	3.907	-0.798	7.44	0.266	-0.0016	111.94	108.27	-1.196	-1.502	0.048	0.062	4.27
CAB2x042/5	0.61	-8.31*	1.46	1.84	0.629	1.352	3.842	-3.55	0.244	-0.346	-824.72	33.08	2.096	1.125	-0.029	-1.029	-1.78
Ndimirakakujax042/5	-1.05	-0.87	-0.09	-2.10	-0.704	-2.204	1.744	-3.47	0.037	-0.224	-485.39	-81.66	-0.28	-2.264	-0.014	1.050	-5.52
RW582x042/5	2.27	-0.20	-0.09	2.67	0.629	1.129	-1.022	-2.73	-0.13	0.37	277.94	38.19	-0.67	3.95*	-0.008	-0.040	-2.38
RW547x042/5	-1.05	-1.09	-0.98	-3.43	-0.592	-5.98*	-1.07	2.834	0.057	-0.524	171.27	83.6	0.816	-0.66	-0.047	-0.928	-1.99
RW1180x042/5	-0.27	1.68	-0.65	0.67	-0.148	1.796	-2.697	-0.521	-0.002	1.487	654.27	-11.73	-0.767	1.98	-0.009	1.009	6.11
Mafutalax041/1	0.27	-3.70	-1.59	-0.24	-1.148	-0.870	-0.166	-1.61	-0.05	-0.19	29.44	4.505	-0.383	0.64	0.100*	0.181	7.42
CAB2x041/1	-1.27	6.18	-2.81*	-0.40	-1.370	-0.092	1.447	-2.81	-0.33	0.535	-907.2	5.74	-1.23	0.275	0.027	-0.419	5.27
Ndimirakakujax041/1	1.39	-0.70	0.96	0.65	0.629	-0.648	-1.917	2.703	0.037	-0.413	-567.89	45.24	-0.116	1.203	-0.052	-0.851	9.61
RW582x041/1	0.39	-0.37	0.63	0.42	0.629	1.685	1.792	4.274	-0.01	0.181	195.44	39.6	0.043	-1.114	-0.090*	0.100	1.56
RW547x041/1	-0.61	-0.26	1.07	0.15	0.7407	0.907	-2.031	-1.148	0.345	-0.713	88.78	-45.4	-0.69	-0.96	0.014	1.556	-12.77
RW1180x041/1	-0.16	-1.15	1.74	-0.57	0.5185	-0.981	0.875	-1.404	-0.19	1.298	571.78	-62.2	2.376	-0.12	-0.014	-0.594	-14.34
Mafutalax720/12	0.22	-5.09	1.24	-0.10	0.963	-3.037	0.964	-5.83	-0.11	-0.193	241.78	-117.5	1.58	-0.55	-0.021	-0.104	-3.24
CAB2x720/12	0.66	2.13	1.35	-1.43	0.741	-1.259	-5.29*	6.364	0.157	-0.538	-694.89	62.6	-0.867	-1.16	-0.088	2.548	-7.76
Ndimirakakujax720/12	-0.33	1.57	-0.87	1.45	0.074	2.852	0.173	0.772	0.094	-0.415	-355.55	78.19	0.396	0.98	0.050	-0.241	-8.64
RW582x720/12	-2.67*	0.57	-0.53	-3.10	-1.259	-2.815	-0.770	-1.54	-0.07	0.178	407.78	-102.7	0.626	-1.88	0.106*	-0.238	-7.04
RW547x720/12	1.67	1.35	-0.09	3.28	-0.148	5.074	3.099	-1.68	-0.28	-0.716	301.11	-18.7	-0.12	0.677	-0.001	-0.743	18.47*
RW1180x720/12	0.44	-0.53	-1.09	-0.10	-0.370	-0.815	1.822	1.925	-0.06	1.295	784.11	-59.05	-1.61	-0.62	-0.024	-0.190	12.82

DTF – days to 50% flowering ; DT75M – days to 75% maturity ; DTFM – days to fully maturity ; NPP – number of pods per plant ; NSP – number of seeds per pod ; 100-SW – 100-seed mass ; GY – grain yield ; pphenols – total polyphenols ; Zn – zinc ; Fe – iron ; * $p<0.05$

6.3. Discussion

Iron contents varied from 65 to 140 ppm among the 27 genotypes studied. Zinc contents varied from 41.9 to 74.7 ppm, while total polyphenol contents varied from 0.85 to 45.23 mg of gallic acid equivalent (GAE) per g of dry common beans. Variations in iron and zinc contents were significant among the testers. Iron contents varied from 65.4 ppm for tester 041/1 to 121.9 ppm for 720/12. Zinc contents varied from 42.6 ppm for tester 042/5 to 74.7 ppm for 720/12, while total polyphenols varied from 0.85 to 1.71 mg GAE g^{-1} of dry common beans. Giuberti *et al*. (2019), in their study on nutrients' and antinutrients' seed contents in common bean lines carrying mutations affecting seed composition, found that iron contents varied from 83.77 to 91.37 ppm, zinc contents from 34.36 to 37.94 ppm and total polyphenols from 8.037 to 12.800 mg GAE g^{-1} of dry common beans among these testers. For Carvalho *et al*. (2012), zinc contents varied from 32.6 to 70.2 ppm. The difference in polyphenols can be attributed to the difference in the methods of determination used. While Giuberti *et al*. (2019) treated 50 mg of grinded seeds per sample with 2 ml of 70% MeOH solution according to the method reported by Taga *et al*. (1984), in this study, 1 g of grinded common bean seeds was diluted with deionized water (ten-fold) and polyphenol determination was based on colorimetry method as described by Singleton *et al*. (1999).

Among females, RW582 and RW1180 had the highest iron amounts, while CAB2 had the least polyphenol content. These observations might be explained by the color of the seed coat. Wu *et al*. (2004), reported that beans with red and purple seed coats contain more polyphenols, while white seeded beans contain low amounts of polyphenols. In their study on lipophilic and hydrophilic antioxidant capacities of common foods in the United States, they found that small red beans contained more polyphenols compared to the white beans. Navy beans had 2.23 mg GAE g^{-1} of dry beans. However, black,

pinto, small red and red kidney recorded 8.80, 10.23, 11.85 and 12.47 mg GAE g^{-1} of dry beans respectively. On overall, the small red beans had the highest antioxidant activity out of 100 common foods in comparison including fruits, nuts and vegetables.

Testers had small amounts of polyphenols compared to the lines and hybrids. This finding is consistent to the breeding goal of testers used in this study. These materials were bred at Research Centre for Genomics and Bioinformatics (CREA), Italy, to improve mineral bioavailability by reducing antinutritional factors and particularly phaseolin and lectins (Giuberti *et al*., 2019). Antinutritional factors are linked together by a positive type of correlation such that a reduction in phaseolin and lectins induces a reduction in other factors such as polyphenolics, flavonoids and phytic acid (Campion *et al*., 2013). However, some agronomic plant performance is lost. This is because polyphenols, for instance, are involved in antioxidant activity of a plant and prevent tumor (Yang & Gan, 2018). In addition, according to Yang & Gan (2018) ; Wu *et al*. (2004), polyphenols are involved in increase of resistance against biotic diseases and bacteria particularly. This activity decreases when common beans have lower polyphenol contents. Polyphenols are also associated with anti-inflammatory and chemopreventive effects and involved in anti-diabetic, anti-inflammatory, anti-tumor, anti-mutagenic and anti-atherosclerosis even though they reduce mineral bioavailability in common beans.

Among hybrids, CAB2 x 720/12 had the highest zinc amount, while RW547 x 720/12 and RW1180 x 720/12 had the highest iron amounts. This could be due to the additive genetic effect of male parent 720/12 in progenies. These genotypes can be advanced through selection in early generations.

Genotypes with high iron levels tended to be the ones with high zinc levels. This might be attributed to the positive correlation between these minerals. Iron and zinc were positively correlated ($r=0.40$, $p<0.01$). This indicated that selection for high iron accumulation results in high zinc content in the genotypes studied. Several researchers have reported similar findings. Mbikayi *et al*. (2018), in their study on identification of biofortified beans in Eastern DRC, reported a highly significant positive correlation ($r=0.94$; $P<0.001$) between iron and zinc concentrations in common beans and suggested that genetic factors involved in their expression co-segregate together. In another independent study research on trace minerals in the common bean, Beebe *et al*. (2000) observed a significant positive correlation ($r=0.663$; $p<0.01$) among these two nutrients. They suggested the presence of alleles at specific loci with major effects; hence their allelic variations can be identified. Working on 47 F_2 segregating populations of biofortified common bean varieties for Africa, Kimani & Warsame (2019) found significant positive correlation ($r=0.439$; $P<0.001$) between these nutrients. Tryphone & Nchimbi-msolla (2010) observed a significant positive correlation ($r=0.416$; $P<0.001$) between these nutrients using 90 collected common bean genotypes at Sokoine University in Tanzania. Zacharias *et al*. (2012), reported, in their study a significant positive correlation between these minerals. Blair *et al*. (2009) added that these minerals are represented by a similar total number of quantitative trait locus (QTL) colocalized together and reported that inheritance of these nutrients is controlled by additive genes. Welch & Graham (2004) reported a reduction of iron and zinc bioavailability in common beans with the high level of polyphenols. However, the type of correlation between the polyphenol levels and each of these minerals was not significant. The non-significant types of correlation might be attributed to the lack of replications of observations. Due to high financial charges and low resources, no

replication was considered in this study. But for Giuberti *et al*. (2019), dense iron and zinc common beans are associated with low levels of antinutritional factors such as polyphenols and phytates.

Broad-sense heritability was important (H^2>0.70) for iron and polyphenol levels except for zinc. For this trait, broad-sense heritability was moderate (66.1%). This would indicate the importance of additive genetic effects over the environmental variations, particularly in inheritance of the two first traits and thereby suggest the importance of recurrent selection as the best approach to develop high performing common bean genotypes.

Unlike Mukai (2017) who observed in her study on inheritance and characterization of cooking time, seed iron and zinc in common bean, high levels of both narrow and broad-sense heritability (0.71 and 0.99) for zinc, in this study, the magnitude of broad- and narrow-sense heritability was 66.1 and 0% for zinc concentration. This suggested the importance of dominance gene effects in inheritance of this trait. Possobom *et al*. (2018) found broad-sense magnitudes varying from 67.21 to 90.03%, while the magnitude of narrow-sense heritability was 29.15% particularly among the lines of middle American gene pool. Dominance effects are important in controlling the inheritance of this character. They also reported that inheritance pattern of zinc contents in these seeds is quantitative.

The magnitude of narrow- and broad-sense heritability was 0.1 and 70.88% for iron concentration. Parents-offspring regression analysis showed a moderate heritability explained up to 9.42% by linear model. This indicated the importance of both dominance and additive genetic effects in inheritance of this character and that heterosis breeding as well as recurrent methods would be the best approaches for varietal

development. However, Mukai (2017), in her study, found both narrow and broad-sense heritability high (0.89 and 0.99). In their study on genetics of iron and zinc concentrations in six generations of two populations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2), Lamptey *et al*. (2023) reported that both additive and dominance gene effects are important in determining the expression of high iron and zinc levels. They found broad-sense heritability ranging from moderate to high (62 to 82% for iron and 60 to 74% for zinc) and narrow-sense heritability ranging from moderate to high for iron (53 to 75%) and from low to moderate for zinc (21 to 46%). In their study on genetics of iron and zinc in Andean common bean seeds, Zemolin *et al*. (2016) found low magnitude of narrow-sense heritability (19.04 %) for iron and moderate (63.60 %) for zinc. Both additive and dominance genetic effects are important in inheritance of iron and zinc.

The magnitude of narrow- and broad-sense heritability was 0.27 and 80.9% for polyphenolic levels in common bean genotypes. Parents-offspring regression estimate of heritability also showed the same observation (low narrow-sense heritability) indicating the importance of dominance effects and suggesting heterosis breeding toward development of high performing common bean varieties.

Heterosis as well as recurrent selection would be the appropriate breeding methods toward increasing iron and zinc concentrations and decreasing polyphenolic levels in common bean.

High-parent heterosis varied among hybrids. The best performing hybrids were those with negative or reduced heterosis percentages regarding the level of polyphenols, and positive and increased heterosis for iron and zinc concentrations in the seeds (Acquaah, 2012). Hybrid Ndimirakakuja x 042/5 would be the high zinc performing genotype to advance, while hybrids Ndimirakakujax041/1, Mafutalax041/1 and Mafutalax042/5

would be the high iron performing genotypes. Even though no hybrid with negative heterosis percentage for total polyphenols was found, hybrid RW582x041/1, with the least heterosis coefficient would be the best promising genotype toward reduced polyphenolic levels.

Findings indicated no significant GCA effects among parents for iron, zinc and polyphenols. However, Mukai (2017) found significant GCA (positive and negative) for most of parental lines used in her study except one parent (CAL96). Differences in our findings might be attributed to the genotypic variability but also the mating design used. In the present study, a line x tester mating design was used, while in her study all the combinations (except reciprocals) were allowed.

Among hybrids, RW582 x 041/1, with negative significant SCA effects for polyphenols, should be advanced in a breeding programme aimed at decreasing total polyphenolic levels in Butembo and Lukanga. On the other hand, hybrid RW547x720/12 should be advanced if the aim of a breeding programme is to increase iron levels in common bean seeds.

6.4. Conclusion

Testers were genotypes with the least polyphenolic concentrations. Tester 720/12 combined high iron and zinc levels. The lines performed better for iron and zinc levels than the testers, except the tester 720/12. Four genotypes (RW582 x 041/1, CAB2 x 720/12, CAB2 x 042/5 and RW547 x 042/5) had reduced polyphenol levels among hybrids. Hybrid CAB2 x 720/12, in addition, had also high zinc level. Hybrids RW547 x 720/12 and RW1180 x 720/12 had the highest iron amounts.

Additive and dominance gene action were important for iron, zinc and polyphenol accumulation suggesting that both recurrent and heterosis breeding methods are viable

approaches to develop dense iron and zinc common bean genotypes with the least polyphenolic amounts.

CHAPTER 7

7. GENETIC DIVERSITY AND ASSOCIATION MAPPING OF GRAIN IRON AND ZINC CONCENTRATION IN COMMON BEAN

7.1.Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important sources of macronutrients such as proteins, carbohydrates and micronutrients such as iron and zinc for millions of people living in areas of the world where prevalence of undernourishment is the highest. These areas include Sub-Sahara Africa and the Caribbean where 31.4 and 16.5% of undernourishment prevalence have been reported (FAO, 2017 ; Petry *et al*., 2013).

Micronutrient deficiencies affect more than two billion people globally. Deficiencies can be caused by low intakes of minerals and vitamins or factors such as poor diet, low mineral bioavailability (Caproni *et al*., 2020). Iron deficiency is the most important. De Benoist *et al*. (2008) reported that 42% of pregnant women, 30% of preschool children and 12.7% of men older than 15 years are anemic. Zinc is another important mineral whose deficiency causes impairment of growth regulation and easy development of infections. For Welch & Graham (2004), its deficiency is as common as iron deficiency.

Biofortification of common bean represents an important strategy to reduce iron and zinc deficiencies, most particularly in DRC where this crop plays a key role as a diet. Other strategies include food diversification, supplementation and fortification (De Benoist *et al*., 2008 ; Caproni *et al*., 2020).

Iron and zinc biofortification in common bean is a long-lasting strategy and can be achieved through genetic approach based on conventional methods of selection or based on modern biotechnology (Caproni *et al*., 2020). Selection relying on phenotypic

observation is time-consuming and is influenced by environmental factors but an effective genetic selection can be used on a large scale without requiring further investment.

Genetic diversity in iron and zinc concentrations in common bean has been studied by various researchers (Delfini *et al*., 2021 ; Kimani & Warsame, 2019 ; Amongi *et al*., 2018 ; Mulambu *et al*., 2017 ; Tryphone & Nchimbi-msolla, 2010 ; Welch & Graham, 2004). Many studies have revealed significant environmental influences for these nutrients (Gelaw *et al*. 2023; Bulyaba *et al*., 2020 ; Blair *et al*., 2011).

To circumvent environmental influences, stable molecular markers closely associated with iron and zinc should be identified through a Genome-wide association analysis (GWAS). The latter is a method used to map QTL associated with many traits of interest. The model used can also have an influence on the association made. Using bi-parental population, Blair *et al*. (2009, 2010) identified few quantitative trait loci (QTL). Furthermore, using an Andean recombinant inbred line population of 100 common bean lines derived from a cross between a Colombian cream-mottled climbing bean with high iron and zinc levels and an Argentinean cream seeded climbing bean with low iron and zinc levels in three different environments, Blair *et al*. (2011) identified nine QTL on five linkage groups. Through the means of GWAS, Caproni *et al*. (2020), using 192 homozygous common bean genotypes, identified some molecular SNP markers co-located on Pv01 chromosome. Gelaw *et al*. (2023) identified 43 QTL associated with iron and zinc levels using 11,480 SNP markers and 289 common bean genotypes tested at Haramaya and Melkassa in Ethiopia. These markers were located on chromosomes 1, 6, 10 and 11.

The method used for GWAS is another critical factor for QTL analysis. For instance, Bradbury *et al*. (2007) stated that general linear model (GLM) does not address inflated false-positive leading to spurious association. However, a mixed linear model (MLM) as well as Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) hold great promise toward control of inflated false-positive rates, suboptimal prediction accuracies as well as extensive computational requirements (Lipka *et al*., 2012) ; (Huang *et al*., 2018). According to the later authors, BLINK identifies more associated SNPs than GLM. Furthermore, Wang & Zhang (2021) added that BLINK model eliminates the restriction assuming that causal genes are distributed across the genome boosting the statistical power.

Using GLM on 96 common bean genotypes collected in India, Mahajan *et al.* (2017) identified 13 simple sequence repeat markers associated with iron, zinc and protein contents on all the linkage groups except chromosomes 4, 8 and 11. Using multi-locus mixed model (MLMM), Klaudija *et al*. (2019) cited by Gelaw *et al*. (2023) identified one locus on chromosome 3 and another on chromosome 6 for iron and zinc. Gunjača *et al*. (2021) reported no quantitative trait nucleotide for seed iron using a panel of 174 genotypes, representing Croatian common bean landraces, and 6,311 high-quality DArTseq-derived SNP markers.

Furthermore, in their study on meta-QTL analysis of seed iron and zinc in common bean, Izquierdo *et al*. (2018) identified 12 putative genes related to mineral transport or storage. These genes involve products that have previously been reported in iron and zinc acquisition such as ZIP, FRO and NA (Ishimaru *et al*., 2005 ; Vert *et al*., 2002), translocation within plant such as ZIP, FRO, NA and MATE (Grotz & Guerinot, 2006

; Ishimaru *et al*., 2005 ; Rogers *et al*., 2009 ; Vert *et al*., 2002) and storage in seeds such as NRAMP (Thomine *et al*., 2003).

The objective of this study was to identify QTL associated with seed iron and zinc concentration using high density DArTSeq SNP markers. In addition, this study tries to propose putative genes controlling iron and zinc in common bean.

7.2.Materials and methods

7.2.1. Experimental materials

This study involved a panel of 183 iron- and zinc-biofortified common bean genotypes, comprising selections from second-generation biofortified lines (Chapter 4), CIAT breeding lines, and five local varieties. Field trials were conducted in Butembo and Lukanga, eastern Democratic Republic of the Congo, from March to July 2023. Description of sites has been discussed in the section 4.2.1.

7.2.2. Experimental design and crop management

Genotypes were tested in an augmented design arranged in three blocks where only local varieties were replicated in blocks. Each block comprised 61 biofortified genotypes. A plot was represented by a single row containing 10 hills 30 cm apart each from another. A row was 60 cm apart from another and the distance between blocks was 1 m. Characteristics of soils of the study sites were presented in Table 5.1. All the management practices as presented in the section 4.2.3 were conducted.

7.2.3. Determination of iron (Fe) and zinc (Zn)

Iron and zinc contents were determined by Inductively Coupled Plasma (ICP) based-technique as discussed in section 4.2.5 at the Department of Land Resource

Management and Agricultural Technology (LARMAT), Faculty of Agriculture, University of Nairobi between November and December 2023.

7.2.4. DNA extraction and genotyping

Common bean seeds samples were packaged and sent to SEQART AFRICA located at International Livestock Research Institute in Nairobi for Genotyping. The procedure for seed packaging was that proposed by SEQART. DNA extraction was done from leave tissue of two weeks old seedlings. The leaf samples were stored at -80°C and extraction and isolation of DNA were done as by a modified CTAB-based method using Nucleomag Plant DNA extraction kit. The genomic DNA extracted was in the range of 50-100µg/µl. The genomic DNA quality and quantity were checked on 0.8% agarose. Libraries were constructed according to Kilian *et al*. (2012) DArTSeq complexity reduction method through digestion of genomic DNA using a combination of PstI _MseI enzymes and ligation of barcoded adapters and common adapter followed by PCR amplification of adapter-ligated fragments. Libraries were sequenced using Single Read sequencing runs for 138 cycles. Next generation sequencing was carried out using NovaseqX.

SEQART AFRICA uses genotyping by Sequencing (GBS) DArTseq™ technology, which provides rapid, high quality and affordable genome profiling, even from the most complex polyploid genomes. DArTseq markers scoring was achieved using DArTsoft14 which is an in-house marker scoring pipeline based on algorithms. Two types of DArTseq markers were scored, SilicoDArT markers and SNP markers which were both scored as binary for presence/absence (1 and 0, respectively) of the restriction fragment with the marker sequence in genomic representation of the sample. Both

SilicoDArT markers and SNP markers were aligned to the reference genomes of Common_bean_v9, to identify chromosome positions.

The markers derived from DArTSeq were filtered out to remove lower quality SNPs. SNPs with ≥80% missing data and MAF <5% and genotypes with ≥30% missing data were pruned from the analysis.

7.2.5. Data analysis

7.2.5.1.Phenotypic variation

Descriptive statistics together with one-sample T test was computed for both locations and combined across locations using statistix, 8th version. Frequency distribution of germplasm was performed using Statistical Package for Social Sciences, 15th edition.

7.2.5.2.Population structure and kinship analysis

Population structure was analyzed using LEA package in R statistical software and the estimation of the optimum number of subpopulations was realized based on sparse Non-Negative Matrix Factorization (sNMF) to provide STRUCTURE-like output. The analysis was conducted using 10 ancestral populations (K=10), with 10 repetitions for each cross-entropy criterion and K value. The ideal number of ancestral populations was determined by plotting the least cross-entropy as a function of K using the plot function of LEA. The admixture proportions on the genotypes were displayed by a barplot plot of the ancestry coefficients (Q matrix) created for the second run of K. Kinship matrix was developed in R statistical software by multiplying SNP matrix with its transpose and dividing the product by the number of markers.

7.2.5.3.Association analysis

This analysis was performed using Bayesian-information Linkage-disequilibrium Iteratively Nested Keyway (BLINK) implemented in GAPIT software (Wang & Zhang, 2021) to detect marker-trait association found between the SNP markers and each of the iron and zinc concentrations. Population structure, in this model, was considered as a fixed effect and the kinship among individuals as the variance-covariance structure of the random effect. Associations between a marker and iron and/or zinc content were presented in Manhattan plots by adjusting the raw *p*-value using the Bonferroni error rate control method. The quantile-quantile (Q-Q) plots of the observed and expected *p*-values were helpful to test the adequacy of a fitted normal straight line to the markers (Delfini *et al*., 2021).

The pairwise linkage disequilibrium (LD), necessary to determine the resolution of association mapping, was generated based on the adjusted pairwise r^2 values across the genome using TASSEL software where r^2 indicated the correlation of markers with the QTL. The LD decay was plotted using the basic functions in R statistical software.

7.2.5.4.Search for candidate genes

Candidate genes were screened using the *Phaseolus vulgaris v2.1* reference genome, which was retrieved via the *Phytozome*, version 13 database, using the *biomaRt* package in R. Putative genes were screened on this reference genome within a window of 600 kb upstream and downstream of significant SNPs. This was based on the fact that the LD decayed at about 600 kb (576,879 kb). Annotation with the Gene Ontology was done to select genes with known putative functions that furthermore were referred to as candidate genes.

7.3.Results

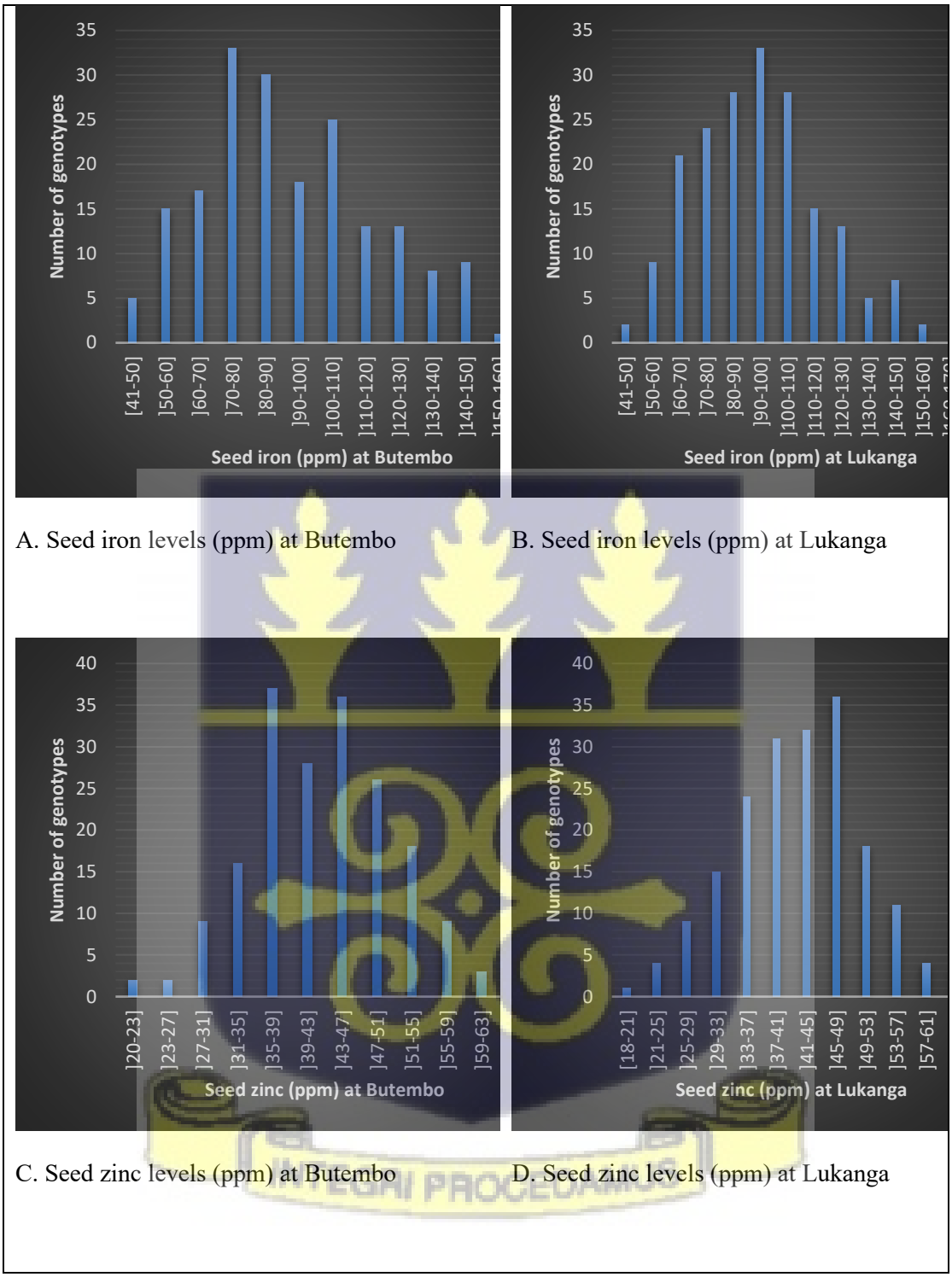
7.3.1. Phenotypic variation

Significant variations ($p<0.001$) were observed among study germplasm for iron and zinc in both sites and across sites (Table 7.1). Iron levels were slightly higher at Lukanga than at Butembo, while zinc levels were slightly higher at Butembo than at Lukanga. Iron levels varied from 41.0 to 169.7 ppm at Butembo and from 43.3 to 174.3 ppm at Lukanga. Zinc levels varied from 20.1 to 63.9 ppm at Butembo and from 18.0 to 62.9 ppm at Lukanga. In combined locations, iron amounts ranged from 43.3 to 172.2 ppm and zinc amounts from 19.1 to 63.4 ppm. Variability was large for iron (25.4%) than for zinc (18.2%). The negative skewness coefficient observed for zinc at Lukanga showed that at this location data as well as the mean, median and mode for this trait tended to be concentrated at the left side of the distribution curve (Figure 14), the mode being greater than median and the later greater than the mean. The peak of distribution curve was high for iron only at Lukanga.

Table 7. 1 : Descriptive statistics of common bean germplasm evaluated for iron and zinc at Butembo and Lukanga

Trait	Location	Min	Mean	Max	Mode	Median	Skewness	Kurtosis	CV(%)	p-val
Iron	Butembo	41.0	92.1	169.7	74.9	87.6	0.4	-0.4	28.2	0.0
	Lukanga	43.3	93.9	174.3	105.6	91.9	0.5	0.1	25.5	0.0
	Across sites	43.3	93.0	172.0	109.1	91.1	0.4	-0.0	25.4	0.0
Zinc	Butembo	20.1	42.8	63.9	43.9	43.1	0.03	-0.23	19.2	0.0
	Lukanga	18.0	41.7	62.9	45.6	42.1	-0.04	-0.21	20.8	0.0
	Across sites	19.1	42.3	63.4	47.0	42.6	0.05	-0.0	18.2	0.0

The frequency distribution of iron and zinc at Butembo and Lukanga was skewed in Figure 7.1.



A. Seed iron levels (ppm) at Butembo

B. Seed iron levels (ppm) at Lukanga

C. Seed zinc levels (ppm) at Butembo

D. Seed zinc levels (ppm) at Lukanga

Figure 7. 1 : Frequency distribution of 188 common bean genotypes for seed iron (A & B) and zinc (C & D) at Butembo and Lukanga

7.3.2. Population structure

The optimum subpopulation constructed through population structure of 188 study genotypes was observed at k=4 (Figure 7.2). Four hypothetical ancestral populations were figure out. More than 40% of genotypes were grouped in one subpopulation. The remaining genotypes were considered as admixture.

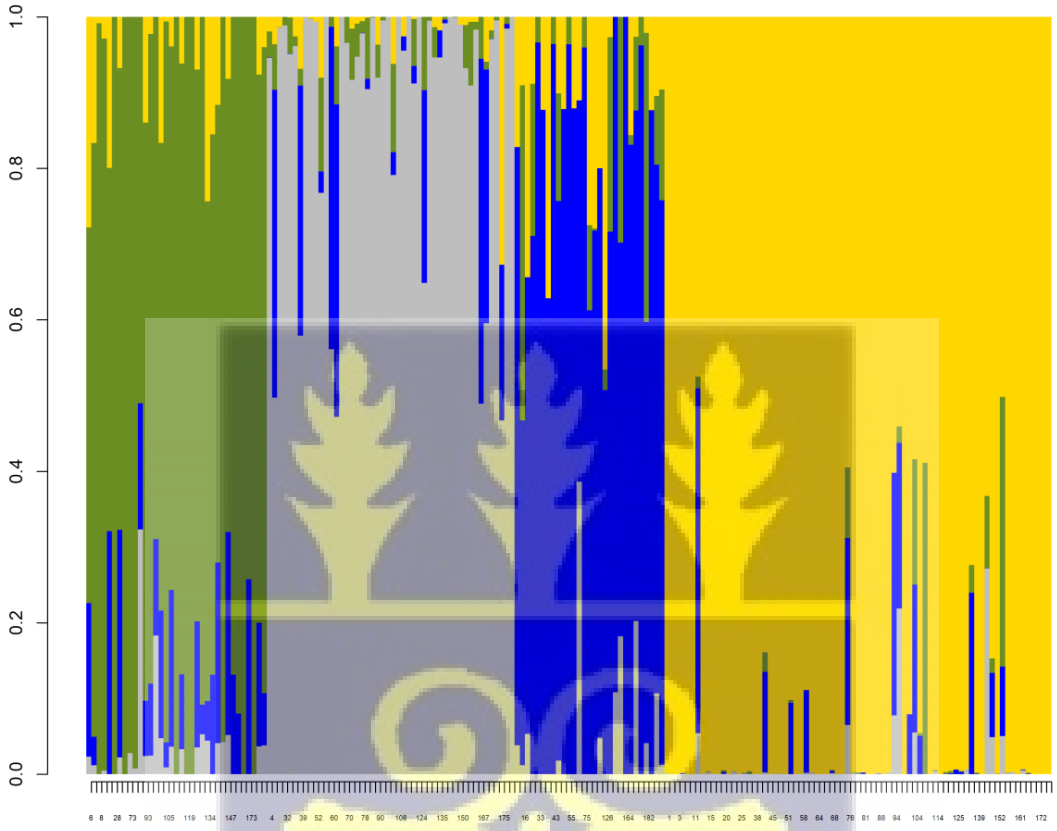


Figure 7. 2 : Population structure for 188 common bean genotypes where each color represents a subpopulation

7.3.3. Linkage disequilibrium

Linkage disequilibrium (LD) indicated the extent of association. In the present study, R^2 values varied from 0 to 1 for 11,880 SNP markers used.

The critical threshold level (R^2) was set at 0.1, while the observed average LD decay was 576.879 kbp indicating that 576.879 kbp would be used as confidence interval of a QTL from a significant SNP found (Figure 7.3).

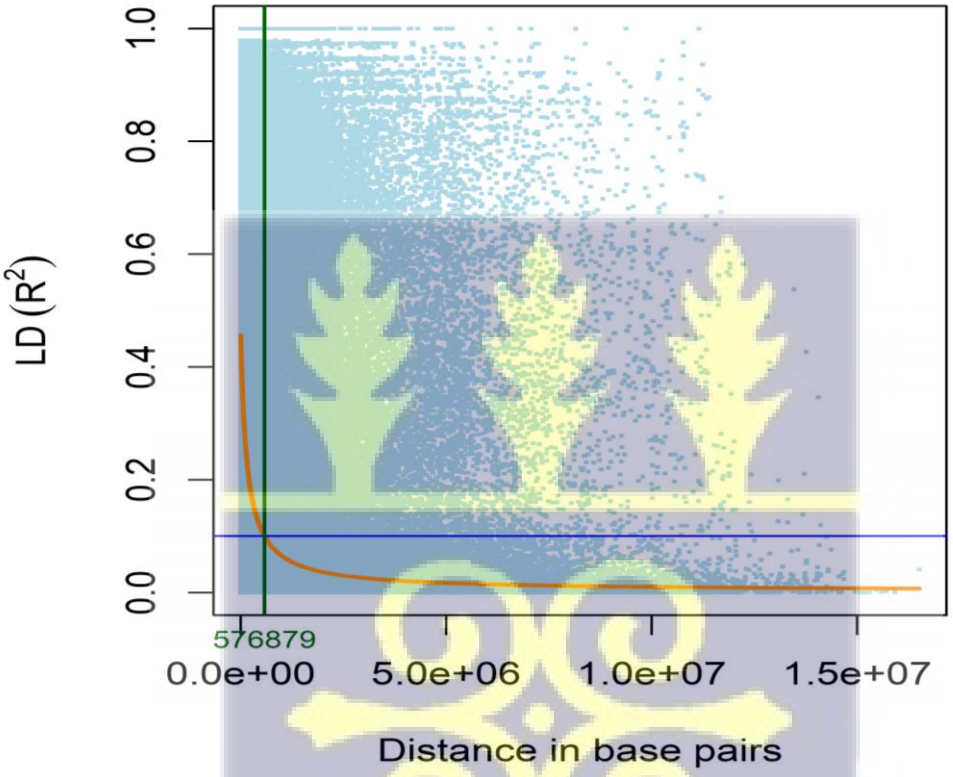


Figure 7. 3 : Linkage disequilibrium (LD) decay and R^2

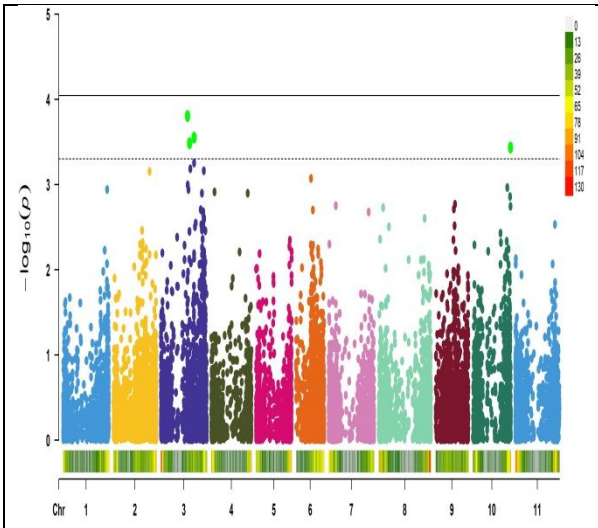
7.3.4. Association analysis

Association between phenotypic and genotypic data is presented in Manhattan plots where the X-axis is the genomic position of the SNPs in the genome and the Y-axis the negative log base 10 of the *p*-values (Figure 7.4).

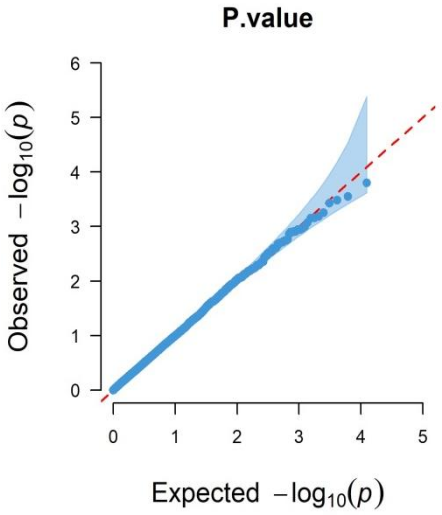
The QQ plot indicated that observed marker trait association (MTA) *p*-values were close to the expected distribution where the four subpopulations generated from population structure were used as covariate.

Markers were distributed across the 11 common bean chromosomes. A MTA was declared significant when at least its $-log_{10}(p)$ was superior or equal to 4.1. A total of four QTL associated with seed iron and zinc contents were identified at Lukanga and across Butembo and Lukanga. Two QTL strongly associated respectively with seed iron and zinc at Lukanga resided on chromosomes 1 and 6 respectively. Across locations, the two QTLs strongly associated with seed iron and zinc were identified on chromosomes 3 and 9. However, none of the QTL was significant at any linkage group at Butembo.

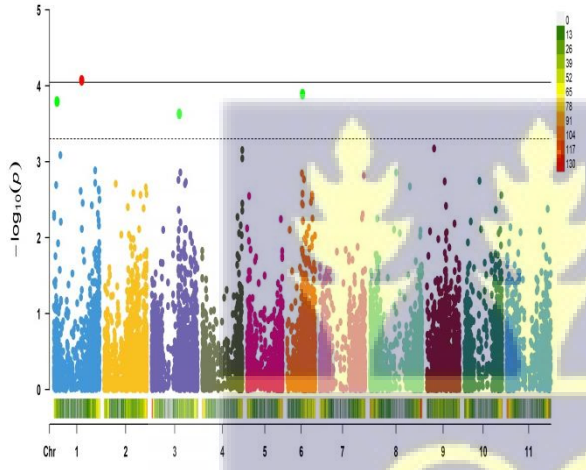
To declare distinct QTL within each chromosome, the distance at which LD decayed to the critical threshold value was used as the given confidence interval. Therefore, QTL found between the range from -576,879 to +576,876 bp were declared as the same QTL and inherited together.



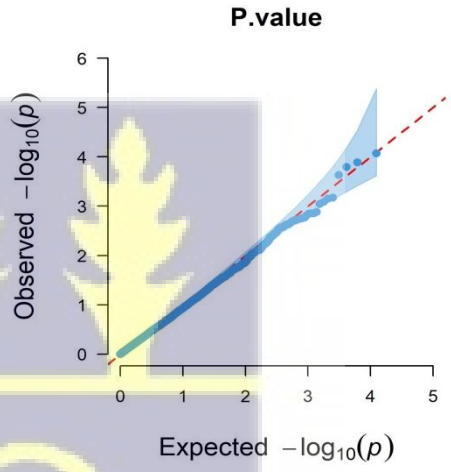
Manhattan plot for Fe at Butembo



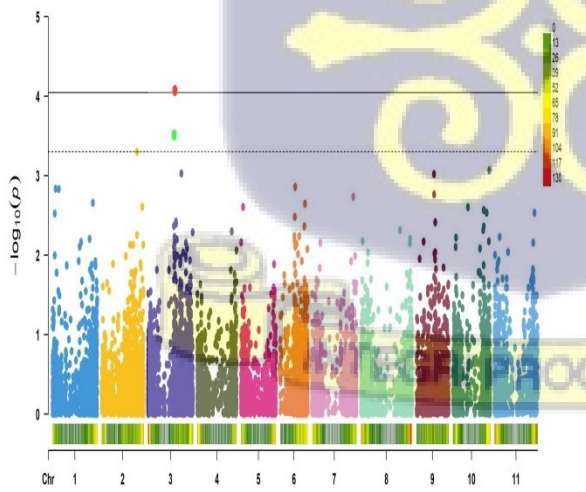
QQ plot of p-values at Butembo



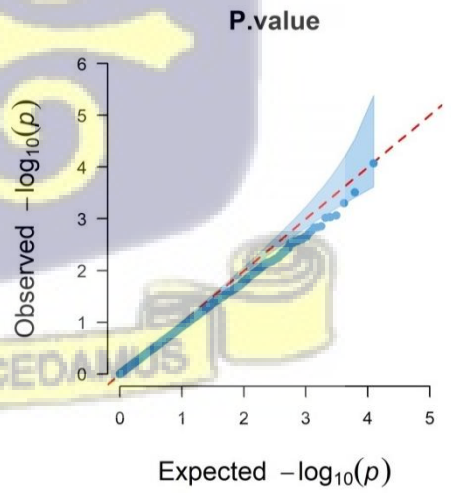
Manhattan plot for Fe at Lukanga



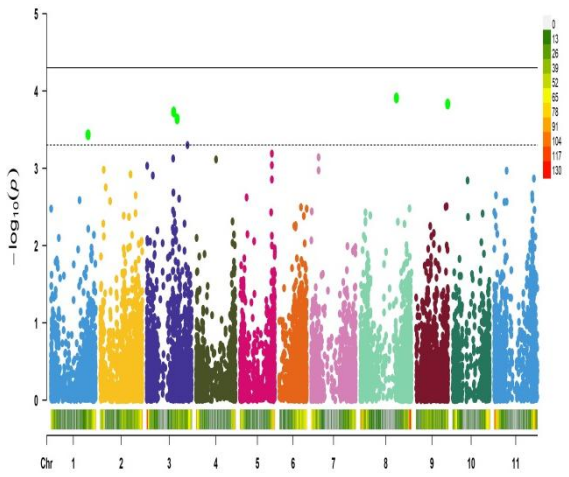
QQ plot of p-values at Lukanga



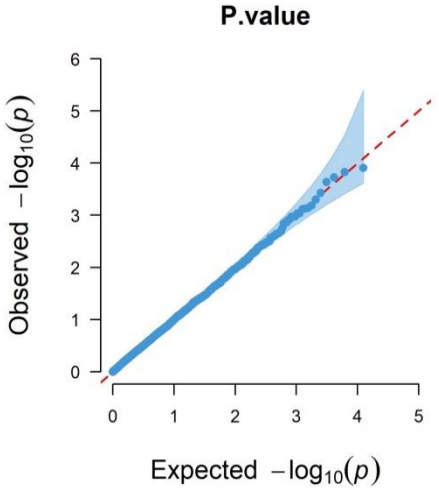
Manhattan plot for Fe across sites



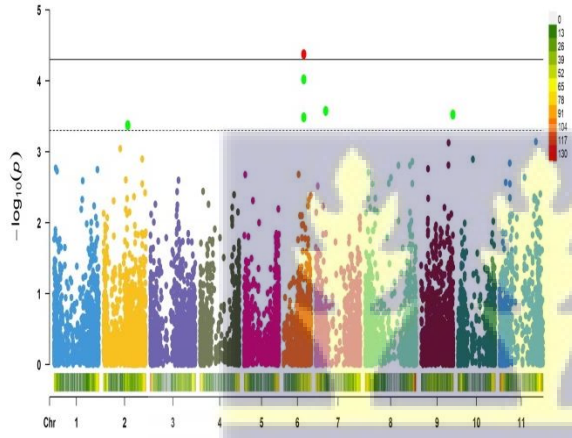
QQ plot of p-values across sites



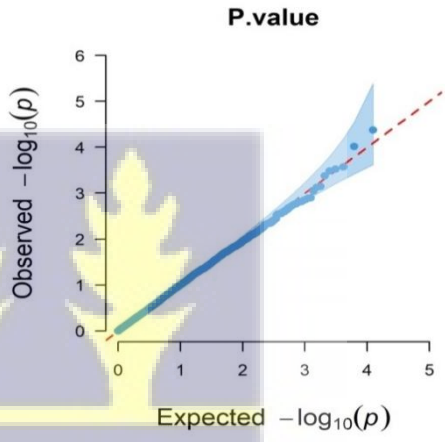
Manhattan plot for Zn at Butembo



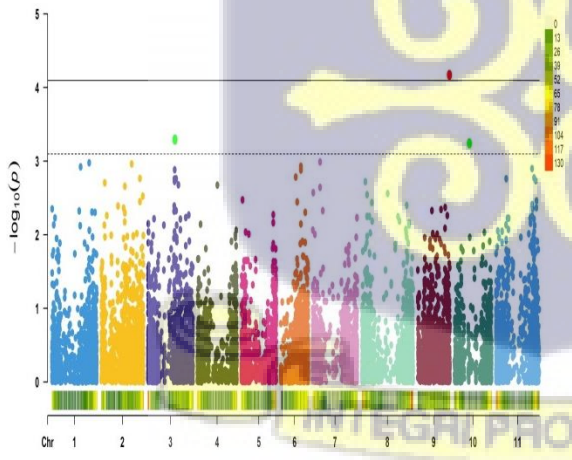
QQ plot of p-values at Butembo



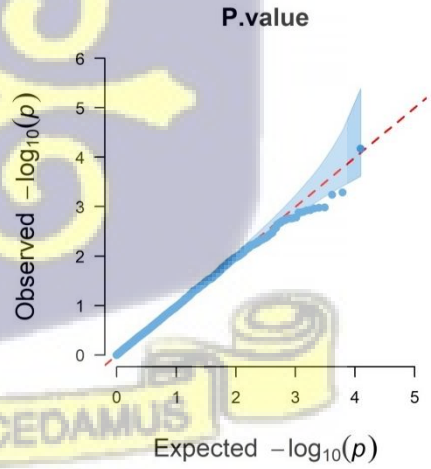
Manhattan plot for Zn at Lukanga



QQ plot of p-values at Lukanga



Manhattan plot for Zn across sites



QQ plot of p-values across sites

Figure 7. 4 : Manhattan plots for the genome-wide association for iron and zinc in common bean (left) and quantile-quantile (QQ) plots of p-values (right)

7.3.5. In silico analysis of candidate genes controlling iron and zinc in common bean

Putative genes controlling iron (Fe) and zinc (Zn) were searched on significant QTLs of linkage groups 1, 3, 6 and 9. Annotation on the *Phaseolus vulgaris v2.1* reference genome indicated more than 100 genes with various gene ontologies in different chromosomes and which would be inherited together. Based on information on known putative functions of genes involved in Fe/Zn uptake, translocation and storage in plants, 13 candidate genes with different IDs located on the above four linkage groups were proposed controlling Fe and Zn in common bean (Table 7.2). These genes belong to four families.

Table 7. 2 : Putative genes controlling iron and zinc in common bean

Chromosome position	SNP position (bp)	Gene ID	Function
1	31,748,006	Phvul.001G112300	Cation H^+ antiporter 21-related (Bernardazzi, 2022)
3	31,311,230	Phvul.003G125300	Cation H^+ antiporter 19 (Bernardazzi, 2022)
		Phvul.003G120500	ABC transporter G (Morita *et al*., 1998 ; Izquierdo *et al*., 2018)
		Phvul.003G120400	ATP-Binding Cassette transporter
		Phvul.003G122400	ABC transporter B (Morita *et al*., 1998 ; Izquierdo *et al*., 2018)
		Phvul.003G122700	ABC transporter B (Morita *et al*., 1998 ; Izquierdo *et al*., 2018)

		Phvul.003G122600	ABC transporter B (Morita *et al*., 1998 ; Izquierdo *et al*., 2018)
		Phvul.003G122500	ABC transporter B (Morita *et al*., 1998 ; Izquierdo *et al*., 2018)
6	23,480,643	Phvul.006G126600	Homeobox-Leucine ZIPPER protein HDG11 (Grotz & Guerinot, 2006)
		Phvul.006G126500	Homeobox-Leucine ZIPPER protein HDG11 (Grotz & Guerinot, 2006)
		Phvul.006G128600	Homeobox-Leucine ZIPPER protein ATHB-14 (Grotz & Guerinot, 2006)
9	36,952,281	Phvul.009G248400	ABC transporter (Morita *et al*., 1998) ; (Izquierdo *et al*., 2018)
		Phvul.009G250100	MATE efflux family protein (Izquierdo *et al*., 2018)

7.4.Discussion

Population structure and the kinship were used as a fixed effect and the variance-covariance structure of the random effect for marker trait association. The number of subpopulations was low compared to what Gelaw *et al*. (2023) found in their study. In fact, they observed six subpopulations, while in this study, four subpopulations were observed. The difference might be attributed to the size of the study population which was high compared to the size used in this study. They used an inter-gene pool population of 289 genotypes. Another reason might be attributed to the autogamous attribute of common bean. This attribute narrows down the existing diversity.

Both the LD decay and r^2 were important in estimation of LD. LD decay was used as a function of physical distance in base pairs (bp) or kilo base pairs (kbp). As proposed by Gelaw *et al*. (2023), the important number of subpopulations and admixture in this study, could be the reason for high LD. In the present study, the LD decay was 576,879 bp at r^2 of 0.1, while in their study, the LD decay was set at 232.58 kbp.

Correlation for Fe and Zn is important to explain the consistency in QTL detection and suggested that seed Fe/Zn level is a stably heritable character. For Blair *et al*. (2010), the overlap in the QTL for these minerals could be explained by the highly significant correlations between these minerals. Previously, Beebe *et al*. (2000) reported that the positive correlation between these minerals might indicate a physiological relationship between uptake, translocation or accumulation of both minerals.

Fewer QTL for Fe and Zn were found in this study. Unlike Gelaw *et al*. (2023) who identified a panel of 53 QTL, in the present study, four significant QTL were found at Lukanga and across Butembo and Lukanga. Two QTL strongly associated with seed Fe were found on Chr1 and Chr3, while the remaining two QTL associated with Zn were found on Chr6 and Chr9. The fewer QTL observed might be an effect of analyzing an intra-gene pool population whose size is narrowed. This is in line with Cichy *et al*. (2009) who found a moderate number of QTL in Andean x Andean cross on Chr1, 6 and 11. Gelin *et al*. (2007), using a narrow population from a cross between two navy beans from the Mesoamerican gene pool, found only one QTL for Zn and none for Fe.

Common bean, as well as other plants, acquire Fe and Zn from the rhizosphere. Fe uptake goes through two different strategies in plants to adapt to Fe-deficient environments. Liang (2022) mentioned reduction-based strategy named “Strategy I” and chelation-based strategy known as “Strategy II”. From the model described in

Arabidopsis, to enable Strategy I, protons are extruded into the rhizosphere to reduce the soil pH and increase Fe solubility. In fact, for Grotz & Guerinot (2006), Fe is a thousand fold more soluble and available for every one unit drop in pH. The movement requires genes involved in H^+ exchange. Genes Phvul.001G112300 and Phvul.003G125300 were identified on Chr1 and Chr3 and are involved in H^+ exchange and uses H^+-ATPase to enable this exchange. These are isoforms of the electroneutral Na^+/H^+ exchanger (NHE) gene. Bernardazzi *et al*. (2022) reported that the NHE, due to secondary active transport $Na^+/K^+/H^+$ ATPase, is involved in H^+ exchange and this system has been identified in all domain of life. Their relatives, AHA H^+-ATPases, have been characterized in Arabidopsis (Morrissey & Guerinot, 2009). In addition, Bernardazzi *et al*. (2022) reported that NHE proteins are available in Golgi apparatus, plasma membrane and endosomes in various cell types.

Most candidate genes associated with iron and zinc uptake were located on Chr3. These genes belong to the ATP-binding cassette (ABC) family. It has been observed in the *Cyanobacteria synechocystis* sp. PCC 6803, that Fe^{+3} is transported by an ABC transporter, FutABC (Katoh *et al*., 2001). In addition, Grotz & Guerinot (2006), based on characterization of two members of the heavy metal ATPase genes family in Arabidopsis, demonstrated that metal ATPase proteins function in Zn homeostasis.

According to Morita *et al*. (1998), ABC genes represent one out of four groups of MATE family genes that code proteins involved in translocation of cell xenobiotics and metabolic waste products. For Izquierdo *et al*. (2018), ABC and multidrug and toxic compound extrusion (MATE) genes products are involved in export of an Fe chelator allowing the movement of Fe in the plant cell.

A significant QTL was also found on linkage group b06 for Zn at Lukanga. Blair *et al*., (2010), based on determination coefficients varying from 9.57 to 55.17, found high values of determination coefficients for QTL on this linkage group. Three homeobox-Leucine ZIPPER genes, Phvul.006G126600, Phvul.006G126500 and Phvul.006G128600, were identified on Chr6. They encode a class II ZIP protein required in regulation of meristematic activity in various tissues. This protein might also be involved in Zn uptake. In Arabidopsis, Grotz & Guerinot (2006) reported that ZIP proteins can functionally complement a yeast strain defective in Zn uptake. In planta, these authors reported that mRNAs of ZIP proteins accumulate in response to Zn deficiency. Furthermore, according to Izquierdo *et al*. (2018), ZIP proteins as well as NRAMP and YSL gene products may be involved in transport of metals in plants including Fe. Ishimaru *et al*. (2005) ; Vert *et al*. (2002) identified ZIP genes that were involved in mineral uptake, transport to leaves and translocation to seeds in rice and Arabidopsis respectively. Furthermore, Cichy *et al*. (2009), working on QTL analysis for mineral levels in an Andean bean population, observed that gene products associated with Fe and Zn on Chr6 contributed 14 to 36% and 12 to 39% to the phenotypic variation respectively for Fe and Zn.

The presence of an ATP-binding cassette (ABC) transporter gene, Phvul.009G248400, that belongs to the subfamily of ABC and MATE family (Morita *et al*., 1998) was identified on Chr9. Members of this subfamily utilize ATP as the source of energy. According to Izquierdo *et al*. (2018) and Rogers *et al*. (2009), products of this subfamily export a transmembrane protein that evolves as a specific Fe chelator allowing the movement of Fe in the plant. In addition, Phvul.009G250100 was another MATE efflux family gene found on this chromosome. These genes are of particular interest as members of this family as they have been successfully used to increase mineral

concentration in several crops such as soybean (Rogers *et al*., 2009), wheat (Borg *et al*., 2012) and rice (Goto *et al*., 1999).

7.5.Conclusion

Markers were distributed across the 11 common bean chromosomes. A total of four QTLs associated with seed iron and zinc contents were identified at Lukanga and across Butembo and Lukanga. Two QTLs strongly associated with seed iron were located on chromosomes 1 and 3, while the remaining two QTLs associated with zinc were located on chromosomes 6 and 9.

Annotation on the *Phaseolus vulgaris v2.1* reference genome indicated more than 100 genes, with different gene ontologies, located on different linkage groups (1, 3, 6 and 9) which would be inherited together. A total of 13 genes that belong to four families and located on the above four linkage groups were proposed as candidate genes controlling Fe and Zn in common bean. This was based on information on known putative functions of genes involved in Fe/Zn uptake (ZIP, FRO and NA genes), translocation (ZIP, FRO, NA and MATE genes) and storage in plants (NRAMP genes). One gene (Phvul.001G112300), an isoform of the electroneutral Na^+/H^+ exchanger (NHE) gene, involved in H^+ exchange was identified on chromosome 1. Seven genes (Phvul.003G125300, Phvul.003G120500, Phvul.003G120400, Phvul.003G122400, Phvul.003G122700, Phvul.003G122600 and Phvul.003G122500) that belong to NHE and ABC transporter gene families were identified on chromosome 3. Three genes (Phvul.006G126600, Phvul.006G126500 and Phvul.006G128600) that belong to ZIP gene family were identified to chromosome 6 and finally two genes (Phvul.009G248400 and Phvul.009G250100) that belong to ABC transporter and MATE gene families were identified on chromosome 9.

CHAPTER 8

8. CONCLUSIONS, RECOMMENDATIONS AND CONTRIBUTIONS TO KNOWLEDGE

8.1.Conclusions

The aim of this work was to determine farmers' varietal preferences, constraints and perceptions of iron and zinc biofortified common beans, cooking time and quality of high performing second-generation biofortified genotypes and to assess the performance and yield stability of selected iron and zinc biofortified common bean genotypes. In addition, the study was extended to the determination of the mode of inheritance of seed iron, zinc and polyphenols and on identification of QTLs associated with seed iron and zinc in common bean accessions and local varieties cultivated in DRC.

Farmers preferred high yielding varieties that mature earlier and with a good resistance to abiotic stresses. Important constraints to common bean production in Lubero and Beni territories included low yields, pests and diseases damages, changes in climatic occurrences, poor soil fertility, wandering of animals and low farm sizes due to intense insecurity particularly in Beni territory. Iron and zinc biofortified common beans are not known in these territories. Farmers knew that iron is involved in blood structure and wish to exploit varieties with high levels of iron and zinc.

Selected second-generation biofortified common bean genotypes had high hydration coefficients and cooked faster than local varieties. Cooking time varied from 73 to 170 minutes. However, the split percentages of cooked beans were high for biofortified lines compared to local varieties. Sensory characters such as taste and flavour of cooked beans of selected biofortified genotypes varied from slightly pleasant to excellent, while the starchiness was judged clear and slightly clear.

Genotypes varied significantly for agronomic and quality characters, except number of seeds per pod. Biofortified genotypes RK 11, BF08-07-22 and BF08-26-162 combined iron and zinc amounts significantly higher than those for the high iron and zinc checks. Broad-sense heritability and genetic gain under selection were high for pod length. Heritability was moderate for 100-seed mass and seed iron and genetic gain was low for all other traits. Based on AMMI stability values, biofortified genotypes BF08-14-96B, BF08-7-19B and BF08-14-51C were the most stable and yielded more than 1,800 kg ha^{-1}.

Dominance genetic variance was important to control the inheritance of accumulation of total polyphenols. In addition to dominance, additive genetic effects were important underlying iron and zinc in seeds of common bean. Hence, both heterosis and recurrent breeding approaches are important in developing high performing genotypes with reduced polyphenolics and increased iron and zinc levels.

Four QTLs associated with seed iron and zinc were identified. These QTLs were located on chromosomes 1 and 3 for iron and on chromosomes 6 and 9 for zinc. Based on the putative functions of genes involved in iron and zinc acquisition, translocation and storage in plants, 13 candidate genes were proposed controlling the genetic basis of iron and zinc in common bean. These genes belong to four families : NHE, ABC, ZIP and MATE gene families.

8.2.Recommendations

The following recommendations could be made from the results of the studies carried out :

- A biofortification programme that includes farmers is needed. Lubero and Beni territories would likely improve the adoption of improved varieties to increase farmers' economic returns and reduce effects of iron and zinc deficiencies.
- The 124 selected biofortified should be assessed for nutritional nutrients remaining in seeds after and before cooking.
- Biofortified common bean genotype G4-24A that combined high grain yield, seed iron and zinc and genotypes RK 11, BF08-07-22 and BF08-26-162 that combined high iron and zinc should be advanced as the fast-track iron and zinc biofortified genotypes and subjected to iron and zinc bioavailability assessment.
- The most stable and high yielding biofortified genotypes BF08-14-96B, BF08-7-19B and BF08-14-51C should be advanced through validation processes across contrasting environments.
- Heterosis and recurrent breeding approaches should be considered to develop performing genotypes with low amounts of polyphenols and high levels of iron and zinc in common bean seeds.
- Cross location QTL analysis should be studied to assess stability of QTL across years and locations. Further validated loci and genes could be used in common bean breeding to accelerate delivery of dense iron and zinc common bean types.
- Mapping of discovered genes should be important for further genetic studies toward increasing iron and zinc in common bean through modern biotechnology approaches.

8.3.Contributions to knowledge

This study makes significant contributions to the understanding and advancement of iron and zinc biofortification in common bean cultivation in DRC, particularly in the North-Kivu province.

It successfully identified promising common bean genotypes that exhibit both high yield potential and enhanced mineral content, particularly iron and zinc. These genotypes represent a strategic opportunity to improve agricultural productivity while simultaneously addressing nutritional deficiencies. By integrating yield and nutritional traits, the selected lines offer better returns for farmers and contribute to the broader goals of sustainable food systems in DRC.

Beyond agronomic performance, the findings serve as a cross-cutting intervention to reinforce national and regional strategies aimed at combating malnutrition. The provision of iron and zinc biofortified common bean genotypes directly supports efforts to reduce micronutrient deficiencies, especially among vulnerable populations. This aligns with global and local nutrition policies and highlights the role of agricultural research in delivering tangible health benefits through crop improvement.

Moreover, the study contributes to strengthening regional agricultural innovation systems and extension services. By generating locally adapted, nutrient-rich genotypes, it equips policymakers and practitioners with evidence-based recommendations tailored to specific agroecological zones. This enhances the relevance and impact of agricultural interventions, fostering inclusive development and resilience across farming communities.

The identification of QTLs associated with iron and zinc accumulation in common bean genotypes represents a pivotal advancement in biofortification research. This breakthrough opens new avenues for genomic selection, providing a robust framework to accelerate the breeding and dissemination of nutritionally superior varieties in DRC. By harnessing molecular tools and predictive breeding models, researchers can significantly reduce the time required to develop and release improved cultivars. This

approach not only enhances breeding efficiency but also ensures that genetic gains are rapidly translated into tangible benefits for farmers and consumers. Ultimately, it marks a transformative step toward modernizing bean improvement programmes and reinforcing the role of agriculture in addressing micronutrient deficiencies and food insecurity.

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Appendix 1 : Mixed questionnaire

Farmers'preferred common bean varieties, perceptions on Fe and Zn accumulation and other constraints facing common bean production in Eastern DRC

Questionnaire No.		Axis	
Date of interview		Village	
Territory		GPS data	

A. Biographic data

1. Name of household head……

2. Name of respondent ……

3.

a) Gender	1. Male	2. Female	
b) Marital status	1. Single	2. Married monogamous	3. Married polygamous
	4. Divorced	5. Widowed	
c) Education level	1. No formal education	2. Primary classes	3. High school
	4. University		
d) Age	1. Below 25	2. Between 25-35	3. Between 36-45
	4. Between 46-55	5. Beyond 55	

4. Relation of respondent to the household head ----------

5. What is the size of your land? ---------- Acres

6. What is the size of land allocated to common bean production?

7. Which crops have you been growing for the last 5 years?

	Crop	Rank	Acreage	Purpose*
1				
2				
3				
4				

*Purpose: 1. Cash crop (income) 2. Food 3. Cash and Food crop 4. Security/saving 5. Livestock feed 5. Others

B. Common beans production information

i) if common bean is not among the crops grown in 5 above

1. Have you ever grown common beans? 1. Yes 2. No

2. If yes, why did you stop? ----------

3. If no, why? ----------

ii) If common bean is among the crops grown in 5 above

4. Which cropping system do you use for common beans? 1. Mono crop (common bean only) 2. Intercrop,

5. If intercropped, with which crops? 1. ----------

2. ----------

3. ----------

6. What are reasons for intercropping?

7. What spacing did you use?

8. Which of the following inputs do you use in common bean production?

Input	Specific type used	Frequency of application	Amount used (per plant/area)	Reason for not using
Manure				
Fertilizer				
Pesticides				
Fungicides				
Other (specify)				

6. What means do you use to access information common bean farming? What kind of information your gathered from the listed sources. Rank these sources in terms of their usefulness.

	2021		
Means/sources	Frequency of access to information	[1]Rank the usefulness of means	
1=Radio			
2=fellow farmers			
3=neighbor			
4=group members			
5=newspapers			
6= SMS from phones			
7=bill boards			
8=information from village			
9= other (specify)			

1 Rank of usefulness: 1=very useful ; 10=least useful

C. Major common bean varieties grown in Eastern DRC

1. Which variety/varieties do you grow and what are their good and bad attributes

	Variety	Good traits	Bad traits
1			
2			
3			
4			

2. What is the source of your planting material 1. Neighbouring farmers 2. Agro vet 3. Market 4. Research institutions 5. Seed companies 6. Others (specify)

3. Have you ever heard about improved varieties of common bean ? 1. Yes 2. No

4. If yes; from which source ? ----------

5. If yes; have you ever planted the improved common bean varieties? 1. Yes 2. No

6. Where did you get your planting materials from? ----------

7. If yes; how do you compare the improved varieties to traditional varieties?

Criteria	Comparison	
	Improved varieties	Traditional varieties
Yield		
Maturity		
Pest and disease tolerance		
Drought tolerance		
Quality traits		
Nitrogen fixation		
Intercropping compatibility		
Seed viability		
Low shattering ability		
Adaptability		
Seed colour		
Others (specify)		

8. Reasons for not planting improved varieties?

……

9 Are you willing to plant improved varieties or you are content with the local variety? 1. willing 2. Content

10. What are the most important traits you look for when selecting a common bean variety?

	Selection criteria	Rank
1	Yield	
2	Maturity	
3	Pest and disease resistance	
4	Drought tolerance	
5	Quality traits	
6	Nitrogen fixation	
7	Intercropping compatibility	
8	Seed viability	
9	Low shattering ability	
10	Standability	
11	Seed availability	
12	Seed colour	
13	Others (specify)	

D. Common bean Productivity, utilization constraints and marketing

1. What is the bean productivity in the last cropping season?

Bean variety	Area planted (acres)	Weight (kg)	Source of seeds	Was fertilizer applied (0=no ; 1=yes)	At planting ? (kg) & type (0=no ;1=yes)	At top dressing (kg) & type (0=no ;1=yes)	Price/Unit

2. What constraints do you face in common bean production?

	Constraint	Rank	Suggested solution
1			
2			
3			
4			
5			

3. During the last 1-2 years, have you experienced any reduction in common beans yields? 1. Yes 2. No . Give evidence if yes……

……

4. In the last 3-5 years, did you experience yield reduction? 1. Yes 2. No . Give evidence if yes ……

……

5. What are your means of getting access to the market?

		Distance (Km)		[1]Means of travel		Period (min)	
		Current period	Before 2021	Current period	Before 2021	Current period	Before 2021
Input market	Small market						
	Big market						
Output market	Small market						
	Big market						
Distance/time to main road							

[1]*1=walking ; 2=bicycle ; 3=motorbike ; 4=public vehicle ; 5= private car ; 6=other (to specify)*

6. During the last season, how have you utilized harvests from your farm of common bean? (please precise the quantities with unit of measure)

[1]Purpose	Qty produced (total)	Qty consumed, donated or gifted	Quantity sold	Qty wasted or spoilt	Price/unit	Qties sold through group network	[2]How marketed ?	[3]Who keeps the money	Months when sold

[1]*Purporse: 1=for sale only ; 2=for food only ; 3= food but sale in emergency case ; 4=food but sale when has plenty ; 5= food and sale ; 6=others (to specify) ……*

[2]*How marketed: 1=individuality ; 2=collectively through group networking ; 3= self and group*

[3]*Who keeps money after sale: 1=wife ; 2=husband ; 3=child (girl) ; 4=child (boy) ; 5=others (to specify) ……*

7. Give information on the sales of common bean for the last season

Qty sold	[1]To who you mostly sell ?	[2]Where was the sale done?	[3]Mode of sale	Distance to market	[4]Means of transport to market	Period of year when beans are high costly	Period of year when beans are low costly

[1]*Who do you mostly sell to: 1=local trader ; 2=trader from far ; 3=other farmers ; 4=others (to specify) ……..*

[2]*Where was the sale done?: 1=urban market ; 2=local market ;3= roadside new the farm ; 4=on farm ; 5=other (to specify)……..*

[3]*What was the Mode of sale: 1=credit only ; 2=cash only ; 3=both*

4Whats were the means of transport to market: 1=walking ; 2=bicycle ; 3=motorbike ; 4=public vehicle ; 5= private car ; 6=other (to specify)……

8. Have you ever tried to organize yourself with other farmers so to sell common beans in groups? 1. Yes 2. No

9. If yes, when did you start working in group networking?……..

10. If yes,

With whom	Qties sold collectively	Frequencies of sales	[1]What markets ?	[2]What differences in received prices?	Distance to market/selling point	[3]Means of transport

[1]*What markets: 1=urban market ; 2=local market ;3= roadside new the farm ; 4=on farm ; 5=other (to specify) ……..*

[2]*What differences in received prices? : 1=better ; 2=lower ; 3=no difference*

[3]*Means of transport: 1=walking ; 2=bicycle ; 3=motorbike ; 4=public vehicle ; 5= private car ; 6=other (to specify)……*

E. Perceptions on Zn and Fe concentration in common bean

1. Have you heard about mineral concentration in common beans? 1. Yes 2. No

2. If yes which ones? 1. Fe 2. Zn 3. Others (specify)

3. If yes, what benefits should play these minerals in common bean recipes?.....

4. Do you know some common bean lines with high Zn and Fe concentrations reported? 1. Yes 2. No

5. If yes, which ones?

	Varieties with reported high Zn and Fe concentration	Rank
1		
2		
3		

6. Do you cultivate common bean lines with reported high Zn and Fe concentration?

1. Yes 2. No

7. If yes which ones?

8. Where did you get them? 1. Neighbouring farmers 2. Agro vet 3. Market 4. Research institutions 5. Seed companies 6. Others (specify)

9. What are the constraints faced when cultivating these varieties?

	Constraints	Rank
1		
2		

3		
4		

10. Which benefits are you getting when cultivating the available varieties with high Fe and Zn

	Benefits	Rank
1		
2		
3		

11. Do you wish to exploit more improved varieties towards high Fe and Zn concentration together with other key traits 1. Yes 2. No

12. Have you heard that Zn plays a role in inhibition of replication of viruses? 1. Yes 2. No

13. Have you heard that Fe is involved in blood structure of an organism? 1. Yes 2. No

Appendix 2 : Hydration coefficient, cooking time (minutes) and integrity of beans (%) of 124 high performing second-generation biofortified genotypes and five local common bean varieties in DRC

N°	Genotype	Hydration coefficient	Cooking time	Integrity of beans
	Biofortified lines			
1	BF08-26-69	1.886	100	97.9
2	BF08-01-45A	1.976	116	83
3	BF08-13-102	2.043	100	92.1
4	KMA13-28-5	1.543	110	98.6
5	BF08-14-51A	3.373	106	96.5
6	BF08-01-21	1.986	73	90
7	KMA13-22-27	1.671	120	97.8
8	BF08-3-23B	1.971	112	94.4
9	MV-14	1.943	93	97.8
10	BCB11-509	2.6	125	82.9
11	BF08-07-74A	1.99	100	96.1
12	G4-585	1.614	141	100
13	BF08-14-20	2.1	118	98.6
14	BF08-13-38	2.013	137	97.7
15	KMA13-10-05	2.125	120	98.8
16	BF08-16-21	2.0125	110	97.7
17	MBC23	1.875	131	89
18	BF08-7-112D	2.014	168	99.4
19	BF08-26-68B	1.986	140	100
20	BF08-16-36A	2.015	133	94.7
21	NAIN DE KYONDO	1.943	113	21.3
22	BF08-7-114	1.986	133	95.4
23	BF08-16-76	1.88	120	98.1
24	BF08-13-44A	2.028	101	97
25	G2333(B)	1.471	129	98.5
26	BF08-1-77	2.029	110	98.6
27	BF08-14-24	1.843	118	100
28	BF08-03-13	1.942	123	96
29	BF08-16-67B	1.986	129	98
30	BF08-1-18C	1.857	128	86.1
31	BF08-14-116	1.743	150	98.7
32	BF08-07-74C	1.99	100	96
33	BF08-03-22	1.943	122	96
34	BF08-13-47	1.793	105	95.35
35	BF08-13-44B	2.03	99	99
36	RK14	2.1	95	82.4
37	BF08-16-67A	1.98	131	97.8
38	GLP585	1.614	142	100
39	BF08-1-60	2	105	96.6
40	BF08-1-47A	1.871	116	98.6
41	BF08-1-29	2.029	111	98.6

42	SR6	1.912	135	95
43	BF08-01-50	1.946	116	83
44	BF08-36-53	1.946	75	98.7
45	BF08-14-153A	3.32	101	89.7
46	BCB11-492	2.45	127	82.9
47	BF08-13-43A	1.753	105	95.35
48	KMA-21-19	1.427	151	82
49	BF08-14-96A	3.3	105	94.7
50	BF08-13-170	1.793	105	95.35
51	BF08-14-102	3.423	102	92.7
52	BF08-14-51B	3.253	108	96.7
53	BF08-01-18A	2.002	114	84
54	BF08-14-96B	3.338	105	94.7
55	BF08-14-51C	3.353	106	96.7
56	BF08-01-45B	1.986	115	83
57	KMA13-28-21	1.414	170	82.2
58	BF08-03-12B	1.981	117	98.8
59	BF08-1-80	1.871	115	98.6
60	RK11	2.1	95	82.4
61	BF08-03-13B	1.944	121	96.1
62	BF08-1-49B	2	104	96.6
63	KMA13-22-19	1.786	156	95.4
64	BF08-03-05	1.929	103	97.4
65	KMA13-05-21	1.793	105	95.35
66	BF08-14-153B	3.353	98	86.7
67	BF08-7-19B	2.14	148	95.3
68	BF08-13-18D	1.793	105	95.35
69	BF08-16-82B	1.6	116	98.5
70	BF08-01-18B	1.976	116	83
71	KMA13-23-22	1.427	153	81
72	BF08-3-23A	1.971	112	94.4
73	KMA13-10-18	1.406	149	81.4
74	BF08-7-19A	2.13	149	95.5
75	BF08-1-51	2.03	109	98.6
76	RK12B	2.2	94	82.2
77	BF08-07-112D	1.96	104	95.5
78	MC20	2.03	117	96.8
79	BF08-16-14	1.6	116	98.5
80	BF08-07-116	1.96	102	95.5
81	KMA13-23-21	1.406	150	81.4
82	BF08-16-52	1.6	120	98.5
83	G4-24A	1.6	116	90.5
84	BF08-01-90	1.961	118	83
85	BF08-03-20	1.6	120	98.6
86	BF08-1-60B	1.95	109	96.6
87	BF08-1-27	2.01	111	98.6

88	KENYA UMOJA	1.943	118	77.7
89	BF08-01-47B	1.986	75	90
90	BCB11-433	2.00	118	93.3
91	BF08-01-92	2.056	75	94
92	BCB11-372	2.3	130	84.3
93	BF08-07-74B	1.96	102	95.5
94	BF08-16-18E	1.61	116	98.5
95	BF08-03-12A	1.961	119	98.4
96	SR9	1.897	139	95
97	BF08-01-62B	1.976	116	84
98	BF08-01-01	2.115	94	90
99	SER12	1.987	136	95
100	KMA13-13-70	1.406	151	81.4
101	MEX54	1.877	129	95
102	BCB11-303	2.5	127	92.9
103	BF08-01-62A	1.966	115	85
104	BF08-16-92	1.71	116	98.5
105	BCB11-138	2.4	131	85.3
106	BF08-7-80	1.926	133	95.4
107	RK6	2.2	100	83.1
108	BF08-26-162	1.98	123	92.5
109	RK12A	2.1	95	82.4
110	BF08-16-31	1.78	117	98.6
111	KMA13-03-35	1.406	151	81.4
112	MV1	1.903	92	97
113	BF08-16-68	1.88	118	98.1
114	BF08-01-49A	1.976	116	86
115	BF08-07-22	1.986	133	95.4
116	MC29A	1.90	124	95
117	BCB11-342	2.356	129	84.3
118	BF08-7-75	2.115	94	90
119	KMA13-32-28	1.506	145	82.4
120	BF08-01-54	1.976	116	84
121	BF08-1-30	1.99	109	96.6
122	KMA13-19-33	1.506	143	84.4
123	BF08-16-36B	2.013	136	95
124	BF08-13-43B	1.693	112	95.35
	Mean	**2.00**	**117.85**	**92.2**
	Local lines			
125	IKINIMBA	1.729	139	99.3
126	KALANGITI	1.72	144	99.1
127	MAFUTALA	1.9	130	99.8
128	OBUSOSERA	2	120	93
129	DEMAI	1.89	139	98.5
	Mean	**1.8**	**134.4**	**97.9**

Appendix 3 : Days to 50% flowering (DTF), Days to 75% (DT75M) and 100% (DTFM) maturation of 160 biofortified genotypes and five local common bean varieties in DRC

	DTF		DT75M		DTFM	
Genotypes	**No. Days**	**Group**	**No. Days**	**Group**	**No. Days**	**Group**
BCB11-138	43.8	c-k	90.8	a-k	98.7	b-l
BCB11-303	43.0	g-k	88.0	c-k	95.5	f-l
BCB11-342	44.2	c-k	92.8	a-h	100.7	b-k
BCB11-372	43.7	d-k	88.3	c-k	98.8	b-l
BCB11-433	45.0	c-k	90.8	a-k	98.2	b-l
BCB11-492	43.7	d-k	86.7	f-k	95.5	f-l
BCB11-509	41.7	j-l	86.3	f-k	94.8	g-l
BF08-01-01	48.3	a-g	94.3	a-g	102.2	b-i
BF08-01-18A	48.0	a-g	93.8	a-g	99.3	b-l
BF08-01-18B	48.5	a-g	96.3	a-e	104.2	b-g
BF08-01-21	45.3	b-k	95.3	a-f	105.3	a-f
BF08-01-45A	44.7	c-k	97.0	a-e	106.3	a-f
BF08-01-45B	48.5	a-g	96.7	a-e	104.0	b-g
BF08-01-47B	48.2	a-g	95.2	a-f	102.3	b-h
BF08-01-49A	49.0	a-g	96.2	a-e	106.3	a-f
BF08-01-50	48.3	a-g	95.5	a-e	103.0	b-h
BF08-01-54	48.3	a-g	95.8	a-e	105.7	a-f
BF08-01-62A	47.0	a-h	97.8	a-c	105.7	a-f
BF08-01-62B	46.7	a-j	95.8	a-e	103.0	b-h
BF08-01-90	47.0	a-h	95.7	a-e	106.0	a-f
BF08-01-92	48.7	a-g	94.2	a-g	103.2	b-h
BF08-03-05	49.8	a-e	95.5	a-e	102.3	b-h
BF08-03-12	45.8	b-k	96.3	a-e	104.5	b-f
BF08-03-12B	45.0	c-k	94.5	a-f	102.2	b-i
BF08-03-13	46.2	b-j	96.5	a-e	106.7	a-f
BF08-03-13B	50.5	ab	96.5	a-e	105.2	a-f
BF08-03-20	48.3	a-g	97.0	a-e	106.5	a-f
BF08-03-22	47.7	a-h	96.7	a-e	104.7	b-f
BF08-07-112D	46.8	a-i	94.5	a-f	103.5	b-g
BF08-07-116	47.3	a-h	95.0	a-f	104.5	b-f
BF08-07-22	48.2	a-g	92.8	a-h	101.0	b-k
BF08-07-74A	46.8	a-i	95.2	a-f	105.8	a-f
BF08-07-74B	47.3	a-h	95.7	a-e	102.5	b-h
BF08-07-74C	48.7	a-g	94.7	a-f	104.8	b-f
BF08-1-18C	46.8	a-i	96.3	a-e	103.2	b-h
BF08-1-27	48.2	a-g	96.3	a-e	104.2	b-g
BF08-1-29	49.2	a-g	95.0	a-f	105.7	a-f
BF08-1-30	47.2	a-h	95.0	a-f	102.3	b-h
BF08-13-102	46.8	a-i	95.2	a-f	108.2	a-d
BF08-13-170	48.2	a-g	93.2	a-h	101.2	b-k
BF08-13-18D	48.3	a-g	94.8	a-f	102.8	b-h

BF08-13-38	45.2	c-k	93.3	a-h	102.0	b-j
BF08-13-43A	50.2	a-c	96.0	a-e	103.7	b-g
BF08-13-43B	45.7	b-k	93.8	a-g	102.3	b-h
BF08-13-44	45.8	b-k	95.2	a-f	107.5	a-e
BF08-13-44B	49.5	a-f	95.5	a-e	101.0	b-k
BF08-13-47	47.0	a-h	97.2	a-e	105.0	b-f
BF08-14-102	46.7	a-j	89.7	b-k	99.0	b-l
BF08-14-116	46.7	a-j	94.5	a-f	102.2	b-i
BF08-14-153A	47.0	a-h	93.8	a-g	102.5	b-h
BF08-14-153B	49.0	a-g	93.2	a-h	102.0	b-j
BF08-14-20	50.2	a-c	97.2	a-e	108.3	a-d
BF08-14-24	46.0	b-k	93.7	a-g	101.2	b-k
BF08-14-51A	47.0	a-h	93.8	a-g	106.7	a-f
BF08-14-51B	43.7	d-k	92.5	a-h	101.2	b-k
BF08-14-51C	48.0	a-g	94.8	a-f	104.0	b-g
BF08-1-47A	45.0	c-k	94.3	a-g	103.2	b-h
BF08-14-96A	47.2	a-h	94.2	a-g	79.2	m
BF08-14-96B	45.2	c-k	94.7	a-f	102.2	b-i
BF08-1-49B	48.2	a-g	96.3	a-e	103.0	b-h
BF08-1-51	48.7	a-g	94.7	a-f	103.5	b-g
BF08-1-60	47.0	a-h	95.7	a-e	105.7	a-f
BF08-1-60B	47.2	a-h	95.5	a-e	106.3	a-f
BF08-16-14	47.3	a-h	94.2	a-g	105.5	a-f
BF08-16-18E	45.8	b-k	95.2	a-f	103.5	b-g
BF08-16-21	48.7	a-g	92.5	a-h	99.8	b-l
BF08-16-31	49.3	a-g	97.2	a-e	103.3	b-g
BF08-16-36A	47.3	a-h	94.5	a-f	104.2	b-g
BF08-16-36B	47.2	a-h	95.2	a-f	104.5	b-f
BF08-16-52	48.5	a-g	95.7	a-e	109.2	a-c
BF08-16-67A	47.3	a-h	94.8	a-f	103.2	b-h
BF08-16-67B	49.2	a-g	97.5	a-e	107.0	a-f
BF08-16-68	47.7	a-h	95.7	a-e	103.8	b-g
BF08-16-76	49.8	a-e	95.2	a-f	105.0	b-f
BF08-16-82B	48.2	a-g	95.0	a-f	103.0	b-h
BF08-16-92	48.0	a-g	97.7	a-d	105.5	a-f
BF08-1-77	47.5	a-h	96.5	a-e	106.0	a-f
BF08-1-80	48.5	a-g	95.2	a-f	103.0	b-h
BF08-26-162	47.0	a-h	93.8	a-g	102.5	b-h
BF08-26-68B	44.8	c-k	90.5	a-k	101.3	b-k
BF08-26-69	48.8	a-g	94.7	a-f	106.8	a-f
BF08-3-23A	47.7	a-h	95.0	a-f	104.2	b-g
BF08-3-23B	48.2	a-g	95.5	a-e	108.2	a-d
BF08-36-53	48.5	a-g	94.7	a-f	102.8	b-h
BF08-7-112D	48.2	a-g	93.0	a-h	102.2	b-i
BF08-7-114	47.8	a-h	94.2	a-g	104.5	b-f
BF08-7-19A	48.8	a-g	95.0	a-f	102.7	b-h
BF08-7-19B	48.0	a-g	95.0	a-f	118.3	a

BF08-7-75	48.3	a-g	96.5	a-e	104.7	b-f
BF08-7-80	49.5	a-f	95.7	a-e	105.5	a-f
CAB2	49.5	a-f	96.5	a-e	108.0	a-d
CODLMV059	52.8	a	100.8	a	109.7	ab
DEMAI	41.9	i-k	83.8	i-k	93.0	i-l
G2333(B)	48.3	a-g	93.2	a-h	103.2	b-h
G4-24A	42.2	h-k	91.3	a-k	98.7	b-l
G4-585	46.7	a-j	92.0	a-i	103.7	b-g
GLP585	47.0	a-h	93.8	a-g	103.8	b-g
HM21-7	41.7	j-l	91.7	a-k	100.3	b-l
ICYANA	50.5	ab	96.2	a-e	104.7	b-f
IKINIMBA	41.1	kl	85.1	h-k	92.8	j-l
KAB06F2-8-12	42.0	h-k	88.2	c-k	96.7	d-l
KAB06F2-8-27	44.2	c-k	92.2	a-i	101.0	b-k
KALANGITI	41.8	jk	83.6	jk	92.4	k-l
KENYA UMOJA	45.0	c-k	90.5	a-k	96.8	d-l
KMA13-03-35	49.8	a-e	94.0	a-g	102.2	b-i
KMA13-05-21	47.3	a-h	94.0	a-g	102.2	b-i
KMA13-10-05	45.7	b-k	89.3	b-k	99.2	b-l
KMA13-10-18	46.2	b-j	90.0	b-k	100.0	b-l
KMA13-13-70	48.8	a-g	92.8	a-h	103.8	b-g
KMA13-19-33	48.0	a-g	96.0	a-e	103.2	b-h
KMA13-21-19	43.5	e-k	85.0	h-k	95.5	f-l
KMA13-22-19	45.2	c-k	91.2	a-k	99.0	b-l
KMA13-22-27	46.8	a-i	92.5	a-h	102.2	b-i
KMA13-23-21	46.2	b-j	92.8	a-h	101.8	b-j
KMA13-23-22	47.5	a-h	87.0	e-k	94.8	g-l
KMA13-28-21	45.5	b-k	92.7	a-h	100.5	b-k
KMA13-28-5	46.8	a-i	92.3	a-h	104.7	b-f
KMA13-32-28	48.3	a-g	93.5	a-g	100.7	b-k
MAC49	47.0	a-h	96.7	a-e	106.5	a-f
MAC74	50.0	a-d	98.0	a-c	118.3	a
MAFUTALA	45.2	c-k	87.0	f-k	95.0	g-l
MC20	45.8	b-k	92.3	a-h	98.7	b-l
MC29A	44.7	c-k	94.2	a-g	103.2	b-h
MCB23	48.8	a-g	91.5	a-k	99.2	b-l
MEX54	47.5	a-h	87.2	d-k	94.5	g-l
MIB465	44.7	c-k	88.5	c-k	95.5	f-l
MV1	50.2	a-c	97.8	a-c	104.5	b-f
MV-14	47.8	a-h	97.3	a-e	108.8	a-c
NAIN DE KYONDO	50.2	a-c	94.0	a-g	103.2	b-h
NAMULENGA	48.2	a-g	94.3	a-g	105.0	b-f
NDIMIRAKAKUJA	44.7	c-k	92.5	a-h	101.2	b-k
NGWINxCAB2 21311	51.5	ab	97.2	a-e	106.3	a-f
OBUSOSERA	43.0	h-k	86.1	g-k	94.0	h-l
RK11	44.8	c-k	91.0	a-k	99.0	b-l
RK12A	44.5	c-k	87.5	c-k	95.8	e-l

RK12B	49.2	a-g	92.2	a-i	99.3	b-l
RK14	45.3	b-k	93.2	a-h	97.8	c-l
RK6	44.3	c-k	90.5	a-k	97.7	c-l
RUGANDURA	44.7	c-k	94.3	a-g	102.0	b-j
RW1180	42.0	h-k	90.2	b-k	95.5	f-l
RW267	45.5	b-k	87.2	d-k	94.3	g-l
RW298	44.0	c-k	89.0	c-k	97.0	d-l
RW3006	47.2	a-h	96.3	a-e	104.0	b-g
RW439	42.7	h-k	87.2	d-k	92.8	i-l
RW547	45.7	b-k	88.8	c-k	97.7	c-l
RW582	35.5	l	81.2	k	88.7	l-m
RW693	43.3	f-k	87.8	c-k	94.7	g-l
RW744	43.2	f-k	86.0	g-k	92.0	k-l
RW805	45.8	b-k	95.8	a-e	105.2	a-f
RW849	45.7	b-k	87.5	c-k	96.8	d-l
RW880	45.0	c-k	86.5	f-k	94.2	g-l
RW896	46.2	b-j	86.3	f-k	92.5	j-l
RW942	42.5	h-k	86.8	f-k	94.8	g-l
RWR1180	42.0	h-k	84.2	h-k	91.7	k-l
RWR2154	45.8	b-k	86.8	f-k	95.7	f-l
RWR2245	42.3	h-k	85.0	h-k	94.5	g-l
RWV2359	50.8	ab	99.7	ab	106.3	a-f
RWV2887	49.2	a-g	97.3	a-e	106.5	a-f
SER12	49.2	a-g	93.8	a-g	103.0	b-h
SR6	43.2	f-k	89.2	b-k	98.5	b-l
SR9	45.0	c-k	91.8	a-j	100.7	b-k
UBR(92)25	46.0	b-k	93.2	a-h	100.3	b-l
UGK116	43.3	f-k	87.0	f-k	94.5	g-l
UGK72	48.7	a-g	94.3	a-g	109.3	a-c
ZKA93-10m/95	41.2	j-l	89.3	b-k	94.3	g-l

Appendix 4 : Number of pods per plant (NPP), pod length, seeds per pod and 100-seed weight (100-SW) of 160 biofortified genotypes and five local common bean varieties in DRC

	NPP		Pod length		Seeds per pod		100-SW	
Genotypes	Pods	Group	(cm)	Group	Seeds	Group	(g)	Group
BCB11-138	5.5	c	11.3	E-S	4.7	a	36.2	i-C
BCB11-303	6.6	c	10.3	X-3	3.7	a	36.8	h-A
BCB11-342	8.1	bc	11.7	t-M	5.0	a	37.3	h-y
BCB11-372	6.6	c	8.7	89.+	3.2	a	47.6	a-f
BCB11-433	6.4	c	8.6	9.+-*	2.8	a	44.8	b-i
BCB11-492	9.9	abc	12.6	a-n	4.4	a	39.1	f-t
BCB11-509	6.1	c	12.4	d-v	3.6	a	37.8	h-w
BF08-01-01	11.9	abc	11.5	z-Q	6.0	a	31.2	s-I
BF08-01-18A	14.1	abc	12.6	a-p	6.2	a	34.5	l-D
BF08-01-18B	9.0	abc	12.2	h-z	5.8	a	32.6	o-E
BF08-01-21	10.8	abc	12.8	a-j	6.6	a	34.5	l-D
BF08-01-45A	11.5	abc	12.2	h-y	6.2	a	27.5	C-L
BF08-01-45B	11.7	abc	12.7	a-l	6.2	a	34.6	k-D
BF08-01-47B	18.5	a	12.8	a-k	7.1	a	28.4	A-K
BF08-01-49A	10.2	abc	13.1	a-c	7.4	a	30.3	u-I
BF08-01-50	13.5	abc	12.6	a-p	5.7	a	31.7	s-G
BF08-01-54	16.5	abc	11.7	s-K	5.9	a	34.5	l-D
BF08-01-62A	10.5	abc	11.6	v-M	5.4	a	31.2	s-I
BF08-01-62B	12.3	abc	12.7	a-l	6.1	a	30.1	u-I
BF08-01-90	10.2	abc	12.9	a-h	7.0	a	32.8	n-E
BF08-01-92	12.4	abc	12.2	h-y	6.3	a	37.2	h-z
BF08-03-05	18.1	a	11.9	m-F	6.0	a	28.6	z-K
BF08-03-12	13.1	abc	11.9	m-E	5.9	a	36.5	h-B
BF08-03-12B	11.9	abc	12.1	k-D	5.9	a	35.8	j-C
BF08-03-13	13.7	abc	12.7	a-k	6.6	a	33.0	n-E
BF08-03-13B	16.9	abc	12.9	a-i	6.7	a	34.0	l-D
BF08-03-20	12.2	abc	12.8	a-j	5.5	a	34.4	l-D
BF08-03-22	9.0	abc	12.3	f-x	5.7	a	32.0	p-F
BF08-07-112D	12.6	abc	11.8	p-H	5.7	a	35.6	j-C
BF08-07-116	12.1	abc	11.4	z-Q	5.9	a	31.5	s-H
BF08-07-22	13.3	abc	12.2	h-y	5.8	a	29.5	v-I
BF08-07-74A	13.2	abc	12.1	h-B	5.7	a	30.8	s-I
BF08-07-74B	15.1	abc	12.1	k-D	6.3	a	31.3	s-I
BF08-07-74C	13.2	abc	12.4	d-v	6.2	a	36.9	h-A
BF08-1-18C	11.6	abc	12.8	a-k	6.7	a	34.1	l-D
BF08-1-27	12.7	abc	12.8	a-j	6.1	a	30.7	t-I
BF08-1-29	12.9	abc	12.2	h-z	5.4	a	32.5	o-E
BF08-1-30	13.1	abc	12.7	a-l	6.3	a	32.9	n-E
BF08-13-102	10.1	abc	12.2	h-y	6.3	a	34.6	k-D
BF08-13-170	9.1	abc	12.5	a-r	7.0	a	29.9	u-I
BF08-13-18D	10.4	abc	13.1	a-e	6.8	a	33.4	m-E

BF08-13-38	14.3	abc	13.1	a-c	7.2	a	32.3	p-F
BF08-13-43A	10.9	abc	12.4	b-s	5.9	a	32.7	o-E
BF08-13-43B	8.1	abc	11.6	x-O	5.2	a	31.2	s-I
BF08-13-44	16.3	abc	12.8	a-j	6.5	a	31.1	s-I
BF08-13-44B	9.1	abc	11.3	D-R	5.8	a	30.7	t-I
BF08-13-47	9.1	abc	12.4	c-t	6.4	a	31.4	s-I
BF08-14-102	10.8	abc	11.1	H-V	6.5	a	26.8	D-L
BF08-14-116	10.5	abc	10.9	M-Y	5.6	a	31.4	s-I
BF08-14-153A	13.4	abc	13.2	ab	6.8	a	37.4	h-x
BF08-14-153B	13.9	abc	12.0	l-E	5.6	a	28.5	z-K
BF08-14-20	11.3	abc	12.1	i-B	6.2	a	31.8	r-F
BF08-14-24	10.1	abc	11.7	s-L	6.0	a	29.4	v-I
BF08-14-51A	12.8	abc	13.1	a-d	6.6	a	32.8	n-E
BF08-14-51B	12.2	abc	12.5	a-q	6.9	a	31.3	s-I
BF08-14-51C	16.4	abc	13.0	a-g	6.4	a	33.5	m-D
BF08-1-47A	10.9	abc	11.3	D-S	6.4	a	29.4	v-I
BF08-14-96A	8.0	bc	12.4	b-s	6.0	a	29.9	u-I
BF08-14-96B	11.8	abc	12.6	a-o	6.6	a	29.3	v-I
BF08-1-49B	18.1	a	12.7	a-l	6.5	a	34.6	k-D
BF08-1-51	10.4	abc	11.7	s-L	5.4	a	34.5	l-D
BF08-1-60	11.9	abc	12.3	f-x	6.2	a	31.8	r-F
BF08-1-60B	11.9	abc	13.3	a	6.6	a	35.0	j-D
BF08-16-14	10.4	abc	11.7	u-M	6.4	a	30.4	t-I
BF08-16-18E	9.6	abc	12.7	a-m	6.3	a	32.6	o-E
BF08-16-21	18.3	a	11.7	s-K	7.3	a	28.9	x-J
BF08-16-31	12.1	abc	11.6	w-N	6.2	a	33.0	n-E
BF08-16-36A	11.8	abc	12.7	a-l	5.9	a	32.8	o-E
BF08-16-36B	10.1	abc	12.4	b-s	6.0	a	32.5	o-E
BF08-16-52	13.1	abc	12.6	a-o	6.5	a	33.2	m-E
BF08-16-67A	9.5	abc	13.1	a-f	6.4	a	32.8	n-E
BF08-16-67B	10.4	abc	11.6	w-N	3.3	a	54.7	a
BF08-16-68	10.3	abc	11.9	m-F	6.2	a	47.7	a-f
BF08-16-76	13.3	abc	11.3	D-S	6.6	a	24.5	G-L
BF08-16-82B	14.4	abc	12.4	d-v	5.9	a	29.7	u-I
BF08-16-92	9.5	abc	11.2	F-T	4.7	a	31.8	r-F
BF08-1-77	16.6	abc	12.3	e-w	6.0	a	32.8	o-E
BF08-1-80	12.9	abc	12.9	a-h	6.7	a	34.6	k-D
BF08-26-162	12.0	abc	10.4	U-3	5.5	a	30.3	u-I
BF08-26-68B	18.7	a	9.8	2-5	5.0	a	26.2	E-L
BF08-26-69	10.3	abc	12.4	d-v	5.4	a	30.2	u-I
BF08-3-23A	10.3	abc	12.6	a-o	6.5	a	34.5	l-D
BF08-3-23B	14.2	abc	12.4	c-v	6.5	a	32.5	o-E
BF08-36-53	10.7	abc	12.4	c-t	5.5	a	31.6	s-H
BF08-7-112D	12.1	abc	12.5	a-r	5.5	a	39.4	f-s
BF08-7-114	9.6	abc	12.6	a-p	6.0	a	33.7	m-D
BF08-7-19A	14.2	abc	12.8	a-k	6.6	a	33.2	m-E
BF08-7-19B	15.1	abc	12.5	b-s	6.2	a	31.0	s-I
BF08-7-75	9.8	abc	12.3	g-x	5.8	a	31.4	s-I

BF08-7-80	10.3	abc	11.8	r-J	6.4	a	30.1	u-l
CAB2	10.3	abc	10.2	Y-3	5.5	a	29.8	u-l
CODLMV059	8.1	abc	10.9	N-Z	4.5	a	48.1	a-e
DEMAI	8.1	bc	9.2	6-8	5.4	a	24.9	G-L
G2333(B)	9.3	abc	9.7	3-6	6.0	a	24.7	G-L
G4-24A	5.6	c	11.4	C-R	3.8	a	43.3	c-j
G4-585	14.8	abc	10.8	P-1	6.6	a	26.3	E-L
GLP585	8.9	abc	10.2	Y-3	6.6	a	25.2	F-L
HM21-7	6.2	c	10.9	N-Z	4.0	a	35.6	j-C
ICYANA	9.1	abc	10.9	M-Y	5.4	a	42.5	c-l
IKINIMBA	9.9	abc	8.9	7-9.	4.7	a	26.5	E-L
KAB06F2-8-12	5.2	c	11.5	y-P	3.6	a	41.8	c-m
KAB06F2-8-27	17.9	ab	11.7	s-K	4.4	a	50.0	a-d
KALANGITI	7.4	c	9.3	5-7	5.0	a	22.3	J-L
KENYA UMOJA	5.5	c	11.0	K-X	3.1	a	40.5	e-q
KMA13-03-35	9.1	abc	11.9	n-G	5.8	a	29.3	v-l
KMA13-05-21	8.5	abc	11.4	B-R	5.8	a	32.2	p-F
KMA13-10-05	13.8	abc	11.3	D-S	5.5	a	25.8	E-L
KMA13-10-18	16.1	abc	9.1	6-9	5.4	a	26.6	D-L
KMA13-13-70	12.6	abc	11.1	G-V	6.1	a	33.2	m-E
KMA13-19-33	8.8	abc	11.6	x-O	5.6	a	33.9	l-D
KMA13-21-19	6.7	c	10.6	S-2	5.5	a	28.5	z-K
KMA13-22-19	10.2	abc	11.0	J-W	5.5	a	34.1	l-D
KMA13-22-27	15.2	abc	11.9	o-G	4.6	a	35.7	j-C
KMA13-23-21	15.6	abc	11.7	u-M	7.9	a	32.6	o-E
KMA13-23-22	5.7	c	10.3	W-3	3.8	a	34.1	l-D
KMA13-28-21	10.1	abc	11.0	I-V	5.3	a	36.4	h-B
KMA13-28-5	9.4	abc	10.4	V-3	7.2	a	25.2	F-L
KMA13-32-28	15.0	abc	10.2	Y-3	5.3	a	29.6	v-l
MAC49	8.2	abc	11.7	s-K	4.6	a	50.3	a-c
MAC74	6.5	c	11.8	q-I	4.7	a	43.2	c-k
MAFUTALA	11.5	abc	9.5	4-6	5.4	a	25.6	F-L
MC20	8.1	abc	10.4	T-3	4.4	a	37.8	h-w
MC29A	8.1	abc	11.0	L-Y	5.5	a	35.8	j-C
MCB23	12.5	abc	12.2	h-A	6.3	a	33.2	m-E
MEX54	6.8	c	9.5	4-7	4.0	a	37.6	h-w
MIB465	6.6	c	8.3	.+-*	5.7	a	22.5	I-L
MV1	10.2	abc	12.4	c-u	5.7	a	32.0	q-F
MV-14	16.3	abc	12.1	h-B	6.1	a	32.4	p-F
NAIN DE KYONDO	11.3	abc	10.4	U-3	4.9	a	25.0	F-L
NAMULENGA	11.6	abc	10.0	1-4	4.5	a	36.5	h-B
NDIMIRAKAKUJA	11.2	abc	8.9	7-9.+	4.7	a	28.4	A-K
NGWINxCAB2 21311	7.0	c	10.2	Z-3	3.9	a	40.7	e-p
OBUSOSERA	9.9	abc	9.0	7-9.	4.8	a	26.6	E-L
RK11	10.1	abc	10.9	N-Z	3.8	a	37.3	h-y
RK12A	13.4	abc	11.0	K-X	5.3	a	36.4	h-B
RK12B	5.0	c	10.8	P-2	3.5	a	38.0	h-v
RK14	6.6	c	10.6	R-1	4.0	a	41.1	e-o

RK6	6.3	c	10.7	Q-1	3.5	a	35.9	j-C
RUGANDURA	9.9	abc	9.1	6-9.	6.5	a	31.6	s-H
RW1180	4.8	c	10.8	O-Z	3.6	a	40.4	e-r
RW267	7.5	bc	8.9	7-9.	4.7	a	23.0	H-L
RW298	10.4	abc	9.8	2-5	3.5	a	52.7	ab
RW3006	9.9	abc	12.1	j-C	4.8	a	50.0	a-d
RW439	5.6	c	10.2	Z-3	4.9	a	27.9	B-L
RW547	6.9	c	9.0	6-9.	5.0	a	19.4	L
RW582	10.5	abc	11.1	G-U	5.0	a	36.9	h-A
RW693	7.6	bc	8.2	+-*	4.6	a	21.6	J-L
RW744	7.8	bc	7.9	-*	4.2	a	29.2	w-I
RW805	9.2	abc	7.8	*	4.7	a	35.6	j-C
RW849	6.8	c	9.1	6-9	5.1	a	26.5	E-L
RW880	6.7	c	8.1	+-*	4.8	a	23.5	G-L
RW896	6.7	c	9.1	6-9.	5.5	a	20.2	J-L
RW942	8.4	abc	10.0	1-4	5.4	a	20.1	KL
RWR1180	7.8	bc	10.4	T-3	3.5	a	32.8	n-E
RWR2154	7.1	c	8.6	9.+-*	3.5	a	47.4	a-f
RWR2245	8.2	abc	10.1	Z-3	3.4	a	38.3	g-u
RWV2359	8.7	abc	11.3	E-S	4.5	a	44.9	b-h
RWV2887	13.8	abc	11.8	q-H	4.4	a	46.6	a-g
SER12	8.8	abc	11.5	z-Q	6.4	a	31.9	q-F
SR6	7.6	bc	12.3	h-x	5.1	a	31.4	s-I
SR9	15.7	abc	10.2	Y-3	5.5	a	32.7	o-E
UBR(92)25	9.0	abc	8.7	7-9.+	4.9	a	29.8	u-I
UGK116	11.3	abc	11.4	A-Q	5.5	a	28.7	y-K
UGK72	9.0	abc	10.8	P-Z	3.9	a	41.5	d-n
ZKA93-10m/95	6.7	c	9.0	6-9.	3.8	a	41.1	e-o

Appendix 5 : Yield, Iron (Fe) and zinc (Zn) levels of 160 biofortified genotypes and five local common bean varieties in DRC

Genotypes	Yield (kg ha^{-1})	Groups	Fe (ppm)	Groups	Zn (ppm)	Groups
BCB11-138	917.8	fgh	103.1	defghijklmnopqrstu	42.6	abcdefghi
BCB11-303	952.4	efgh	90.5	ijklmnopqrstuvwxyzABCDEFGHI	41.5	abcdefghij
BCB11-342	2308.0	bcd	101.1	defghijklmnopqrstuv	50.6	abcdef
BCB11-372	1147.8	defgh	77.9	stuvwxyzABCDEFGHIJK	42.8	abcdefghi
BCB11-433	1253.2	cdefgh	77.3	tuvwxyzABCDEFGHIJK	52.1	abcde
BCB11-492	2096.7	bcde	91.1	ijklmnopqrstuvwxyzABCDEFG	45.4	abcdefghi
BCB11-509	591.8	h	74.5	uvwxyzABCDEFGHIJK	50.3	abcdef
BF08-01-01	1578.2	bcdefgh	91.6	ijklmnopqrstuvwxyzABCDEFG	37.6	defghij
BF08-01-18A	2186.2	bcde	71.4	uvwxyzABCDEFGHIJK	48.0	abcdefg
BF08-01-18B	1400.6	cdefgh	92.3	hijklmnopqrstuvwxyzABCDEFG	44.1	abcdefghi
BF08-01-21	2037.3	bcdef	72.6	uvwxyzABCDEFGHIJK	46.3	abcdefghi
BF08-01-45A	1785.3	bcdefgh	94.1	fghijklmnopqrstuvwxyzABCD	35.5	efghij
BF08-01-45B	1618.3	bcdefgh	131.2	bcdefgh	43.5	abcdefghi
BF08-01-47B	2818.3	b	93.2	hijklmnopqrstuvwxyzABCDE	41.0	abcdefghij
BF08-01-49A	1799.6	bcdefgh	66.1	vwxyzABCDEFGHIJK	31.5	fghij
BF08-01-50	2001.2	bcdef	71.1	vwxyzABCDEFGHIJK	19.1	j
BF08-01-54	2186.1	bcde	52.0	HIJK	37.8	defghij
BF08-01-62A	1424.7	cdefgh	63.7	vwxyzABCDEFGHIJK	30.8	fghij
BF08-01-62B	1621.3	bcdefgh	101.2	defghijklmnopqrstuv	48.1	abcdefg
BF08-01-90	971.4	efgh	78.8	rstuvwxyzABCDEFGHIJK	42.5	abcdefghi
BF08-01-92	1689.9	bcdefgh	103.8	defghijklmnopqrstu	46.8	abcdefghi
BF08-03-05	1843.2	bcdefg	69.6	vwxyzABCDEFGHIJK	40.8	abcdefghij
BF08-03-12	1587.1	bcdefgh	125.2	bcdefghijkl	42.0	abcdefghij
BF08-03-12B	919.9	fgh	87.5	lmnopqrstuvwxyzABCDEFGHIJ	37.9	defghij
BF08-03-13	2546.8	bcd	121.0	bcdefghijklmno	44.5	abcdefghi
BF08-03-13B	2051.0	bcdef	83.9	nopqrstuvwxyzABCDEFGHIJ	39.9	bcdefghij
BF08-03-20	1486.7	cdefgh	68.4	vwxyzABCDEFGHIJK	33.0	efghij
BF08-03-22	1217.2	cdefgh	76.9	uvwxyzABCDEFGHIJK	50.1	abcdef
BF08-07-112D	1546.4	bcdefgh	146.7	ab	50.5	abcdef
BF08-07-116	1664.7	bcdefgh	101.0	defghijklmnopqrstuv	46.6	abcdefghi
BF08-07-22	2265.6	bcde	148.2	ab	63.0	ab
BF08-07-74A	1811.2	bcdefgh	144.1	ab	45.8	abcdefghi
BF08-07-74B	2315.1	bcd	99.2	defghijklmnopqrstuvw	50.5	abcdef
BF08-07-74C	1773.8	bcdefgh	95.3	defghijklmnopqrstuvwxyzABC	37.7	defghij
BF08-1-18C	1396.2	cdefgh	82.8	opqrstuvwxyzABCDEFGHIJ	45.4	abcdefghi
BF08-1-27	2167.2	bcde	74.9	uvwxyzABCDEFGHIJK	35.9	defghij
BF08-1-29	2023.4	bcdef	102.9	defghijklmnopqrstu	36.9	defghij
BF08-1-30	1803.6	bcdefgh	89.7	ijklmnopqrstuvwxyzABCDEFGHI	41.7	abcdefghij
BF08-13-102	1589.9	bcdefgh	80.1	qrstuvwxyzABCDEFGHIJK	42.2	abcdefghij
BF08-13-170	1181.0	defgh	172.0	a	55.0	abcd
BF08-13-18D	1517.6	bcdefgh	109.7	cdefghijklmnopqrs	46.5	abcdefghi
BF08-13-38	2304.0	bcd	100.1	defghijklmnopqrstuvw	53.0	abcde
BF08-13-43A	1839.3	bcdefg	63.2	vwxyzABCDEFGHIJK	36.9	defghij
BF08-13-43B	1540.6	bcdefgh	93.3	hijklmnopqrstuvwxyzABCDE	41.6	abcdefghij
BF08-13-44	2620.7	bc	86.4	lmnopqrstuvwxyzABCDEFGHIJ	37.5	defghij
BF08-13-44B	1020.2	efgh	58.0	ABCDEFGHIJK	36.8	defghij
BF08-13-47	1164.7	defgh	88.2	klmnopqrstuvwxyzABCDEFGHIJ	57.6	abc
BF08-14-102	1523.0	bcdefgh	59.2	yzABCDEFGHIJK	34.8	efghij
BF08-14-116	1524.5	bcdefgh	89.0	jklmnopqrstuvwxyzABCDEFGHI	39.4	cdefghij

BF08-14-153A	2189.8	bcde	119.7	bcdefghijklmnop	52.8	abcde
BF08-14-153B	1666.8	bcdefgh	140.1	abc	54.5	abcd
BF08-14-20	1744.5	bcdefgh	104.9	defghijklmnopqrstu	43.6	abcdefghi
BF08-14-24	1940.1	bcdef	91.2	ijklmnopqrstuvwxyzABCDEFG	33.6	efghij
BF08-14-51A	1891.6	bcdefg	90.3	ijklmnopqrstuvwxyzABCDEFGHI	47.1	abcdefghi
BF08-14-51B	1931.5	bcdef	51.5	IJK	34.4	efghij
BF08-14-51C	1864.7	bcdefg	84.9	mnopqrstuvwxyzABCDEFGHIJ	37.4	defghij
BF08-1-47A	1957.7	bcdef	128.1	bcdefghij	38.5	defghij
BF08-14-96A	1468.8	cdefgh	53.2	GHIJK	24.5	hij
BF08-14-96B	2190.9	bcde	57.9	ABCDEFGHIJK	28.9	fghij
BF08-1-49B	2106.9	bcde	56.2	CDEFGHIJK	37.9	defghij
BF08-1-51	1164.5	defgh	117.1	bcdefghijklmnopqr	46.5	abcdefghi
BF08-1-60	2055.2	bcdef	92.7	hijklmnopqrstuvwxyzABCDEF	55.7	abcd
BF08-1-60B	1348.1	cdefgh	99.3	defghijklmnopqrstuvw	47.5	abcdefgh
BF08-16-14	1224.1	cdefgh	63.0	vwxyzABCDEFGHIJK	33.1	efghij
BF08-16-18E	1617.5	bcdefgh	128.3	bcdefghi	44.4	abcdefghi
BF08-16-21	2451.3	bcd	59.9	xyzABCDEFGHIJK	34.6	efghij
BF08-16-31	1835.8	bcdefgh	111.4	cdefghijklmnopqrs	60.1	abc
BF08-16-36A	1972.3	bcdef	91.6	ijklmnopqrstuvwxyzABCDEFG	39.1	cdefghij
BF08-16-36B	2208.1	bcde	105.4	defghijklmnopqrstu	44.7	abcdefghi
BF08-16-52	1438.4	cdefgh	113.7	bcdefghijklmnopqrs	36.4	defghij
BF08-16-67A	1591.0	bcdefgh	122.1	bcdefghijklmn	29.3	fghij
BF08-16-67B	2439.6	bcd	98.1	defghijklmnopqrstuvwxy	45.7	abcdefghi
BF08-16-68	1327.5	cdefgh	103.5	defghijklmnopqrstu	39.3	cdefghij
BF08-16-76	1323.2	cdefgh	108.0	cdefghijklmnopqrstu	48.8	abcdef
BF08-16-82B	2109.4	bcde	118.8	bcdefghijklmnopq	50.7	abcdef
BF08-16-92	1313.0	cdefgh	142.4	abc	57.4	abc
BF08-1-77	2335.9	bcd	109.1	cdefghijklmnopqrs	42.8	abcdefghi
BF08-1-80	1883.9	bcdefg	88.3	klmnopqrstuvwxyzABCDEFGHIJ	44.3	abcdefghi
BF08-26-162	1311.0	cdefgh	145.8	ab	58.4	abc
BF08-26-68B	2140.8	bcde	57.7	BCDEFGHIJK	30.9	fghij
BF08-26-69	1591.5	bcdefgh	86.5	lmnopqrstuvwxyzABCDEFGHIJ	48.0	abcdefg
BF08-3-23A	1421.8	cdefgh	95.7	defghijklmnopqrstuvwxyzAB	40.3	abcdefghij
BF08-3-23B	2036.5	bcdef	134.3	abcd	47.1	abcdefghi
BF08-36-53	1949.3	bcdef	104.9	defghijklmnopqrstu	47.1	abcdefghi
BF08-7-112D	2289.0	bcd	71.0	vwxyzABCDEFGHIJK	33.9	efghij
BF08-7-114	1772.2	bcdefgh	80.6	pqrstuvwxyzABCDEFGHIJK	36.2	defghij
BF08-7-19A	1966.2	bcdef	76.2	uvwxyzABCDEFGHIJK	45.8	abcdefghi
BF08-7-19B	1916.4	bcdef	95.4	defghijklmnopqrstuvwxyzAB	48.0	abcdefg
BF08-7-75	2136.1	bcde	126.7	bcdefghijk	45.9	abcdefghi
BF08-7-80	2326.8	bcd	87.1	lmnopqrstuvwxyzABCDEFGHIJ	47.5	abcdefgh
CAB2	1467.0	cdefgh	101.6	defghijklmnopqrstuv	54.3	abcd
CODLMV059	1381.4	cdefgh	101.6	defghijklmnopqrstuv	49.3	abcdef
DEMAI	765.4	gh	112.7	cdefghijklmnopqrs	37.6	defghij
G2333(B)	1173.7	defgh	80.6	pqrstuvwxyzABCDEFGHIJK	47.1	abcdefgh
G4-24A	4699.7	a	133.7	abcde	52.3	abcde
G4-585	1680.6	bcdefgh	141.4	abc	55.2	abcd
GLP585	1167.9	defgh	61.0	wxyzABCDEFGHIJK	36.6	defghij
HM21-7	1025.6	efgh	53.8	FGHIJK	34.3	efghij
ICYANA	1776.9	bcdefgh	104.8	defghijklmnopqrstu	42.7	abcdefghi
IKINIMBA	1147.4	efgh	77.6	tuvwxyzABCDEFGHIJK	37.9	defghij
KAB06F2-8-12	990.3	efgh	105.1	defghijklmnopqrstu	48.3	abcdef
KAB06F2-8-27	2039.3	bcdef	109.1	cdefghijklmnopqrs	37.7	defghij
KALANGITI	703.6	h	71.2	vwxyzABCDEFGHIJK	35.7	efghij
KENYA UMOJA	1390.1	cdefgh	94.9	efghijklmnopqrstuvwxyzABCD	35.0	efghij

KMA13-03-35	1184.3	defgh	107.5	cdefghijklmnopqrstu	38.3	defghij
KMA13-05-21	1432.7	cdefgh	59.5	yzABCDEFGHIJK	45.1	abcdefghi
KMA13-10-05	1360.3	cdefgh	43.3	K	32.9	efghij
KMA13-10-18	1759.2	bcdefgh	63.4	vwxyzABCDEFGHIJK	31.4	fghij
KMA13-13-70	1824.4	bcdefgh	93.8	ghijklmnopqrstuvwxyzABCDE	35.8	defghij
KMA13-19-33	2170.7	bcde	68.6	vwxyzABCDEFGHIJK	24.7	ghij
KMA13-21-19	1441.7	cdefgh	54.9	EFGHIJK	36.0	defghij
KMA13-22-19	1278.5	cdefgh	88.3	klmnopqrstuvwxyzABCDEFGHIJ	35.9	defghij
KMA13-22-27	1256.7	cdefgh	106.4	cdefghijklmnopqrstu	43.5	abcdefghi
KMA13-23-21	2304.4	bcd	89.3	ijklmnopqrstuvwxyzABCDEFGHI	27.7	fghij
KMA13-23-22	1151.8	defgh	115.9	bcdefghijklmnopqrs	48.9	abcdef
KMA13-28-21	1763.9	bcdefgh	86.7	lmnopqrstuvwxyzABCDEFGHIJ	37.6	defghij
KMA13-28-5	1820.6	bcdefgh	114.4	bcdefghijklmnopqrs	49.9	abcdef
KMA13-32-28	2225.6	bcde	76.0	uvwxyzABCDEFGHIJK	36.1	defghij
MAC49	2532.3	bcd	85.8	mnopqrstuvwxyzABCDEFGHIJ	35.8	defghij
MAC74	1578.4	bcdefgh	103.8	defghijklmnopqrstu	46.2	abcdefghi
MAFUTALA	1513.2	cdefgh	105.7	defghijklmnopqrstu	38.7	defghij
MC20	1033.3	efgh	93.0	hijklmnopqrstuvwxyzABCDE	50.2	abcdef
MC29A	1701.8	bcdefgh	107.0	cdefghijklmnopqrstu	51.3	abcde
MCB23	2185.4	bcde	84.1	nopqrstuvwxyzABCDEFGHIJ	51.8	abcde
MEX54	874.4	fgh	110.8	cdefghijklmnopqrs	33.8	efghij
MIB465	683.4	h	98.7	defghijklmnopqrstuvwx	47.6	abcdefgh
MV1	1422.2	cdefgh	77.8	stuvwxyzABCDEFGHIJK	42.7	abcdefghi
MV-14	2062.2	bcdef	71.2	uvwxyzABCDEFGHIJK	32.7	efghij
NAIN DE KYONDO	1583.5	bcdefgh	96.8	defghijklmnopqrstuvwxyzA	40.4	abcdefghij
NAMULENGA	1597.1	bcdefgh	120.9	bcdefghijklmno	37.1	defghij
NDIMIRAKAKUJA	1269.5	cdefgh	97.7	defghijklmnopqrstuvwxy	36.5	defghij
NGWINxCAB2 21311	2001.3	bcdef	108.9	cdefghijklmnopqrs	48.9	abcdef
OBUSOSERA	948.2	fgh	77.3	uvwxyzABCDEFGHIJK	32.3	fghij
RK11	1691.0	bcdefgh	148.4	ab	63.4	a
RK12A	1964.8	bcdef	108.3	cdefghijklmnopqrst	47.4	abcdefgh
RK12B	1120.9	efgh	132.4	bcdefg	57.3	abc
RK14	1372.4	cdefgh	49.6	JK	39.9	bcdefghij
RK6	1157.7	defgh	113.5	bcdefghijklmnopqrs	51.5	abcde
RUGANDURA	1328.0	cdefgh	66.4	vwxyzABCDEFGHIJK	44.0	abcdefghi
RW1180	1077.8	efgh	56.7	BCDEFGHIJK	36.4	defghij
RW267	668.0	h	114.5	bcdefghijklmnopqrs	42.1	abcdefghij
RW298	2455.2	bcd	74.9	uvwxyzABCDEFGHIJK	38.5	defghij
RW3006	1590.7	bcdefgh	92.4	hijklmnopqrstuvwxyzABCDEF	43.8	abcdefghi
RW439	1224.5	cdefgh	101.7	defghijklmnopqrstuv	50.4	abcdef
RW547	712.9	gh	89.1	jklmnopqrstuvwxyzABCDEFGHI	38.5	defghij
RW582	1199.1	cdefgh	83.7	nopqrstuvwxyzABCDEFGHIJ	48.3	abcdef
RW693	1073.5	efgh	71.6	uvwxyzABCDEFGHIJK	32.8	efghij
RW744	876.3	fgh	93.7	ghijklmnopqrstuvwxyzABCDE	31.0	fghij
RW805	1163.7	defgh	83.1	nopqrstuvwxyzABCDEFGHIJ	40.6	abcdefghij
RW849	1447.8	cdefgh	94.4	fghijklmnopqrstuvwxyzABCD	34.6	efghij
RW880	1002.4	efgh	86.1	mnopqrstuvwxyzABCDEFGHIJ	35.7	efghij
RW896	1045.3	efgh	76.7	uvwxyzABCDEFGHIJK	30.6	fghij
RW942	908.2	fgh	97.4	defghijklmnopqrstuvwxyz	36.9	defghij
RWR1180	773.5	fgh	78.0	rstuvwxyzABCDEFGHIJK	38.3	defghij
RWR2154	1225.8	cdefgh	78.8	rstuvwxyzABCDEFGHIJK	50.7	abcdef
RWR2245	929.1	fgh	68.5	vwxyzABCDEFGHIJK	23.7	ij
RWV2359	2690.9	b	63.0	vwxyzABCDEFGHIJK	29.5	fghij
RWV2887	2331.0	bcd	133.2	abcdef	47.1	abcdefghi
SER12	1778.9	bcdefgh	121.6	bcdefghijklmno	45.7	abcdefghi

SR6	973.7	efgh	132.8	bcdefg	38.9	cdefghij
SR9	1536.6	bcdefgh	90.7	ijklmnopqrstuvwxyzABCDEFGH	37.3	defghij
UBR(92)25	1149.6	defgh	58.4	zABCDEFGHIJK	38.3	defghij
UGK116	1578.6	bcdefgh	90.9	ijklmnopqrstuvwxyzABCDEFGH	43.8	abcdefghi
UGK72	1704.7	bcdefgh	56.0	DEFGHIJK	34.1	efghij
ZKA93-10m/95	874.3	fgh	123.3	bcdefghijklm	48.8	abcdef