DIVERSITY OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) VARIETIES IN IRON AND ZINC CONTENTS FROM COLLECTIONS IN MAJOR BEAN GROWING AREAS OF TANZANIA

BY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2008

ABSTRACT

Two experiments were conducted to determine the diversity of common bean *(Phaseolus vulgaris* L.) varieties in Fe and Zn contents. In total, 90 varieties were collected from major bean growing Regions of Tanzania. Twenty varieties were randomly selected for field experiment, while all 90 varieties were used for screen house experiment at SUA. In the screen house experiment, a Completely Randomized Design with three replications was used. The field experiment was conducted at SUA-Morogoro and Madiira-Arusha. A randomized complete block design with 20 varieties replicated three times. Seeds and leaves from both experiments were collected, dried, ground and the powder was used for Fe and Zn determination. Variation in Fe and Zn contents was observed among varieties in both seeds and leaves, and the best varieties were identified. Results have shown a positive and highly significant ($P \leq 0.001$) correlation between leaves and seeds Fe and Zn, suggesting that genetic factors for increasing Fe are co-segregating with genetic factors for increasing Zn. Levels of Fe and Zn concentration among varieties at two sites was very different emphasizing the effect of both the environment and genotype. However, Leaves of the studied varieties have shown to have above average Fe and Zn contents thus forming a good source of the micronutrients in areas where bean leaves are consumed as vegetables. From this study, it is recommended that varieties that were found having high amount of Fe and Zn be used as a gene source in future breeding work, meanwhile, farmers should use those varieties for consumption and production.

DECLARATION

I, **GEORGE MUHAMBA TRYPHONE**, do hereby declare to neither the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor concurrently being submitted for a degree award in any other University.

..... 25. 01. 2008 .....

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The above declaration is confirmed

..... 25/01/2008 .....

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ACKNOWLEDGMENT

First and foremost this dissertation owes much to my supervisor, Prof. Susan Nchimbi-Msolla of the Department Crop Science and Production, Sokoine University of Agriculture (SUA) for her generous support, guidance and valuable assistance during this study. I thank The Rockefeller Foundation for sponsoring my studies. Heartfelt appreciations are due to Prof. Amon P. Maerere for his positive plans in providing scholarship on behalf of The Rockefeller Foundation for my postgraduate studies. God's glory be onto you always! I also acknowledge SUA Bean Project for supplementing research funds.

Many thanks to the Uyole and Selian Agricultural Research Institutes and SUA Bean Project for providing me with some of the seeds which I used in my research. In addition, the assistance provided by ARI-Selian and SUA, where research was conducted especially Mr. Kisamu at ARI-Selian for assisting in managing the field experiment at Madiira-Arusha. Mr. Marekera, G.P. for assistance in laboratory work.

I feel indebted to my mom and all members of my family for their prayers, encouragement and necessary support. I can't forget to register my heartfelt thanks to Dr. Winnie P. Rwamugira for her close guidance and encouragement. I am also grateful to all members of staff and postgraduate students in the Department of Crop Science and Production SUA, who assisted me in one way or another, both morally and technically during the writing of this dissertation. I will always appreciate their support.

DEDICATION

To Almighty God

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CABI	Commonwealth Agricultural Bureau International
CEC	Cation exchange capacity
CIAT	Centro Internacional de Agricultura Tropical (Internacional Centre for Tropical Agriculture)
CuZnSOD	Copper-zinc superoxide dismutase
CV	Coefficient of variation
DAP	Days after planting
DAS	Days after sowing
DMRT	Duncan's multiple range test
DNA	Deoxyribose nucleic acid
FAO	Food and Agriculture Organisation
Fe	Iron
NS	Not significant
OC	Organic carbon
p.p.m	Parts per million
RNA	Ribonucleic acid
SA	Sulphate of ammonia
SE	Standard error of the mean
SUA	Sokoine University of Agriculture
TSP	Triple super-phosphate
USDA	United States Department of Agriculture
Zn	Zinc

CHAPTER ONE

1.0 INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a crop of considerable importance in the world as a grain legume and as a vegetable. It is one of the most principal food and cash crop legumes grown in the tropical world and most of the production takes place in developing countries (Hillocks *et al.*, 2006). It is an interesting crop from the point of view of the consumer, farmer and processor. For the consumer, bean is important for its good nutritive composition. It is a source of calories, proteins, dietary fibers, minerals and vitamins (Shree, 1999; Hillocks *et al.*, 2006). For a farmer, the crop contributes nitrogen to the soil, while dry seed and fresh pods attract a high market price. For the processor, common bean has many possibilities such as canned or frozen grain and pod. It complements cereals and other carbohydrate rich foods in providing near perfect nutrition to people of all ages, and in addition a regular intake of bean helps to lower cholesterol and cancer risks (Shree, 1999).

Increasing the amount of micronutrients in plant foods for human consumption is a challenge that is particularly important in developing countries. Cereal grains and some legumes are the primary and least expensive source of Ca, Fe, and Zn for the population in these countries; however, intakes do not satisfy their mineral requirements (Welch and Graham, 1999). Common bean is also an important source of dietary minerals with the potential to provide all 15 of the essential minerals required by man. However, the concentrations will vary in response to both genetic and environmental factors (Grusak, 2002). The concentrations of certain minerals

especially Fe, Zn, and Ca are low relative to animal food products (Anderson and Allen, 1994).

Recent reports indicate that Fe deficiency is the most prevalent micronutrient problem in the world affecting over 2 billion people, many of whom depend on beans as their staple food (Welch *et al.*, 1999). Forty percent of Fe intake in developing countries is derived from legumes and cereals (Rosado *et al.*, 2007). Food legumes in general contain appreciable quantities of iron and other minerals (Beebe *et al.*, 1999; Grusak, 2002). Although legumes are often cited as a complement to cereals in terms of amino acid content, they also make a particularly important contribution to micronutrient nutrition (Beebe *et al.*, 1999).

An underlying cause of and fundamental constraints on solution of the micronutrient problem is that non-staple foods, particularly animal products, tend to be the foods richest in bioavailable micronutrients of which the poor in the developing countries desire to eat, but cannot afford. Their diets consist mostly of staple foods, primarily cereals and legumes; in fact, per capita direct consumption of staple foods in the aggregate varies little according to income level. For the poor, these staple foods are already sources of what micronutrients they are able to consume, particularly minerals (Bouis *et al.*, 2000). In common bean some nutritional traits such as proteins and mineral contents are lower in the cultivated forms as compared to the wild counterparts. Selection for yield and other agronomic traits such as resistance to biotic and abiotic stresses, upright plant architecture, growth habits, lodging resistance, and maturity has been extensively utilized by bean breeders to develop

cultivars with superior performance or to develop cultivars that are adapted to specific environments and /or cropping systems (Tar`an *et al.*, 2002). Up to date, seed mineral content is not a specific selection criterion for plant breeding, although genetic variation for this trait is present in the available germplasm collections (Vreugdenhil *et al.*, 2004).

Genetic diversity for seed mineral concentration has been studied in several legumes, although usually involving the characterization of only a limited number of genotypes. In general, analysis of field grown material has demonstrated comparable ranges of mineral concentrations among seeds of most legume species (Welch and Graham, 2002; Wang *et al.*, 2003). Studies have identified Zn deficiencies in children who consume diets high in cereals (Ranum, 1999), therefore, identifying cultivars with higher amount of Fe and Zn may contribute significantly to the improvement of the micronutrient status of people depending on common bean as major component of their diet. The main objective of the study was to evaluate the genetic variability of iron and zinc concentrations in common bean varieties and bean lines grown in major bean growing areas in Tanzania.

The specific objectives were:

(i) To determine and compare Fe and Zn concentration in seeds and leaves of different bean varieties/bean lines grown in two major bean growing areas in Tanzania.

(ii) To determine the stability of iron and zinc with respect to G x E interactions.

(iii) To determine the influence of seed size and seed yield on the levels of Fe and Zn in seeds of common bean.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Common bean (*Phaseolus vulgaris* L.)

Common bean (*Phaseolus vulgaris* L.) is a leguminous crop, which belongs to the sub-family Papilionoideae of the family Fabaceae (Debouck, 1991). The plant is an annual herb, erect and bushy, 20-60 cm tall or twining with stems 2-3 m long (Duke, 1983).

2.1.1 Botanical characteristics

Most beans are herbaceous annuals, although under tropical conditions some beans may behave as short lived perennials. They may be of determinate or indeterminate growth habit, with pinnately compound trifoliolate leaves. The common bean flower has an elongated twisted keel containing the style and ten stamens.

Beans show a high variation in growth habit which appears to be continuous from determinate bush to indeterminate, extreme climbing types although for simplicity , Singh (1999) classified the bean growth habits into four major classes (type I = determinate upright or bush; type II = indeterminate upright bush; type III = indeterminate prostrate, non climbing and type IV = indeterminate strong climbers) and suggesting a key for their identification based on the type of terminal bud , stem strength, climbing ability and fruiting patterns. Growth and development of common bean is divided into vegetative and reproductive stages. The vegetative stages are defined on the basis of pod and seed characteristics in addition to nodes. Common bean has an epigeal germination which is complete in 7-8 days after planting (DAP).

Its flowering may be initiated as early as ten DAP, although it usually begins between 28 and 40 DAP.

2.1.2 Origin of common bean

The common bean was being domesticated more than 7000 years ago in the two centres of origin-Mesoamerica (Mexico and Central America) and the Andean region but is now cultivated worldwide in diverse environments. In Africa, common beans were introduced some 400 years ago (Leakey, 1970). Scientists believe that dry beans along with maize, squash and amaranth, probably began as weeds in the fields planted to cassava and sweet potatoes in Central America. Over the millennia, farmers grew complex mixtures of bean types as a hedge against drought, disease and pest attacks. This process has produced an almost limitless genetic array of beans with a wide variety of colours, textures and sizes to meet the growing conditions and taste preferences of many different regions (Welch and Graham, 2004).

The genus *Phaseolus* is of American origin and comprises over 30 species (Debouck, 1991). Five of them, namely, *P. acutifolious*, A.(gray or tepary bean) *P. coccineus* L. (runner or scarley bean), *P. lunatus* L. (lima, butter or Madagascar bean), *P. polyanthus* Greenman (year-long bean) and *P. vulgaris* L. (common bean, haricot, navy, french or snap bean) were domesticated (Debouck, 1999; Broughton *et al.*, 2003). Among these species the common bean is the most widely distributed and has the broadest range of genetic resources (Singh, 2001). It is mostly used as food crop throughout the world, especially in Latin America and Africa. Portuguese traders

introduced dry beans in sub-Saharan Africa several centuries ago. Today, the crop is a vital staple in this continent, providing the main source of dietary protein for more than 70 million people. Dry beans are raised mostly by women for subsistence and the market on more than 3.5 million hectares, accounting for a quarter of global output (CIAT, 2001). Production is concentrated in densely populated eastern Africa, the Lake Region and highlands of southern Africa (Beebe *et al.*, 1999), although about 40% of the total production from Africa is marketed at an average annual value of USD 452 million (Hillocks *et al.*, 2006).

2.1.3 Importance of common bean

Among major food legumes, the common bean is the third most important worldwide, surpassed only by soybean [*Glycine max* (L.) Merr.] and peanut (*Arachis hypogea* L.) (Singh, 1999). Common bean is the grain legume consumed in largest quantities in the world and is the one of the most important staple crops for small farmers and the urban poor in many Latin America and African countries (De Araunjo *et al.*, 2003). Due to their substantial iron content, common bean grains play an important role among foods (De Araunjo *et al.*, 2003). This type of bean is the main source of protein and provides the needed calories (up to 30% of the dietary energy), dietary fiber, folic acid (vitamin B), essential inorganic micronutrients (Fe, Zn, Ca, Mg, and Cu) flavones, antioxidants, and anticarcinogenic compounds to the consumers (Moraghan and Grafton, 2003). It is also the main supplier of all the iron the human organism requires for its metabolism (Fairbanks, 1999) and meets 25 percent of the daily requirements of magnesium and copper as well as 15 percent of the potassium and zinc (Beebe *et al.*, 1999). In addition, common beans have an

important market niche in urban areas among the millions of rural poor that have migrated to the cities but still consume their familiar staples, bean fetches good price and generate income to farmers. Common bean can also be produced as a vegetable and as such is one of the world's most horticultural crops (Bouis *et al.*, 2000).

2.2 Organization of bean diversity

The bean genetic diversity is large. There are about 65 000 accessions of *Phaseolus* beans in major germplasm banks, of which more than 90% are *Phaseolus vulgaris*. The Centro Internacional de Agricultural Tropical (CIAT) collections, the largest in the world, includes over 40 000 entries of which 26 500 are cultivated *P. vulgaris*, about 1 300 are wild types of common bean and the rest are distant relatives of the common bean (CIAT, 2001). Intraspecific organization of genetic variation in *P. vulgaris* has been well studied. Two major gene pools, namely, the Mesoamerican and the Andean South American were the first to be organized. The evidence for the existence of these two gene pools was supported by phaseolin seed proteins (Gepts and Bliss, 1986), allozymes, morphological traits (Singh *et al.*, 1991) and DNA markers (Becerra and Gepts, 1994). Later, a third gene pool in the northern Andes (Ecuador and northern Peru) was described (Debouck, 1999) and this one is now considered as the nucleus of bean diversity from which wild beans dispersed both northwards and southwards to form the two geographically distinct gene pools in Mesoamerica and Andean South America (Gepts, 1998). Singh *et al.* (1991) further divided the Mesoamerican and Andean South American gene pools of cultivated beans into six races (three within each centre), which represent the different cultivated forms that developed as a consequence of domestication. Each race has its

own characteristics, ecological adaptation and agronomic traits (Beebe *et al.*, 2000). Cultivated forms of common bean also called landraces are often highly variable in appearance but they can be identified and usually have local names (Zeven, 1998). The genetic diversity of landraces is thought to be the most valuable part of global diversity and is considered of paramount importance for future world production. As reservoirs of useful genetic diversity landraces need to be conserved either *in situ* or *ex situ* (Gomez, 2004).

2.3 Importance of iron and zinc in human diets

2.3.1 Iron

More than any other metal, iron is a key element in the metabolism of all living organisms. The iron-sulfur complex of ferredoxins is required for an early step of photosynthesis. The iron-sulfur complex of aconitase in the tricarboxylic acid cycle intimately links the iron content of cells with energy production via oxidative phosphorylation both carbohydrate and in lipid metabolism (Fairbanks, 1999).

Iron keeps immune system healthy and helps the body to produce energy, it is found in every human cell, primarily linked with protein to form the oxygen carrying molecule hemoglobin. The human body contains approximately 4 grams of iron (Graham *et al.*, 2001). Dietary iron comes in two forms: heme iron and non-heme iron. Heme iron is found only in animal flesh, as it is derived from the hemoglobin and myoglobin in animal tissues. Non heme iron is found in plant foods and dairy products. Iron is the part of heme, which is the active site of electron transport in

cytochromes and cytochrome oxidase. Heme is also the site of oxygen uptake by myoglobin and hemoglobin, thereby providing the means of transporting oxygen to tissues and within muscle cells (Fairbanks, 1999).

Haemoglobin in the root nodules of legumes protects nitrogen-fixing enzymes of symbiotic bacteria from oxidative inactivation. The ammonia formed is important in the synthesis of amino acids and proteins. The amino acids and proteins of legumes are transferred through the food chain to herbivores and hence to human (Fairbanks, 1999).

The ability of red blood cells to carry oxygen is attributed to the presence of iron in the hemoglobin. If human lacks iron, will produce less hemoglobin and therefore supply less oxygen to the tissues. Fe deficiency anaemia and other micronutrient deficiencies affect over 3.5 billion people and due to generalized decrease in the quality of poor people's diets have actually increased over recent decades even in areas where food is not limiting (Blair *et al.*, 2002). Fe deficiency is the most important micronutrient deficiency in the world and a main cause of anaemia, a condition in which the blood contains low levels of red blood cells (FAO, 2006). Fe deficiency causes fatigue, reduces work capacity and weakens the immune system. Severe anaemia also heightens the risky of women dying during childbirth and impairs children's physical growth, mental development and learning capacity (WHO, 2002).

2.3.2 Zinc

Numerous aspects of cellular metabolism are zinc dependent. Zinc plays an important role in growth and development, the immune response, neurological function and reproduction (King and Keen, 1999). Zinc is an essential element for sustaining all life. It is estimated that 3000 of the hundreds of thousands of proteins in the human body contains zinc (King and Keen, 1999). Zinc plays an important role in the structure of proteins and cell membranes. A finger-like structure, known as a zinc finger motif, stabilizes the structure of a number of proteins. For example, copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD), while zinc plays a critical structural role (King and Keen, 1999). It is required for white blood cell immune function, growth and development, protein synthesis, nucleic acid synthesis (for DNA) and function, properly clot blood and ensure sound fetal development. Zinc affects production of insulin, wound healing, growth and repair body tissues, behaviour and learning abilities, taste and smell. Zinc is essential for growth, appetite and normal immune function and is increasingly being recognised as critical in diets of people suffering from HIV/AIDS (Beebe *et al.*, 2000).

Zinc finger proteins have been found to regulate gene expression by acting as transcription factors (binding to DNA and influencing the transcription of specific genes). Zinc also plays a role in cell signalling and has been found to influence hormone release and nerve impulse transmission (Fairbanks, 1999). Recently, zinc has been found to play a role in apoptosis (gene-directed cell death), a critical

cellular regulatory process with implications for growth and development, as well as a number of chronic diseases (Moraghan and Grafton, 1999).

Zn is of the great significance in that it constitutes the building blocks of the plant growth hormones responsible for various enzymes and shoots generation. Zn deficiency in crop plant leads to decreasing tryptophan content and protein synthesis and accumulation of free amino acids, consequently resulting in low yields (Bouis, 2003). Zn affects bean growth by alternation of several physiological processes (Moraghan and Grafton, 2003).

Brain development is stunted by zinc insufficiency in utero and in young (King and Keen, 1999). The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function (Fairbanks, 1999). The bioavailability of Zn in food is important, because failure to consume adequate amounts of Zn or consumption factors that reduce zinc absorption may contribute to the development of Zn deficiency (Gibson, 1994). Zn deficiency has been identified among some low income pregnant women, infants and children in many developing countries as well as in some children in Canada and the USA (Gibson, 1994). In countries where plant foods are the major source of essential minerals, either increasing the amount of bioavailable Zn (Beebe *et al.*, 2000; Islam *et al.*, 2002) or increasing the amount of factors that may promote Zn absorption or decrease the amount of putative ant nutritional factors in beans or other staple plant foods represent important steps in the development of food-based solutions to reduce Zn deficiency (Islam *et al.*, 2002).

2.4 Increasing micronutrient (Fe and Zn) concentrations of the staple crops: Agronomic advantages

Soil is said to be deficient in a nutrient when the addition of a fertilizer containing this nutrient produces better plant growth (Graham and Welch, 1999). However, the amount of a mineral micronutrient added to the soil to produce better growth is usually small compared with the total amount of the mineral found in the soil by complete analysis (Ruel and Bouis, 1998). There are at least 3 agronomic advantages to growing mineral-dense crops:

- Efficiency in uptake of minerals from the soil improves diseases resistance in plants and results in reduced use of fungicides. This is because good nutritional balance is as important to disease resistance in plants as it is in humans (Graham and Welch, 1999). Micronutrient deficiency in plants greatly increases their susceptibility to diseases, especially fungal root diseases of the major food crops (Ruel and Bouis, 1998). Thus, breeding for micronutrient efficiency can confer resistance to root diseases that had previously been unattainable and lower dependence on fungicides.
- Micronutrient-efficient varieties grow deeper roots in mineral-deficient soils and so are better able to subsoil water and minerals (Graham and Welch, 1999). When topsoil dries, roots in the dry soil zone (which are the easiest to fertilise) are largely deactivated and the plant must rely on deeper roots for further nutrition (McCay Buis *et al.*, 1995). Roots of plant genotypes that are efficient in mobilizing nutrients from surrounding minerals are not only more disease resistant but are better able to penetrate deficient sub soils and make use of the moisture and minerals contained in sub soils. This reduces the need

for fertilizers and irrigation. Plants with deeper root systems are more drought resistant.

- Micronutrient-dense seeds are associated with greater seedling vigour that is associated with higher plant yields (Rengel and Graham, 1995). An important function of the seed is to supply the young seedling with minerals until it has developed a root system large enough to take over this role. In nutrient-poor soils, there may not be sufficient seed reserves to last while the extra roots are developed to compensate for the low mineral supply (Bouis *et al.*, 2000; Genc *et al.*, 2000).

2.5 Relationship between application of micronutrients to crops and human health

Current trends in micronutrient malnutrition continue to be increasing in many nations. For example; the global burden of iron deficiency has risen from about 35 percent of the world's population in 1960 to over 50 percent in 2003 (WHO, 2002) and iron deficiency among poor women is increasing at alarming rate in many developing countries and current intervention programmes (i.e. food fortification and supplementation programmes) to alleviate the problem have not proven to be effective or sustainable in many countries (Dornton-Hill, 1999).

This global crisis in micronutrient malnutrition is the result of dysfunctional food systems that cannot deliver enough micronutrients to meet the continual nutritional requirements of all. Because agriculture is the primary source of all micronutrients

for human consumption, agricultural systems must be contributing to this failure to meet nutritional needs (Welch *et al.*, 1997).

Importantly, if agricultural technologies are directed at improving the nutritional quality of food crops, they must encompass a holistic food system perspective (Fig. 1) to ensure that the intervention will be sustainable and adopted by farmers and consumers (Combs *et al.*, 1996). However, these types of interventions are relatively expensive and require sophisticated infrastructures for their creation, management and maintenance to assure compliance. The cost of breeding plants with traits that result in significant accumulations of micronutrients in edible portions of staple foods would be a one-time cost. Once achieved, these traits can be passed on in breeding programmes for future varietals generations and transferred globally to all nations with relatively little additional effort or expense (Bouis, 1999; Welch, 2004).

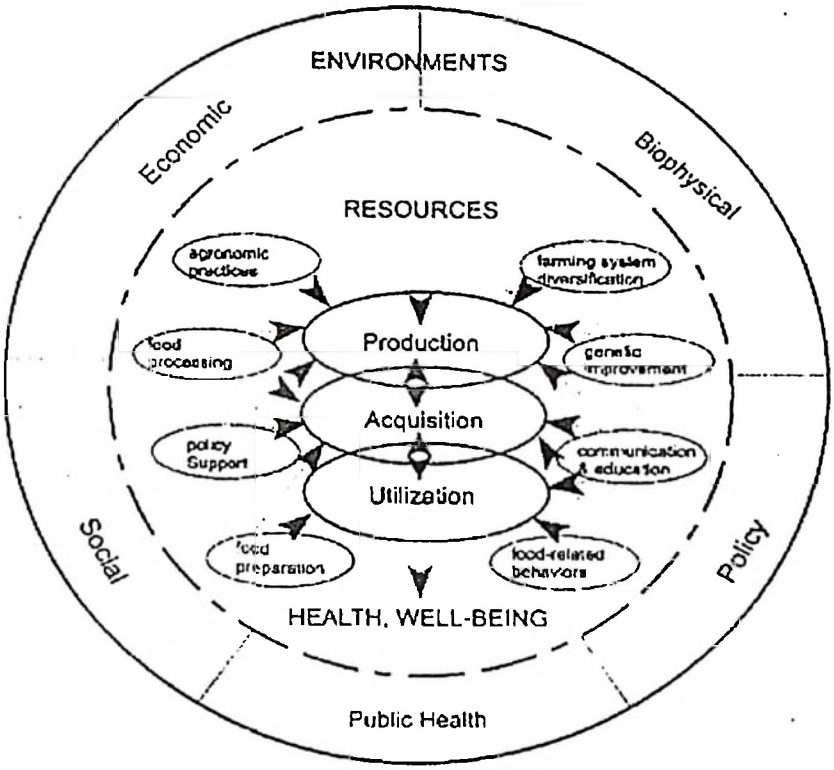

Figure 1: Holistic food system model
Source: Combs *et al.* (1996)

2.6 Genotype x environment interaction

2.6.1 General overview

With regard to the comparison of plant material in a set of multi-environment yield trials, the term genotype refers to a cultivar that is genetically homogenous, such as pure lines or clones, or heterogeneous, such as open pollinated populations rather than to an individual's genetic make up (Ngowi, 2002). The term environment relates to the set of climatic, soil, biotic (insect pests and diseases) and management conditions in an individual trial carried out at a given location (Gebeyehu and Assefa, 2003). When cultivars are compared in different environments, their performance relative to each other may not be the same. One cultivar may have the

highest yield in some environments and a second cultivar may excel in others. Changes in the relative performance of genotypes across different environments are referred to as genotype x environment interaction (Fehr, 1987). The presence of genotype x environment interaction automatically implies that the behaviour of the genotypes in trials (or breeding programs) depend upon particular environment in which they are grown. The performance of genotypes relative to the remaining genotypes grown in different environments may be inconsistent and such inconsistencies result in alterations to the ordering of genotypes from one environment to the next (Hill, 1975). Genotype x environment interaction leading to inconsistency of the best yielding materials across cropping environments, challenges plant breeders and complicates cultivar recommendations. Information on genotype x environment interactions is important to plant breeders in developing and recommending cultivars to suitable environments. Changes in rank among genotypes across environments may consistently limit the effectiveness of selection for superior genotypes across the environments.

2.6.2 Types of interactions

Every factor that is a part of the environment to a plant has the potential to cause differential performance that is associated with genotype x environment interaction. Environmental variables can be classified as either predictable (those under human control such as soil type, planting date) or unpredictable factors including rainfall, temperature, and relative humidity (Allard and Bradshaw, 1964). Genotype x environment interactions can occur in two ways, the first is when difference among genotypes varies without any alteration in their rank while the other is when the rank

among cultivars changes across environments. The change in rank between cultivars results in a genotype x environment interaction and this is the most important interaction for a plant breeder.

2.6.3 Reason for occurrence of genotype x environment interaction

According to Allard and Bradshaw (1964) plant breeders are fully aware that higher plants are dynamic living systems in which change occurs constantly from germination to maturity. The pattern of change is rarely the same from genotype to genotype in one environment or for a single genotype grown in different environments. It has been almost an article of faith from the earliest days of plant breeding that, if we only understood the development pathways by which final products are reached, this would help us improve the efficiency of breeding. Major interaction can be expected when there is wide variation between genotypes for morphophysiological characters conferring resistance to one or more stresses, as determined by climatic, soil, biotic and management factors. Other examples may concern the differential response of genotypes to variable levels of stress, such as low temperature, soil salinity, nutrient deficiency, insect pests, diseases, lodging, grazing or inter specific competition (Annichiarico, 2002a).

2.7 Stability in test varieties

Stability usually refers to a genotype ability to perform consistently, whether at high or low yield levels, across a wide range of environments (Annichiarico, 2002b). There are two obvious ways in which a variety can achieve stability; the variety can be made of a number of genotypes each adapted to somewhat different range of

environments. Second, the individuals themselves may be well buffered so that each member of the population is well adapted to a range of environments (Ngowi, 2002). The reliability of cultivar performance across locations and years can be an important consideration in plant breeding. Some cultivars are adapted to a wide range of environmental conditions while others are more limited in their potential distribution.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Germplasm collections

Bean seeds of different varieties were collected from within the country in four major bean growing regions which are Mbeya, Kagera, Arusha and Morogoro (Fig. 2). Seeds of improved bean varieties were collected from three research Institutes; Sokoine University of Agriculture (SUA), Uyole and Selian Agricultural Research Institutes. The varieties collected were diverse, representing a range in seed types differing in their seed coat colours, seed size, seed shape and other important characteristics such as growth habits. A total of 90 different varieties were collected. There were no specific and strict criteria in choosing which variety and/or cultivar to be collected. Collection was done both from farmers and markets depending on the availability of the seeds in a particular area. Only a half kilogram to one kilogram of seed was sampled.

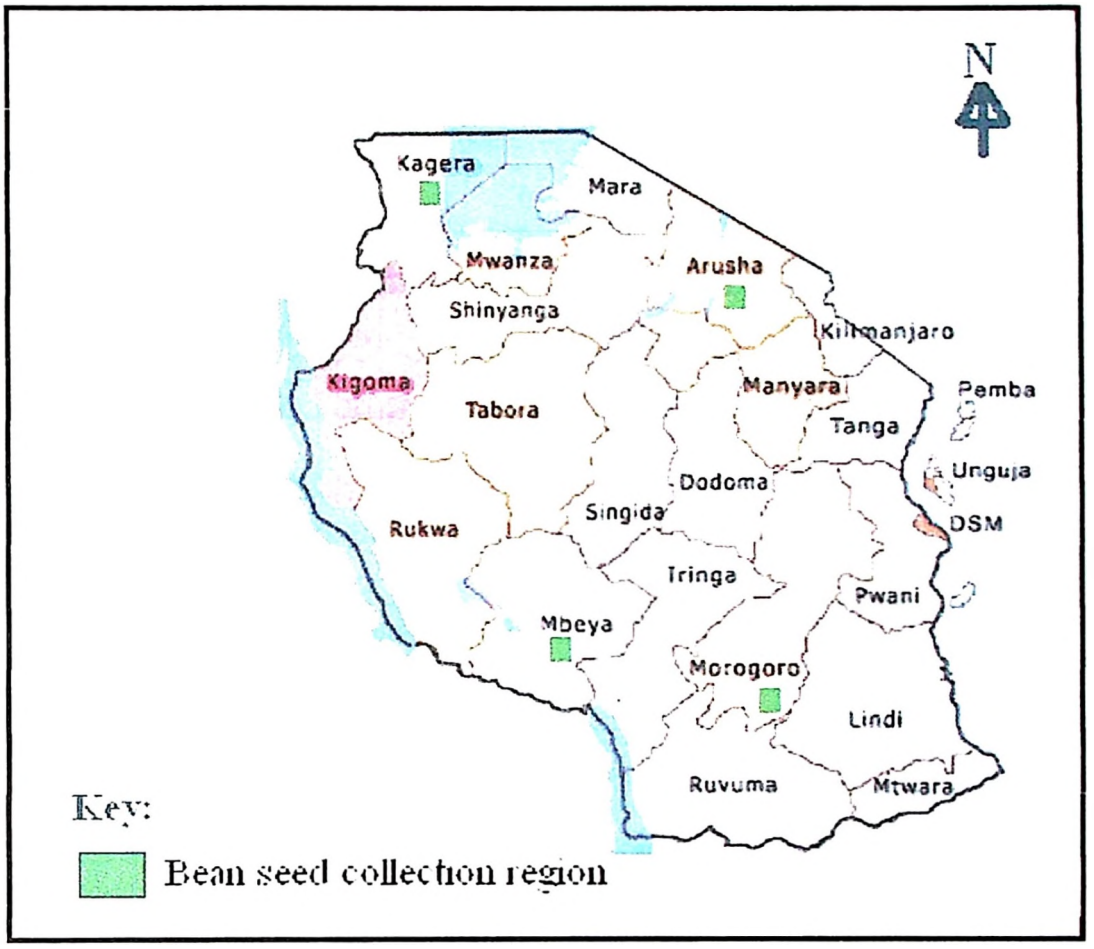

Figure 2: Map of Tanzania showing study area

3.2 Soil sampling and preparation

A bulk soil sample for use in pot experiments and from field experiment sites were collected at a depth of 0-20 cm. The composite soil sample was constituted by twenty sub-samples randomly collected from an area covering about 2 ha. Sub-samples were thoroughly mixed, air-dried and ground to pass through a 6.0 mm. The 6 mm sieved composite soil sample was randomly taken from the soil mass and ground to pass through 2 mm sieve. Then, a 6.0 mm sub-samples was sterilised and thoroughly mixed to give a composite medium for pot experiment study. The 2 mm

and 6 mm sieved composite soil samples were used for laboratory physical and chemical analysis and for the screen house pot experiment, respectively.

3.3 Soil analysis

A sub-sample of the sieved soil was taken for determination of appropriate chemical characteristics. All soil samples were analysed for particle size distribution, soil pH, cation exchange capacity (CEC), exchangeable bases (Ca, K. Mg, and Na), micronutrients (Fe and Zn), organic carbon and available phosphorus. Particle size distribution was determined by the hydrometer method after dispersing the soil samples with sodium hexametaphosphate solution (Day, 1965). Soil textural classes were determined using the USDA textural class triangle (Day, 1965). Soil pH was determined in water at a soil: water ratio of 1:2.5 suspension using pH meter (MacLean, 1982). Available P was extracted using the Bray 1 method (Bray and Kurtz, 1945) and colour was developed by the ascorbic acid of Murphy and Riley (1962). Exchangeable calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry whereas K and Na were extracted using ammonium acetate and analysed by flame spectrophotometry. Cation exchange capacity ($CEC_{pH\ 7}$) was determined with ammonium acetate saturation method at pH 7.0 (Chapman, 1973). Organic carbon (OC) was determined by the Walkley-Black wet combustion method (Tan, 1996) and total N was determined using the Kjeldahl method (Bremner and Mulvaney, 1982). The DTPA extractable Cu, Fe, Mn and Zn were determined by atomic absorption spectrophotometry (Lindsay and Norvell, 1978).

3.4 Experiment 1: Screen house experiment to determine Fe and Zn contents in bean varieties/lines.

The screen house experiment was conducted at SUA in order to screen for Fe and Zn contents in 90 bean varieties/bean lines. The soil was mixed thoroughly with basic nutrients. Iron was not applied since it was above the critical level in soil required for common bean growth. Zinc is affected by P application so to assure that Zn is available to plant it needs to be applied in excess. After mixing soil with nutrients the uniform soil was filled in plastic pots. Each pot was filled with four kg of uniform soil. The pots used were perforated at the bottom to allow drainage. The pots were later arranged in the screen-house on top of meshed steel benches, one metre from the ground. The treatments (common bean varieties/lines) were replicated three times and arranged in a completely randomised design (CRD).

Before sowing bean seeds, the potted soils were watered to 90% of field capacity (FC), and allowed to stay for one day. Four bean seeds were planted in each pot. The soils in the pots were maintained at approximately field capacity during the whole experiment period by watering. Thinning was done one week after emergence and two seedlings were allowed to grow per pot. Two weeks after sowing nitrogenous fertilizer (Urea 46% N) was applied to each pot. A trifoliate leaf was sampled from each plant in each pot at early flowering. Leaf samples were put into paper bags, clearly labelled and oven dried then ground to fine powder using motor and pestle to pass through 0.5 mm sieve for Fe and Zn analysis. After physiological maturity, bean seeds were harvested from each pot into paper bags and air dried. Then, bean seeds were taken to laboratory and ground using sample mill. The powder obtained was

used for determination of Fe and Zn in bean seeds. Iron and zinc was determined using atomic absorption spectrophotometer (AAS) method (section 3.5).

3.5 Plant analyses for Fe and Zn

The atomic absorption spectrophotometer (AAS) method was used to determine iron and zinc content (AOAC, 1995). A total of 0.5 g of ground plant samples were weighed into digestion tubes. Then, 5 ml of 68 % nitric acid was added into each tube and the mixture left to stand overnight. The digestion tubes were then placed in the digestion block and temperature set at 125°C for one hour before they were taken off and cooled. After cooling, 5 ml of 30% hydrogen peroxide (H_2O_2) was added into each tube and heated at about 70°C on digestion block until the reaction stopped. After cooling, 5 ml of 30% H_2O_2 was again added and heated at 70°C. The treatment was repeated until the digest was colourless. The temperature was increased to 180°C and continued digesting to almost dryness and then left to cool. A total of 10 ml of 10% nitric acid was added and the dissolved digest transferred to a 50 ml volumetric flask. The flask was then filled to the mark with distilled water and then mixed. The solution was then ready for determination of iron and zinc.

3.6 Experiment 2: Field experiment

3.6.1 Location of the field study sites

The experiment was conducted concurrently at two sites; Sokoine University of Agriculture (SUA) Horticulture unit and Madiira- a trial site for Selian Agricultural Research Institute (SARI). The experiment was conducted during the dry season from September 2006 to January 2007 under irrigation.

3.6.2 Experimental design

A randomized complete block design (RCBD) was used in this experiment with 20 treatments (bean varieties/bean lines) as shown in Table 1 and each treatment was replicated three times. Single row plot of 4.6 m was used. Two seeds were sown at a spacing of 50 cm x 20 cm between and within row respectively. There were 24 hills, each hill with two plants. A 1.0 m pathway was maintained around the entire experimental area and 1 m between replications.

3.6.3 Land preparation and sowing

Site clearing was done manually followed by harrowing. Different nutrients were applied. P was applied as triple super phosphate (20%P) (25 kg P/ha), N as urea (46%N) (40 kg N/ha) and Zn as zinc sulphate (5 kg Zn/ha). Zn was added despite being at sufficient levels at both sites since application of P might inhibit availability of Zn, in order to make sure that Zn is available has to be applied in excess. All nutrients except N were incorporated in the soil in each plot before sowing. Nitrogenous fertilizer was applied two weeks after emergence. Plots were irrigated and left to drain for one day, the following day bean seeds were sown. After emergence, there was regular irrigation to maintain the moisture content at about field capacity. Frequent weeding was done so as to keep the experimental plots free of weeds for most of the plant growth period. Diseases and insect pests were controlled regularly using appropriate pesticides.

Table 1: Common bean varieties used in experiment 2

Variety	Where obtained	Improved/local	Seed size
Pesa	SUA-Morogoro	Improved	Medium
Bwana shamba	Arusha	Local	Large
Wanja	Mbeya	Local	Medium
Red Wolaita	Arusha	Local	small
Canadian Wonder	Morogoro	Local	Small
Mwanga Chuchu	Kagera	Local	Small
Rosekoko	Kagera	Local	Large
Ranjonomy	Selian-Arusha	Ayt-Selian	Large
Mshindi	SUA-Morogoro	Improved	Medium
Lingot Blanc	Selian-Arusha	Ayt-Selian	Large
Uyole 94	Uyole-Mbeya	Improved	Medium
Uyole 84	Uyole-Mbeya	Improved	Medium
SUA 90	SUA-Morogoro	Improved	Medium
Jesca	Arusha	Improved	Medium
Selian 97	Arusha	Improved	Large
Rojo	SUA-Morogoro	Improved	Medium
Uyole 98	Uyole-Mbeya	Improved	Medium
Lyamungu 90	Arusha	Improved	Large
Uyole 96	Uyole-Mbeya	Improved	Large
Uyole 90	Uyole-Mbeya	Improved	Medium

3.6.4 Leaf sampling

Trifoliate leaves at early flowering were sampled randomly from 10 plants per row plot, dried in oven at 70^0C. The samples were then ground with a tecator 1093 cyclotec sample mill and screened to pass through a 1 mm sieve ready for plant analyses or Fe and Zn determination using a procedure described in section 3.5.

3.6.5 Harvesting

At maturity each row was harvested separately and the pods were threshed manually. Seeds were sun dried, winnowed and packed in paper bags. After packing, the seeds were sampled for weighing to determine 100 seed weight while other samples were ground using sample rotating mill and sieved through 1 mm mesh. The powder obtained was used for Fe and Zn quantification by atomic absorption spectrophotometer. Seed yield was determined by weighing total seed weight per plot (row).

3.7 Data analysis

All data were subjected to analysis of variance (ANOVA) using the MSTATC and IRRISTAT statistical packages. Simple linear correlation between seed Fe and Zn contents, between leaf Fe and Zn contents was performed. Correlation between the mineral contents in leaf and seed was also performed. The correlations of seed Fe content vs. 100 seed weight, Fe in leaf vs. Fe in seed and Zn in leaf vs. Zn in seed sample. The Duncan's Multiple Range Test (DMRT) was used for mean separation.

CHAPTER FOUR

4.0 RESULTS AND DISCUSION

4.1 Physical and chemical properties of soils of the experimental sites

Physical and chemical properties of SUA-horticulture unit and Madiira-Arusha soils are shown in Table 2. The pH of Madiira-Arusha soil, at 6.99 and SUA horticulture unit soil, at 7.33 was rated as being medium and high, respectively (Landon, 1991). The pH of the experimental sites could be considered as optimum for crop production, when other soil and plant factors are not limiting. Nitrogen content in the soil was 0.13% for SUA-horticulture unit and 0.23% for Madiira-Arusha. According to Landon (1991), total N in SUA-horticulture unit soil was rated as low and Madiira-Arusha as medium. Bray 1 (available) P content of the soil was 40.14 mg/kg at SUA horticulture unit and 29.79 mg/kg at Madiira-Arusha soils. Available P from both experimental sites was ranked as medium, implying that the soils were moderately sufficient in P (Landon, 1991). The DTPA extractable Zn observed in the soil was 1.86 mg/kg for SUA horticulture unit and 4.47 mg/kg for Madiira-Arusha. Landon (1991) suggested levels of 0.5-1.0mg/kg to be the critical levels for Zn. Therefore, the concentration of Zn in both sites was rated as sufficient. DTPA extractable Fe in both experiment sites was rated as very high according to Landon (1991) suggesting that the soils were sufficient in Fe.

Table 2: Some physical-chemical characteristics of the experimental soils

	Arusha	Rating/remarks[1]	Morogoro	Rating/remarks[1]
pH in water	6.99	Medium	7.33	High
Organic carbon (%)	2.73	High	1.55	Medium
Total N (%)	0.23	Medium	0.13	Low
Bray-1-P (Mg/kg)	29.79	Medium	40.14	Medium
CEC (cmol(+)/kg)	35.20	High	25.80	High
Exchangeable Ca (cmol(+)/kg)	16.80	High	7.42	Medium
Exchangeable Mg (cmol(+)/kg)	2.99	Medium	3.55	High
Exchangeable K (cmol(+)/kg)	2.07	Very high	0.99	Medium
Exchangeable Na (cmol(+)/kg)	1.11	High	0.51	Medium
DTPA Fe (mg/kg)	71.54	very high	55.44	very high
DTPA Zn (mg/kg)	4.47	Very high	1.86	High
Particle size analysis				
%sand	28		71	
%Silt	31		11	
%Clay	41		18	
Textural class	Clay loam		Sand clay	

[1]Landon (1991)

4.2 Screen house experiment

4.2.1 Days to 50% flowering and days to 85% maturity

Days to 50% flowering and days to 85% maturity for the common bean varieties involved in this study are presented in Appendix 1. Days to 50% flowering differed significantly ($P \leq 0.001$) among common bean varieties. Days to 50% flowering varied from 27 days (Kishoro) to 45 days (Rushesheka) with mean of 35 days. Days to 85% maturity in the common bean varieties was in the range of 53 days (Kihenda ndosho) to 81 days (Shona eigunia), with the average mean of 68 days (Appendix 1). This difference in days to 85% maturity among common bean varieties was

significant ($P \leq 0.001$). The variation in flowering and maturity among different common bean varieties showed that they were genetically diverse in these variables.

4.2.2 Iron and zinc content among common bean varieties

The concentrations of iron and zinc content in the leaves of 90 common bean varieties are shown in Appendix 2. Iron concentrations in leaves varied from 163.7 to 485.6 ppm. The mean for all varieties was 310.5 ppm. The variation among varieties for leaf iron content was significant ($P \leq 0.001$). The leaf iron content among varieties was divided into sub-groups of low, moderate and high. Varieties that had the range of 163.7 to 270 ppm (33.3%) (Low), 271 to 310 ppm (25.6%) (Moderate) and 311 to 485.6 ppm (41.1%) (High).

Zinc contents in leaves exhibited significant ($P \leq 0.001$) differences among common bean varieties. Zinc concentrations varied from 15.7 to 78.3 ppm. The mean for all varieties was 28.0 ppm. Leaf zinc contents among varieties was divided into sub-groups; 15.7 to 22.9 ppm (37.8%) (Low), 23.0 to 29.9 ppm (33.3%) (Moderate) and 30.1 to 78.3 ppm (28.9%) (High). Sufficient ranges of micronutrients in mature leaves are 50-250 ppm for Fe and 25-150 ppm for Zn (Landon, 1991). Results from the studied varieties, suggest that leaves have got more than average content of Fe (mean of 310.5 ppm) and a reasonable content of Zn (mean of 28.0 ppm). These observations imply that leaves can be consumed as vegetable to supply or supplement Fe and Zn requirements in diets, especially varieties that have high levels of these minerals, such as Kanunu, Uyole 90, Bilfa-Uyole, Selian 94, Kyakuponza, Kachele, Kikamba, Lingot blanc, NUA 56, Matawa Red wolaita for iron and Gofta,

Maini, SUA 90, Lyamungu 90, Tibihabwa, Mjunza, RWR ii for zinc. Similar opinion was suggested by Hillocks *et al.* (2006).

Iron contents in seeds differed significantly ($P \leq 0.001$) among the common bean varieties. Iron concentrations in seeds varied from 23.63 to 105.50 ppm. The mean for all varieties was 55.01 ppm. Seed iron content among varieties was divided into subgroups; 23.6 to 42.0 ppm (24.5%) Low), 43.0 to 59.6 ppm (42.2%) (Moderate) and 60.4 to 105.5 ppm (33.3%) (High). Varieties that produced relatively high iron contents are Kasukanywele, Mwamafutala, NUA 30, Kikamba, Kachele, Masusu, Rojo, Tema ekibila, Kakaritusi, Kitebe, Bangaya akateba, Shona eigunia, Kishoro, Canadian Wonder, Selian 2005, Kanunu, Wanja, Canada, Masai red, Kinyobwa and NUA 35. Iron values obtained in this work are similar to or higher than concentrations obtained by other researchers; 61.81- 83.99 mg/kg (Shimelis and Rakshit, 2005) and 41.0-142.0 mg Fe/kg (Guzman-Maldonado *et al.*, 2003).

The varieties significantly (($P \leq 0.001$) differed in their seed zinc contents. Zinc concentrations in seeds varied from 19.00 to 56.13 ppm. The mean for all varieties was 31.44 ppm. Seed zinc contents among bean varieties/lines were divided into sub-groups; 19.00 to 26.90 ppm (22.2%) (Low), 27.00 to 30.00 ppm (33.3%) (Moderate) and 31.00 to 56.13 ppm (44.5%) (High). Varieties Wanja, Red Wolaita, Kakaritusi, Kishoro, Rosekoko, Shona eigunia, Bangaya akatebe, Kikobe, Canada, Rojo, Kyakaragwe, Lingot blanc and Meru accumulated high zinc contents in their seeds. Zinc values obtained are similar to or slightly greater than concentrations of 15.39 to 28.22 mg/kg obtained by Shimelis and Rakshit (2005) who screened eight bean

varieties; 27 to 67 mg Zn/kg by Guzman-Maldonado *et al.* (2003) who screened 120 accessions for Fe and Zn, and of 24 to 57 mg Zn/kg obtained by Hacisalihoglu *et al.* (2005) who screened 35 accessions of common bean.

The variation in both iron and zinc contents found in this study was most likely due to genotypes (varieties). The genetic variability in the tested varieties is likely because the varieties used have all been derived from common bean populations with wide genetic base and therefore differences amongst those traits is very likely. This finding conforms to the results reported by Islam *et al.* (2002) that there is a wide variation in Fe and Zn among bean genotypes. However, the big variation in Fe and Zn concentration observed in this study also finds support from other workers (Islam *et al.*, 2002; White and Broadley, 2005). This variation in common bean varieties suggests that a genetic potential exists to increase concentration of these trace minerals (Fe and Zn) (Oikeh *et al.*, 2003). Furthermore, seed Fe and Zn concentrations behave as quantitative traits and several QTL impacting on them have been identified in bean (Beebe *et al.*, 2000; Guzman-Maldonado *et al.*, 2000; Cichy *et al.*, 2005). Thus, breeding for biofortification of Fe and Zn in seeds of common bean is feasible (White and Broadley, 2005).

4.2.3 Simple correlations for iron and zinc in common bean

Associations among iron and zinc in common bean varieties studied are shown in Table 3. Seed iron was found to be positively correlated but not significant with leaf iron ($r = 0.037$), implying that the amount in leaves can be reflected in seed. Seed zinc was negatively correlated and not significant with leaf zinc ($r = -0.015$). The

results suggest that plant mineral concentrations vary between plant tissues (e.g. leafy structure versus seeds) thereby demonstrating that genetic differences exist, which can contribute to the plant's ability to acquire and sequester minerals in plant. In leaves, iron was found to have a positive and highly significant ($P \leq 0.001$) correlation with leaf zinc ($r = 0.285$). Seed iron was found to have a positive and highly significant ($P \leq 0.001$) correlation with seed zinc ($r = 0.416$). Positive correlation between Fe and Zn in common bean has also been reported by other authors. For example, studies by Gregorio (2002) showed a high positive relationship between Fe and Zn content in common bean seeds who found a very highly significant positive correlation coefficient of 0.52 between the concentrations of Fe and Zn across different genotypes. The positive correlations between each of the two mineral in the leaves and seeds is very important because it indicates that genetic factors for increasing Fe are co-segregating with genetic factors form increasing Zn, therefore selection for one trait will consequently increase levels of another one. Therefore, it implies that selecting for a higher Fe level in bean seeds will also tend to select for increased Zn levels in the seeds (Graham and Welch, 1996; Gregorio, 2002). Common bean supplies significant amounts of minerals to populations in Latin America and Africa, Tanzania in particularly, it is thought that increasing Fe and Zn concentrations in the common bean seeds could increase the dietary intake of Fe and Zn in these regions significantly and the trait be considered in the varieties selection (White and Broadley, 2005). However, to communities the leaves can be consumed as vegetable to the healthy improvement.

Table 3: Simple correlation coefficients among zinc and iron in common bean leaves and seeds

	leaf iron	leaf zinc	seed iron
leaf iron			
leaf zinc	0.285***		
seed iron	0.037	0.006	
seed zinc	-0.025	-0.015	0.416***

*, *** level of significance at 0.05 and 0.001, respectively

4.3 Field experiment

4.3.1 Iron and zinc contents in common bean

The mean concentrations of Fe and Zn in seeds and leaves of the 20 bean varieties are shown in Tables 4 and 5 and figures in Appendices 2 and 3. The iron contents in common bean leaves and seeds grown in Morogoro ranged from 328.8 (Lingot blank) to 700.4 ppm (Uyole 90) and 34.0 (Uyole 94) to 89.1 ppm (Pesa) respectively. In Arusha, iron content ranged from 392.4 (Lingot blank) to 997.7 ppm (Jesca) in leaves and 33.4 (Jesca) to 87.4 ppm (Mshindi) in seeds. Iron content from combined analysis from two sites varied from 360.6 (Lingot blank) to 825.4 ppm (Uyole 90) in leaves and 37.0 (Jesca) to 79.7 ppm (Mshindi) in seed. The concentration of Fe in the leaves was generally higher than the concentration in the seeds. Iron contents in both seeds and leaves of tested varieties exhibited significant ($P \leq 0.05$) differences at both locations. Mean leaf iron content was higher in Madiira-Arusha than in Morogoro. On other hand seed iron content was slightly higher in Morogoro compared to Madiira-Arusha. As with locations, leaf and seed nutrient concentrations were completely different, Jesca having greater concentration of iron in leaves (997.9 ppm) collected in Madiira-Arusha than others but less in seeds (33.4 ppm) while variety Lingot blank had low iron content in leaves for both locations and Uyole 90

had the highest leaf iron content (700.4 ppm) for plants grown in Morogoro. Uyole 90 also showed the highest iron content in bean leaves (825.4 ppm) when the mean of two locations was considered. Seed iron content was highest in Pesa (89.1 ppm) in Morogoro and in Arusha the highest seed iron content was recorded in Mshindi (89.1 ppm) (Table 4). When the mean seed iron contents from two locations was combined, Mshindi also showed the highest seed iron content (79.7 ppm) while Jesca had the lowest (37 ppm). The mean concentration of Fe for two locations in the leaves was 538 ppm and 57.4 ppm in seeds.

Table 4: Mean iron contents in parts per million (ppm) in leaves and seeds of common bean varieties

Varieties	Morogoro		Madiira-Arusha		Combined mean	
	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds
SUA90	482.2ab	46.8 bc	646.6abc	61.6ab	565.9 bc	54.2abc
Jesca	545.0ab	40.6 bc	997.9a	33.4 b	771.4ab	37.0 c
Rojo	362.9 b	67.2abc	660.0abc	53.9ab	511.5 c	60.5abc
Lingot Blanc	328.8 b	54.0abc	392.4 c	54.9ab	360.6 c	54.5abc
Canadian Wonder	508.6ab	61.5abc	686.9abc	53.0ab	596.3 bc	57.2abc
Pesa	558.9 b	89.1a	611.5 bc	44.1ab	485.2 c	71.1ab
Lyamungu 90	559.7 b	81.9ab	626.0abc	41.5ab	492.9 c	61.7abc
Mwanga Chuchu	451.2ab	73.6abc	716.3abc	47.8ab	583.8 bc	60.7abc
Mshindi	418.7ab	72.0abc	607.6 bc	87.4a	513.1 c	79.7a
Wanja	503.5ab	70.4abc	587.3 bc	59.7ab	545.4 bc	65.1abc
Ranjonomby	341.5 b	54.0abc	539.5 c	41.5ab	440.5 c	47.8ab
Selian 97	345.1 b	56.3abc	580.6 bc	60.7ab	462.8 c	58.5abc
Uyole 96	466.5ab	44.3 bc	679.7abc	59.9ab	573.1 bc	52.1abc
Bwana Shamba	411.8ab	59.2abc	680.8abc	55.6ab	546.3 bc	57.4abc
Rosekoko	350.3 b	76.7abc	671.7abc	50.9ab	511.0 c	63.5abc
Uyole 90	700.4a	58.1abc	950.4ab	64.2ab	825.4a	61.1abc
Red Wolaita	389.9ab	65.7abc	695.2abc	50.9ab	542.5 bc	58.3abc
Uyole 98	376.1ab	60.4abc	449.9 c	52.5ab	413.0 c	56.4abc
Uyole 94	461.1ab	34.0 c	504.3 c	60.3ab	482.7 c	47.2 bc
Uyole 84	382.5ab	41.1 bc	689.9abc	47.2ab	536.2 bc	44.1 bc
Mean	427.1	60.8	648.9	54.1	538.0	57.4
CV%	39.5	38.7	29.7	31.2	33.7	35.7
SE±	97.4	13.6	111.3	9.7	50.3	3.3

Means followed by the same letter (s) are not significantly different according to Mean separation by DMRT ($P \leq 0.05$)

Table 5: Mean zinc contents (ppm) in leaves and seeds of common bean varieties

Varieties	Morogoro		Madiira-Arusha		Combined mean	
	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds
SUA90	20.0abc	19.4 c	29.2ab	22.7ab	24.6a	21.1abc
Jesca	23.8abc	24.7 bc	22.8ab	24.0ab	23.3a	24.1abc
Rojo	20.7abc	18.4 c	25.4ab	20.5ab	23.1a	19.5 bc
Lingot Blanc	22.5abc	22.6 bc	24.8ab	21.0ab	23.6a	21.8abc
Canadian Wonder	21.1abc	21.7 bc	28.7ab	17.1 b	24.7a	19.4 bc
Pesa	22.2abc	19.7 c	22.2 b	20.2ab	22.2a	20.0 bc
Lyamungo 90	28.2a	17.6 c	21.7 b	23.1ab	25.0a	20.4 bc
Mwanga Chuchu	19.4abc	23.8 bc	24.0ab	31.9a	21.7a	27.9ab
Mshindi	16.5 c	16.9 c	27.1ab	18.2 b	21.8a	17.5 c
Wanja	20.5abc	22.0 bc	27.6ab	21.1ab	24.0a	21.5abc
Ranjonomby	23.4abc	22.4 bc	27.3ab	20.8ab	25.4a	21.6abc
Selian 97	23.4abc	23.2 bc	25.7ab	27.2ab	24.6a	25.2abc
Uyole 96	22.2abc	24.2 c	24.7ab	21.3ab	23.4a	22.7abc
Bwana Shamba	18.7 bc	19.8 c	27.4ab	27.3ab	23.6a	23.6abc
Rosekoko	22.4abc	23.4 bc	25.4ab	20.1ab	23.9a	21.7abc
Uyole 90	26.3ab	24.5 bc	27.5ab	28.3ab	26.9a	26.4ab
Red Wolaita	21.0abc	37.3 c	27.4ab	21.2ab	24.2a	29.2a
Uyole 98	22.2abc	32.9ab	30.2a	26.0ab	26.2a	29.5a
Uyole 94	16.4 c	26.0ab	25.3ab	19.7 b	20.9a	22.8abc
Uyole 84	19.3abc	26.7 bc	28.7ab	24.2ab	20.9a	25.4abc
Mean	21.6	23.4	26.2	22.8	23.9	23.0
CV%	22.0	25.5	15.3	26.5	18.4	26.2
SE±	2.7	3.4	2.3	3.6	NS	2.0

Means followed by the same letter (s) are not significantly different according to Mean separation by DMRT ($P \leq 0.05$)

However, from combined analysis of variance from two locations, a significant ($P \leq 0.05$) variation among varieties in Fe contents was observed for locations while varieties (genotypes) and genotype x environment interaction components were non-significant (Table 6).

For zinc, bean varieties were significantly ($P \leq 0.05$) different in Zn contents in leaves and seeds. In Morogoro Zn content ranged from 16.4 ppm (Uyole 94) to 28.2 ppm (Lyamungu 90) in leaves and 16.9 ppm (Mshindi) to 37.3 ppm (Red Wolaita) in seeds. Zinc contents in Arusha ranged from 21.8 ppm (Lyamungu 90) to 30.2 ppm (Uyole 98) in leaves and 17.1 ppm (Canadian wonder) to 32.0 ppm (Mwanga Chuchu) in seeds. Lyamungu 90 had the highest contents in Morogoro (28. 2 ppm) while Uyole 94 had the least for leaf content (16.4 ppm). Red Wolaita had the highest seed Zn content for treatments grown in Morogoro (37.3 ppm) whereas Mshindi had the least (26.9 ppm). In Arusha, Uyole 98 had the highest leaf Zn content (30.2 ppm) while Mwanga Chuchu had the highest seed Zn content (31.9 ppm), Canadian Wonder had the least (17.1 ppm) (Table 5).

Table 6: Error mean square for combined analysis of variance for iron and zinc in 20 common bean varieties averaged over SUA and Madiira-Arusha locations

Parameter	Varieties (G)	Locations (E)	Varieties x Locations (GxE)
Iron in leaves	32987.712***	149597.317*	32987.712NS
Iron in seeds	418.950NS	665.540NS	418.950NS
Zinc in leaves	19.201NS	49.523*	19.201NS
Zinc in seeds	34.282*	55.930NS	34.282NS

NS-not significant, *, *** level of significance at 0.05 and 0.001 respectively

Combined data for Zn from two experiment sites ranged from 20.9 ppm (Uyole 94) to 26.9 ppm (Uyole 90) in leaves and 17.5 (Mshindi) to 29.5 ppm (Uyole 98) in seed. There was a significant difference ($P \leq 0.05$) among bean varieties in Zn contents in seeds but not in leaves when the two locations were combined. Results showed that Uyole 98 had the highest Zn contents (29.5 ppm) while Mshindi had the lowest. The mean concentration of Zn in seeds for the combined locations was 23 ppm (Table 5). The results from combined analysis of variance from SUA and Arusha showed a significant ($P \leq 0.05$) variation of zinc contents in seeds. Genotype and genotype x environment interaction components were non-significantly different. In leaves, significant ($P \leq 0.05$) variation of Zn contents was observed for locations while varieties and genotype x environment were non-significantly different (Table 6).

The differences in both Fe and Zn among varieties in different locations, demonstrates that the environmental conditions influence the concentration of iron and zinc in bean leaves and seeds. In order to confirm the environment variance, further evaluation of the traits in several seasons/years and increasing either number of replications or testing environments would be necessary. Iron and zinc concentration differences found in each variety, location and among locations suggest that there is a variety difference in uptake and portioning of nutrients. This is in consonance with studies of De Araujo *et al.* (2003) who observed that no single genotype showed stability for all the traits under different environments. However, high iron and high zinc genotypes will accumulate more of these nutrients than will low Fe and low Zn genotypes grown at the location during the same growing season. The mean seed concentrations of Fe (57.4 ppm) and Zn (23.0 ppm) observed in the

present study are lower and quite close respectively to values reported by other workers. For example, a field study in Ethiopia involving eight varieties of common bean showed mean concentration of Fe of 64.4 ppm (mg/kg); by contrast the mean concentrations of Zn was as low as 21.7mg/kg (Shimelis and Rakshit, 2005). In another field study, the mean concentration of Fe of 10 varieties of common bean grown on a neutral soil in Mexico was 73mg/kg (House *et al.*, 2002). The variations may be attributed to the number of genotypes (varieties), the genotypes selected for the study as well as to the manner in which the beans were grown.

The concentrations of Fe and Zn in 20 varieties grown in two sites were very different (Tables 4 and 5). The differences in levels of the Fe and Zn concentrations in the seed among varieties show that there is a need to test varieties under different environmental conditions in order to select and/or develop varieties that have a high and relatively stable concentration of Fe and Zn in the seed. These observations are supported by the findings by Beebe *et al.* (2004) where the interaction effect between varieties and environment was significant for Fe and Zn. This indicates that both the level of Fe and Zn in the seed, and the stability of Fe and Zn in seed differs among varieties (Mmbaga, 1990; De Araujo, 2003). Studies by Beebe *et al.* (2004) and Moraghan and Grafton (2001b) revealed the same trend reported in the current study; that some varieties have a relatively low concentration of Fe and Zn in the seed in all sites and others have a relatively high concentration in all sites and Moraghan *et al.* (2002) reported that both environment and genotypes affect the Fe and Zn concentrations of common bean seeds. Also, the results from this study finds support from other studies, that the concentration of Fe and Zn in the seed differs between

different varieties of common bean and also that this characteristic is expressed in different environments (Moraghan and Grafton, 2001a).

4.3.2 Correlations of iron and zinc with yield components of common bean

Associations of iron and zinc with yield components among common bean varieties shown in Table 7. It was observed, that concentration of Fe and Zn in the leaves was strongly related ($r = 0.507$, $P \leq 0.001$). The concentrations of Fe and Zn in the seeds were negatively and positively related to seed yield, respectively. The concentrations of iron in seeds correlated positively with seed size. However, concentration of Fe in leaves and concentrations of Zn in both leaves and seeds were negatively related to seed size. These observations are in accordance with the ones observed by Moraghan and Grafton (2001a) that concentrations of Fe and Zn in seed were not significantly positively correlated with seed weight. The concentrations of Fe and Zn in leaves were positively related to seed yield. This implies that there is no negative linkage between seed yield and Fe and Zn density in seeds.

A positive and significant correlation coefficient of 0.495 ($P \leq 0.05$) between seed Fe and Zn was observed in this study. The observed relation between concentration of Fe and Zn in the seeds are in accordance with other studies as like in emmer wheat (Cakmark *et al.*, 2004), in pigeon pea (Høgh-Jensen *et al.*, 2006) and in common bean (Beebe *et al.*, 2000; De Araunjo *et al.*, 2003; Pugesgaard, 2006). A positive ccorrelations between Fe and Zn is indicative in understanding the behaviour of traits and are of value in selecting desired traits in a breeding programme. Significant positive correlations between seed Fe and Zn, leaves Fe and Zn, suggests that

selecting bean seeds for high concentrations of either Zn or Fe may increase the amount of both elements (House *et al.*, 2002) and this is consistent with the findings by Hacisalihoglu *et al.* (2005) that a cultivar with relatively larger seed may show higher nutrient accumulation.

Table 7: Simple correlation coefficients among variables in common bean

	leaf iron	leaf zinc	seed iron	seed zinc
leaf iron				
leaf zinc	0.507***			
seed iron	-0.181	-0.137		
seed zinc	0.179*	0.307	0.495*	
100 seed weight	-0.338	-0.126	0.148	-0.387
seed yield	0.133	0.318	-0.184	0.382

*, **, ***level of significance at 0.05, 0.01 and 0.001, respectively

4.3.3 Yield and yield components

4.3.3.1 Days to 50% flowering and 85% maturity

The vvarieties tested significantly ($P \leq 0.05$) differed in attaining days to 50% flowering in both sites. At SUA and Arusha, variety Lingot Blanc was the earliest while Uyole 84 and Uyole 98 were the latest in flowering at SUA and Arusha, respectively (Tables 8 and 9). There were significant ($P \leq 0.05$) difference in days to 50% flowering among varieties when the 2 locations were combined (Tables 10 and 11). The pooled results revealed that Lingot Blanc flowered earlier while Uyole 98 flowered latest.

Days to maturity was recorded in the two environments (Tables 8, 9 and 10). They did not correspond completely with days to 50% flowering. Uyole 84 matured late in all environments and when results were combined. Results of combined analysis of

variance over two locations indicated highly significant ($P \leq 0.000$) variation on days to 85% maturity among varieties and there was no locations and genotype x environment differences (Table 11).

Table 8: Yield and yield components of the 20 common bean varieties grown at SUA

Varieties	Days to 50% flowering	Days to 85% maturity	Number of seed pod^{-1}	Number of pods plant^{-1}	100 seed weight (grams)	Yield (kg/ha)
Sua90	32.7cd	69.7def	4.7ab	20.7bcde	28.3gh	271.7cde
Jesca	30.3cd	69.3def	3.0e	12.7de	50.8ab	192.9 de
Rojo	32.3cd	71.7cdef	4.0bcd	13.7de	46.1abc	253.2 de
Lingot Blanc	30.0 d	69.0ef	3.0e	13.7de	48.0abc	197.1 de
Canadian Wonder	32.3cd	76.3c	3.7cde	16.0cde	35.9efg	195.4 de
Pesa	31.0cd	72.0cdef	3.0e	15.0cde	42.2cde	189.6 de
Lyamungo 90	32.3cd	68.3f	3.3de	15.0cde	52.5a	264.7cde
Mwanga Chuchu	39.3a	83.0a	3.7cde	23.3bcd	20.4i	179.0 e
Mshindi	31.3cd	69.7def	3.0e	15.7cde	38.1def	179.2 e
Wanja	30.7cd	69.3def	3.0e	10.7e	48.3abc	154.2 e
Ranjonomby	31.0cd	69.0ef	3.3de	22.3bcd	44.2bcd	331.7bcde
Selian 97	33.3cd	72.0cdef	3.3de	17.7bcde	43.0bcde	251.9 de
Uyole 96	33.7cd	75.3cd	4.3bc	20.3bcde	31.7fg	288.1cde
Bwana Shamba	33.0cd	72.3cdef	3.0e	22.3bcd	48.0abc	318.6cde
Rosekoko	32.0cd	69.0ef	3.7cde	19.3bcde	46.9abc	341.1bcde
Uyole 90	38.3ab	77.3bc	5.3a	37.7a	31.0fgh	615.2a
Red Wolaita	40.0a	82.3ab	3.3de	25.7bc	23.8hi	209.0 de
Uyole 98	33.7cd	76.3c	3.7cde	39.0a	35.3efg	507.2ab
Uyole 94	34.7bc	75.0cde	4.0bcd	25.3bc	44.6a-d	451.3abc
Uyole 84	40.3a	84.7a	3.7cde	27.3b	36.8def	382.6 bcd
Mean	33.6	73.6	3.6	20.7	39.8	288.7
CV%	6.7	4.4	14.0	27.8	10.7	34.3
SE±	1.3	1.9	0.3	3.3	2.5	57.1

Means followed by the same letter (s) are not significantly different according to Mean separation by DMRT ($P \leq 0.05$)

Table 9: Yield and yield components of the 20 common bean varieties grown at Madiira-Arusha

Varieties	Days to 50% flowering	Days to 85% maturity	Number of seed pod^{-1}	Number of pods plant^{-1}	100 seed weight (grams)	Yield (kg/ha)
Sua90	32.3fg	70.3cd	5.3abc	13.3abcd	28.0bcd	199.3abc
Jesca	34.7cde	67.0d	4.7bcd	8.7d	39.0abcd	158.1c
Rojo	43.3a	71.0bcd	4.3bcd	15.3abcd	35.5abcd	236.1abc
Lingot Blanc	31.3g	68.3cd	4.0cd	9.3cd	47.3abc	170.2bc
Canadian Wonder	35.0cd	76.0b	5.7ab	10.7bcd	33.8abcd	213.2abc
Pesa	32.7efg	73.0bc	4.7bcd	11.3abcd	38.7abcd	205.7abc
Lyamungo 90	34.0def	70.3cd	3.3d	12.7abcd	48.4ab	202.6abc
Mwanga Chuchu	35.3cd	81.7a	5.7ab	17.3ab	20.5d	210.6abc
Mshindi	33.7def	70.7bcd	4.7bcd	15.3abcd	31.7abcd	224.3abc
Wanja	40.7b	70.3cd	4.0cd	10.7bcd	43.5abc	187.8bc
Ranjonomby	40.0b	69.7cd	4.3bcd	10.0cd	35.3abcd	151.4c
Selian 97	33.7def	73.7bc	5.0bc	14.3abcd	42.2abcd	304.5abc
Uyole 96	35.7cd	72.3bcd	4.7bcd	12.3abcd	50.0ab	288.2ab
Bwana Shamba	34.3cdef	73.0bc	5.3abc	12.7abcd	38.8abcd	265.8abc
Rosekoko	35.3cd	72.3bcd	5.0bc	13.3abcd	47.2abc	308.9abc
Uyole 90	36.3c	82.0a	6.7a	18.0a	25.5cd	302.4abc
Red Wolaita	35.3cd	81.7a	6.7a	17.0ab	29.1bcd	326.3ab
Uyole 98	44.7a	73.3bc	5.0bc	16.0abc	37.0abcd	274.8abc
Uyole 94	35.0cd	73.7bc	4.7bcd	14.7abcd	52.0a	357.1a
Uyole 84	35.0cd	83.3a	5.3abc	13.7abcd	37.0abcd	269.2abc
Mean	35.9	73.7	5.0	13.3	38.0	242.8
CV%	3.1	3.8	15.5	25.4	9.2	33.1
SE±	0.6	1.6	0.4	2.0	2.0	42.4

Means followed by the same letter (s) are not significantly different according to Mean separation by DMRT ($P \leq 0.05$)

Significant ($p \leq 0.05$) interaction observed between genotype x locations for days to 50% flowering have been reported by other researchers (Marandu *et al.*, 1990; Ngowi, 2002; Rutaihwa *et al.*, 2004). These results suggest that besides the presence of genetic differences, flowering duration among varieties varied perhaps due to differences in temperatures at the two sites. This was observed in Morogoro where maturity was early compared to Arusha due to low temperatures in Arusha. The genotype x environment interaction in the variation of days to 50% flowering suggests that some genotypes were poorly adapted to low temperature. This might retard growth and development of common bean varieties. Effects of low temperature have also been observed by other scientists (Ohashi *et al.*, 2000) who found that there is differential decrease in growth and development and consequently flowering duration when temperatures are low.

Variation in maturity is caused by altitude and temperatures, hence the lower the altitude the earlier it flowers and vice versa. Rutaihwa *et al.* (2004) reported similar results suggesting that lack of genotype x environment interactions suggests that this parameter is controlled more genetically than environment also where the varieties are adapted. The whole procedure for release of new varieties in an area, including seed production and distribution, is complicated.

4.3.3.2 Number of seeds per pod and number of pods per plant

Number of seeds per pod and number of pods per plant in common bean varieties in both sites are shown in Tables 8, 9 and 10. There were highly significant differences ($P \leq 0.001$) among bean varieties for number of seeds per pod and number of pods per plant in both locations. At SUA, number of seeds per pod varied from 3.0 to 5.3. At

Madiira-Arusha, number of seeds per pod varied from 3.3 to 6.7. Combined analysis for both locations showed that number of seeds per pod varied significantly among varieties ($P \leq 0.001$) and among locations ($P \leq 0.001$) and there was significant varieties x locations interaction ($P \leq 0.01$) (Table 11). Seed per pod ranged from 3.3 to 6.0 (Table 10). Number of pod per plant at SUA varied from 10.7 to 39.0. At Selian-Arusha, number of pod per plant varied from 8.7 to 18.0. Combined analysis from both locations revealed that number of pod per plant varied from 10.7 to 27.8 (Table 11). Variation in the number of seeds per pod and number of pods per plant among varieties was found to be due to probably less adaptability and stability. Genotype and location interaction was significant ($P \leq 0.001$) in this regard. With respect to locations, varieties varied significantly ($P \leq 0.05$) among themselves, which suggests that the environmental effect had a significant role on how a given variety would perform. Despite the fact that pod per plant and seed per pod is genotype specific, differences in moisture at around grain filling due to heavy and frequent rainfall at the two locations could have led to differential flower drops.

The varieties significantly ($P \leq 0.05$) differed in their 100 seed weight at SUA and Madiira-Arusha (Tables 8 and 9). From combined locations (Table 10), Lyamungu 90 had the largest 100 seed weight and Mwanga Chuchu gave the lowest 100 seed weight at both locations. Lyamungu 90 and Uyole 94 produced the largest seeds at SUA and Arusha respectively. Analysis of variance (Table 11) revealed that there was no significant difference for locations while genotypes and genotype x environment interaction were significantly ($P \leq 0.001$) different for the trait. These results confer with the ones observed by Mduruma and Nchimbi (1991) when

working with common bean found that there were differences in 100 seed weight. Also, the results obtained indicate that there was an appreciable amount of genetic variation for seed yield, yield components among the common bean varieties used in this study. Genetic variation among bean in breeding population for similar traits has been reported by Mduruma and Nchimbi (1991). The differential responses of varieties in 100 seed weights over locations also indicated that environmental factors were not the same. Seed size is an important character in bean breeding because larger seeds have higher consumer acceptance and command higher market prices than small seeds.

4.3.3.4 Seed yield (kg/ha)

The tested varieties exhibited significant ($P \leq 0.05$) differences in yield performance in all locations (Tables 8, 9, 10 and Fig. 2). At SUA the highest yielding variety was Uyole 90 (615 kg/ha) while the lowest yielding variety was Wanja (154.2 kg/ha) followed by Mwanga Chuchu (170 kg/ha) and Mshindi (170.2kg/ha). At Madiira-Arusha the highest yielding variety was Uyole 94 (367 kg/ha) while the lowest yielding variety was Ranjonomby (151.4 kg/ha) followed by Jesca (158.1 kg/ha) and Lingot blanc (170.2 kg/ha). From a combined analysis (Table 10), the results indicates that variety Uyole 90 (442.1 kg/ha) was the highest yielding and the yield was significantly ($P \leq 0.05$) different from that of other varieties. The lowest yielding variety was Wanja (171 kg/ha). The combined analysis also revealed significant ($P \leq 0.001$) differences among varieties and variety x environment interaction but locations were significant at $P \leq 0.05$ (Table 11).

For grain yield, variation due to genotype x location interactions was significant ($P \leq 0.001$). This indicates that in this study the environment played a major role in influencing the trait or yield components measured. Similar results were reported by Moraghan *et al.* (2002) where common bean genotypes were evaluated for two seasons at three locations in Ethiopia. Of the traits measured, differences among varieties for days to 50% flowering, maturity and grain yield were significant across ($P \leq 0.01$) the locations. Genotype x environment interaction was significantly different ($P \leq 0.001$) for 50% flowering and others but not for days to 85% maturity. This implies that in this study the environment played a major role in influencing the traits/variables measured, agreeing with the results by Ngowi (2002).

Table 10: Yield and yield components of the 20 common bean varieties combined over two locations

Varieties	Days to 50% flowering	Days to 85% maturity	Number of seed pod^{-1}	Number of pods plant^{-1}	100 seed weight (grams)	Yield (kg/ha)
Sua90	32.5cde	70.0def	5.0ab	17.0bcd	28.2g	235.5bc
Jesca	32.5cde	68.2f	3.8bcd	10.7d	44.9abcd	175.5c
Rojo	37.8ab	71.3cdef	4.2bcd	14.5bcd	40.8cdef	244.6bc
Lingot Blanc	30.7c	68.7ef	3.5cd	11.5cd	47.6abc	183.6c
Canadian Wonder	33.7cde	76.2bc	4.6bc	13.3bcd	34.8f	204.3c
Pesa	31.8de	72.5cdef	3.8bcd	13.2bcd	40.4cdef	197.7c
Lyamungo 90	33.2cde	69.3def	3.3d	13.8bcd	50.4a	233.7bc
Mwanga Chuchu	37.3ab	82.3a	4.6bc	20.3abc	20.4h	194.8c
Mshindi	32.5cde	70.2def	3.8bcd	15.5bcd	34.9f	201.7c
Wanja	35.7bc	69.8def	3.5cd	10.7d	45.9abcd	171.0c
Ranjonomby	35.7bc	69.3def	3.8bcd	16.2bcd	39.7def	241.5bc
Selian 97	33.5cde	72.8cdef	4.3bcd	16.0bcd	42.6bcde	278.2abc
Uyole 96	34.7bcd	73.8cdef	4.5bcd	16.3bcd	40.9cdef	304.8abc
Bwana Shamba	33.7cde	72.7cdef	4.2bcd	17.5bcd	43.4abcde	292.3abc
Rosekoko	33.7cde	70.7cdef	4.3bcd	16.3bcd	47.1abcd	325.0abc
Uyole 90	37.3ab	79.7ab	6.0a	27.8a	28.3g	442.1a
Red Wolaita	37.7ab	80.0a	5.0ab	21.3ab	26.4gh	267.6bc
Uyole 98	39.2a	74.8bcd	4.3bcd	27.5a	36.1ef	391.0ab
Uyole 94	34.8bcd	74.3bcde	4.3bcd	20.0abc	48.3ab	404.2ab
Uyole 84	37.7ab	84.0a	4.5bcd	20.5abc	36.9ef	325.9abc
Mean	34.8	73.7	4.3	17.0	38.9	265.8
CV%	5.2	4.1	15.2	27.3	10.0	33.9
SE±	1.0	1.7	0.4	2.7	2.0	42.4

Means followed by the same letter (s) are not significantly different according to Mean separation by DMRT ($P \leq 0.05$)

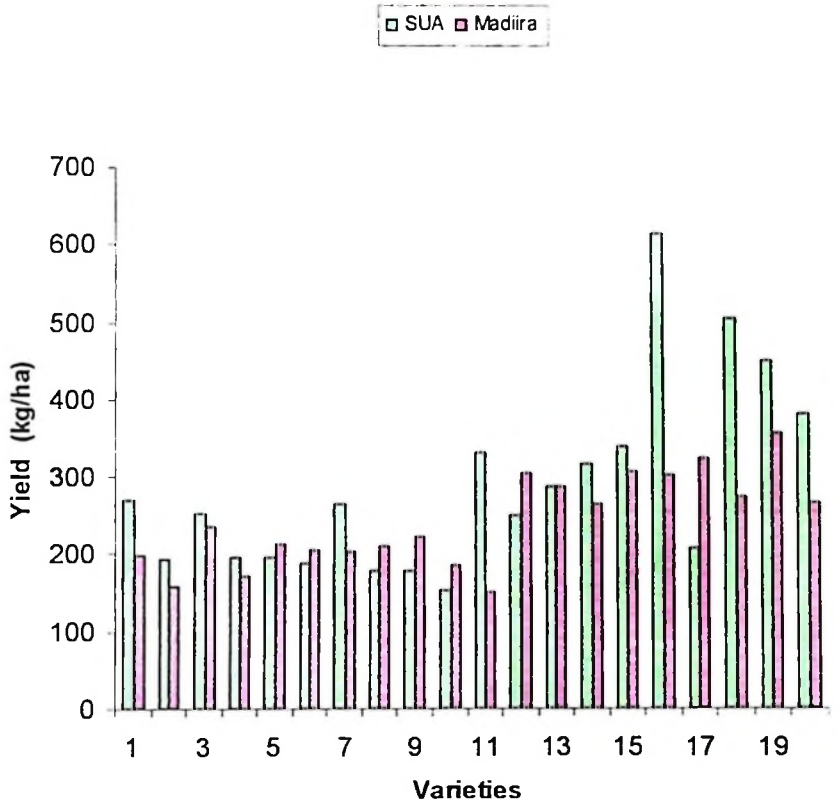

Figure 3: Seed Yield of 20 bean varieties evaluated at SUA and Madiira-Arusha

Table 11: Error mean square for combined analysis of variance (ANOVA) for different characters of 20 common bean varieties averaged over SUA and Madiira-Arusha locations

Parameter	Varieties (G)	Locations (E)	Varieties x Locations (GxE)
Days to 50% flowering	3.204***	0.967***	3.204***
Days to 85 maturity	9.123***	1.167NS	9.123NS
Seeds per pod	0.422***	0.475***	0.422**
Pods per plant	22.227***	26.017***	22.227***
100 seed weight (g)	15.218***	80.829NS	15.218***
Seed yield (kg/ha)	8122.388***	8649.994*	8122.388***

NS-not significant, *, **, *** level of significance at 0.01 and 0.001 respectively

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The findings from screen house suggest that there is Fe and Zn variability among common bean varieties from major bean growing Regions in Tanzania. Varieties Wanja, Kanunu, Kishoro, Shona eigunia, Bangaya akatebe, Mwamikola, Kitebe, Lyamungu 90, Masai red, Canada, Rojo, Lingot blanc, Matawa, Kirundo, Kinyobwa, Bwana shamba, Kakaritusi, Mjunza, Kitebe and Kasukanywele were identified to contain higher levels of Fe and Zn compared to others.

Selecting varieties with higher capacity to accumulate Fe and Zn could contribute significantly to the improvement of micronutrient status of people depending on common bean as a major component of their diet.

The present study has shown that it is possible to identify genetic differences within common bean varieties for Fe and Zn contents as a pre-requisite for breeding aiming at increasing their concentrations.

The levels of Fe and Zn concentration of the 20 varieties in two sites were very different. This shows that testing of improved varieties under different environmental conditions is very important in developing varieties that have relatively stable concentrations of Fe and Zn in the seed. The study has also revealed that some

varieties such as Lingot blanc have low concentration of Fe and Zn in seed in all sites. These emphases the effect of both the genotype and environment effect on Fe and Zn concentrations of common bean seeds. It has also been found that leaves of common bean contains more than average contents of Fe (310.0 ppm) and reasonable content of Zn (28.0 ppm), which implies that they can be advocated in human diets especially in developing countries as source of these micronutrients.

The positive and highly significant correlation between leaf Fe and Zn, and also, between seed Fe and Zn, suggests that genetic factors for increasing Fe are co-segregating with genetic factors for increasing Zn. This means that in the breeding programme, the incorporation of one of these nutrients will not be at the expense of the other.

The study has shown the potential of exploiting the genetic variation in seed concentration of Fe and Zn without the general negative effect on yield of adding new traits. Thus, breeding for higher trace minerals density in seeds will not incur yield penalty. Generally, seed and yield components of the studied varieties were found to be highly influenced by the environment into which they were studied. The data presented in this study will facilitate programme endeavouring to develop high yielding cultivars of beans that are significantly enriched in bioavailable Fe and Zn concentrations.

5.2 Recommendations

From this study, it is recommended that:

(i) Further study on trace mineral (Fe and Zn) inheritance should be carried out through conventional breeding to determine the best selection techniques. While varieties found to have high levels of Fe and Zn be used as a gene source in future breeding work. Meanwhile, farmers should use those varieties for consumption and production.

(ii) Effort should be made to encourage people in bean growing areas to use bean leaves as vegetables.

(iii) Varieties and environment effects and the contrast in behaviour of leaf and seed nutrient concentrations need a more detailed examination of the factors that control uptake and portioning of nutrient supplies in leaves and seeds.

(iv) Since this was a one season evaluation of these varieties, it is suggested that this study be carried out for at least two seasons with additional of replications and locations to further verify the results obtained.

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APPENDICES

Appendix 1: Days to flowering and maturity among 90 common bean varieties/lines

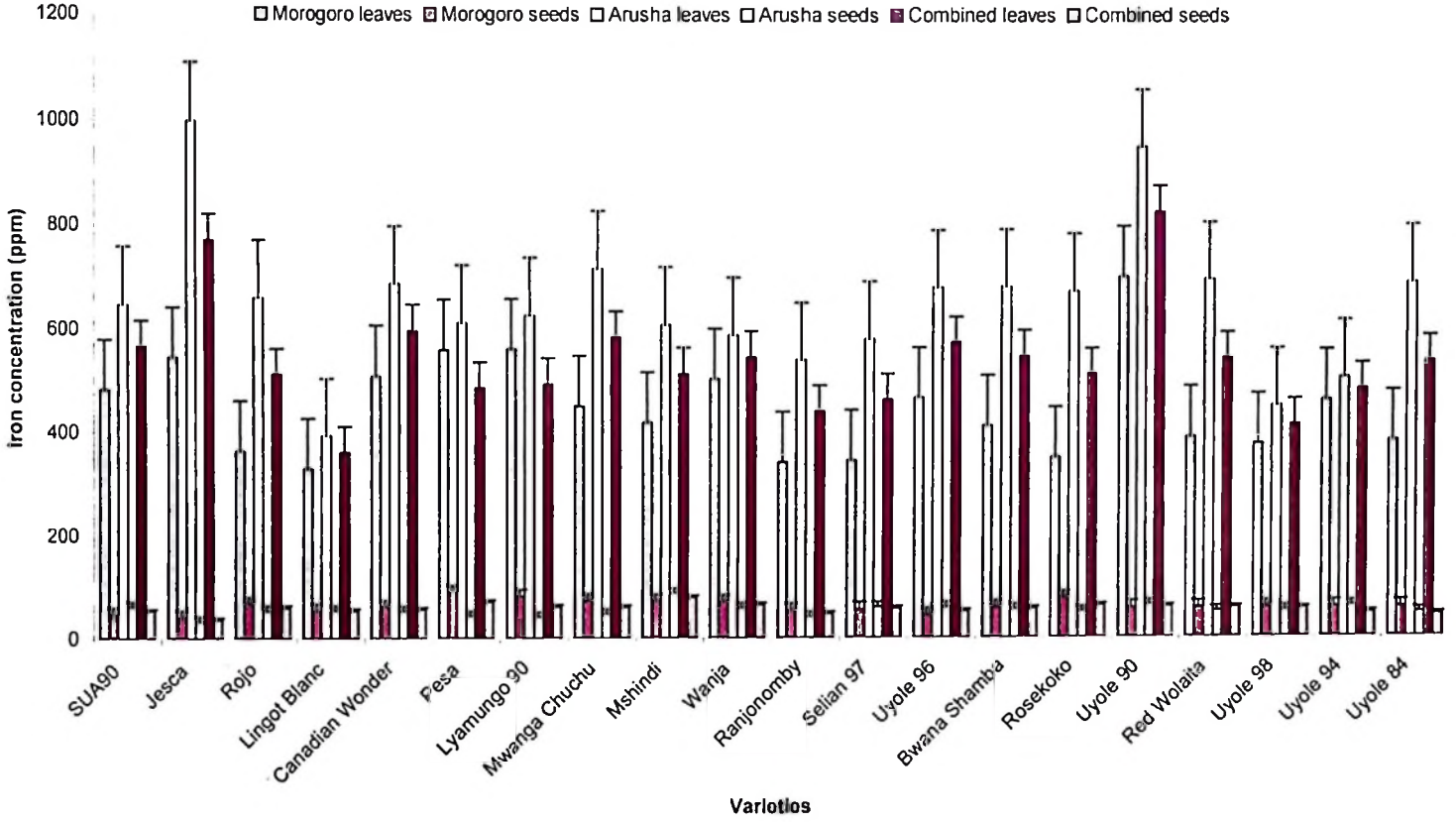
Varieties/Treatments	Days to 50% Flowering	Days to 85% Maturity
Wanja	31.33 i-q	61.33m-x
Kanunu	38.33 a-i	63.67h-v
Selian 2005	36.33 b-l	71.33b-k
Canadian Wonder	31.00 j-q	61.67l-x
Mwasipenjele	29.00 m-q	57.00s-x
Maharage karanga	32.00 g-q	71.67b-j
Bilfa-Uyole	34.67 d-o	66.33e-s
Pesa	30.00k-q	56.67t-x
Selian 97	34.00e-p	62.00k-w
Uyole 98	33.00f-q	66.00e-t
Kihenda ndosho	29.00m-q	56.00u-x
Ngela	34.33e-p	66.33e-s
Bugubugu	33.00f-q	53.00wx
Maharage soya	34.00e-p	63.67h-v
Rushesheka	45.00a	78.33abc
Uyole 90	38.00b-j	67.67d-r
Msafiri	29.67l-q	60.67o-x
Kisapuri	28.00 o-q	60.67o-x
Red Wolaita	39.00a-g	70.67b-m
Awash melka	42.33a-c	66.67e-r
Katunduri	36.33b-l	64.67f-u
Lyamungu 85	38.33a-i	73.00a-h
Kishoro	26.67q	54.67vwx
Rosekoko	34.00e-p	73.33a-g
Shona eigunia	40.33a-e	81.33a
Bangaya akatebe	32.67f-q	64.33f-u
Mwamikola	38.00b-j	74.67a-e
Kitebe	33.33e-q	70.33b-n
Kikobe	34.67d-o	64.00g-v
Lyamungu 90	31.67h-q	61.67l-x
Canada	34.67d-o	71.00b-l
Masai red	39.00a-g	69.33c-p
Chipikupiku	27.33pq	56.67t-x
Selian 94	33.00f-q	59.00r-x
RWR ii	37.33b-j	70.67b-m
Kigoma	27.33pq	55.67u-x
Uyole 84	39.00a-g	79.00ab
Kawanja	36.33b-l	73.67a-f
Mwanga Chuchu	35.33c-n	71.00b-l
Kyaburundi	37.00b-k	69.33c-p
Tibihabwa	34.67d-o	69.67c-o
Mjunza	34.67d-o	64.00g-v
Kakaritusi	36.33b-l	62.67i-v
Tema ekibila	39.67a-f	72.33a-h

Kashoro	27.33pq	62.67i-v
Kailaguju	38.67a-h	69.00c-p
Meru	38.00b-j	75.00a-e
Kyakuponza	32.33g-q	62.33j-v
Kamoshi	36.00b-m	73.00a-h
Bwana shamba	31.67h-q	67.67d-r
Rojo	30.00k-q	64.33f-u
Masusu	28.67n-q	60.00p-x
SUA 90	33.33e-q	61.00n-x
Mwanamwana	34.67d-o	52.67x
Kyakaragwe	33.00f-q	69.67c-o
Mshindi	29.67l-q	59.33q-x
Kinyobwa	31.00j-q	66.67e-r
Kachele	33.00f-q	60.00p-x
Soya fupi	37.00b-k	66.00e-t
Mayoha	37.67b-j	72.00b-i
Jesca	34.67d-o	60.33o-x
Roba 1	35.67b-n	69.33c-p
Zebra	40.33a-e	67.67d-r
White	39.00a-g	75.33a-e
Uyole 94	32.33g-q	71.67b-j
Maini	35.00d-o	62.67i-v
Kablanketi	35.67b-n	66.33e-s
Uyole 96	33.67e-p	61.67l-x
Gofta	35.67b-n	69.33c-p
Kirundo	33.33e-q	62.00k-w
Kikamba	42.67ab	71.00b-l
Matawa	41.67a-d	73.00a-h
Ayenew	34.67d-o	66.33e-s
Ranjonomby	33.67e-p	60.33o-x
NUA 43	36.33b-l	68.67d-q
Ngwakungwaku	36.00b-m	67.33d-r
Lingot blanc	35.33c-n	67.67d-r
NUA 30	36.33b-l	67.33d-r
PVA 8	40.33a-e	76.67a-d
Main de Kyondo	38.67a-h	72.00b-i
MCM 2001	37.33b-j	75.00a-e
NUA35	33.00f-q	64.33f-u
OBA 1	37.00b-k	69.33c-p
Mwamafutala	37.67b-j	79.33ab
K132	37.00b-k	72.00b-i
NUA4	35.33c-n	67.00e-r
MLB49-89A	33.67e-p	67.67d-r
NUA59	36.00b-m	69.33c-p
NUA 56	34.00e-p	66.67e-r
Kasukanywele	34.33e-p	67.33d-r
Mean	34.67	66.704
CV (%)	9.79	6.89
SE±	1.97	2.655

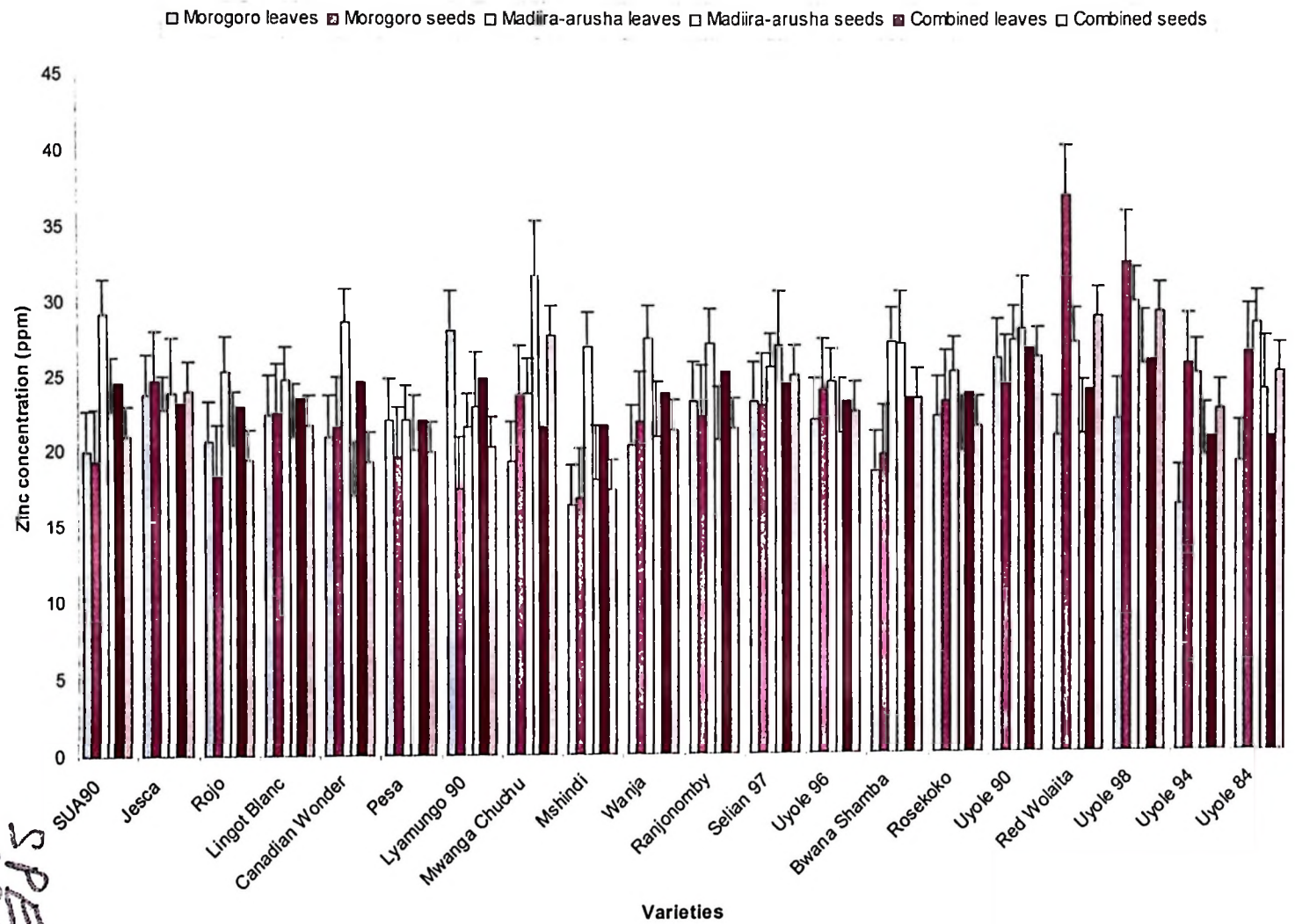
Appendix 2: Concentrations of iron and zinc contents in leaves and seeds of 90 common bean varieties/lines

Varieties/Treatments	Leaf Fe	Leaf Zn	Seed Fe	Seed Zn
Wanja	251.2g-p	21.43i-q	79.87a-e	42.8b-e
Kanunu	485.1a	22.57i-q	76.90a-h	30.63h-r
Selian 2005	361.9a-n	22.60i-q	83.50a-d	27.00j-t
Canadian Wonder	352.0a-o	19.77l-q	71.33a-l	25.50m-t
Mwasipenjele	283.0d-p	21.40i-q	36.00h-n	25.30m-t
Maharage karanga	258.3g-p	23.43h-q	25.10mn	29.97i-s
Bilfa-Uyole	404.4a-g	27.07g-q	40.80e-n	22.43r-t
Pesa	361.3a-n	25.70g-q	50.00d-n	27.33j-t
Selian 97	232.0j-p	20.50j-q	29.93l-n	28.87i-s
Uyole 98	286.2d-p	22.97i-q	37.87f-n	23.13q-t
Kihenda ndosho	381.0a-k	26.33g-q	31.03k-n	26.90j-t
Ngela	329.6a-o	20.67j-q	31.33j-n	19.00t
Bugubugu	326.0b-o	20.27j-q	40.47e-n	23.10q-t
Maharage soya	229.7j-p	22.80i-q	48.30d-n	25.20m-t
Rushesheka	288.0d-p	31.00e-q	23.63n	31.20g-r
Uyole 90	470.6ab	35.17c-l	34.47i-n	24.37o-t
Msafiri	202.3nop	18.33o-q	43.60d-n	25.40m-t
Kisapuri	255.4g-p	27.73g-q	54.90c-n	28.97i-s
Red Wolaita	395.0a-i	35.33c-l	55.83c-n	47.97b
Awash melka	236.1h-p	29.93e-q	60.37b-n	27.07j-t
Katunduri	352.0a-o	31.37e-q	36.27g-n	45.93bc
Lyamungu 85	358.2a-n	31.83e-p	55.00c-n	28.97i-s
Kishoro	339.7a-o	27.97g-q	76.33a-h	44.53bcd
Rosekoko	301.5d-p	22.43i-q	54.70c-n	39.83b-h
Shona eigunia	285.7d-p	21.80i-q	74.57a-i	56.13a
Bangaya akatebe	278.0d-p	22.70i-q	105.50a	46.43b
Mwamikola	287.0d-p	36.97c-i	63.67b-n	35.50e-l
Kitebe	297.3d-p	28.40f-q	71.50a-k	34.93e-m
Kikobe	207.7m-p	36.73c-i	51.67d-n	41.37b-f
Lyamungu 90	264.5g-p	43.20b-f	61.33b-n	32.67f-q
Canada	311.0c-p	24.5h-q	68.17a-l	47.57b
Masai red	260.0g-p	27.83g-q	66.67a-l	33.47f-o
Chipikupiku	290.1d-p	37.03c-i	42.00e-n	26.43k-t
Selian 94	434.8a-e	23.63h-q	40.10e-n	28.83i-s
RWR ii	258.50g-p	40.40b-g	41.53e-n	29.67i-s
Kigoma	357.1a-o	35.47c-k	44.97d-n	25.30m-t
Uyole 84	375.7a-l	36.67c-i	37.83f-n	24.93o-t
Kawanja	300.6d-p	27.57g-q	33.83i-n	29.40i-s
Mwanga Chuchu	256.4g-p	21.93i-q	48.13d-n	35.33e-l
Kyaburundi	391.6a-j	33.70d-o	56.17c-n	36.47d-j
Tibihabwa	347.0a-o	49.13bc	55.80c-n	28.73i-s
Mjunza	376.0a-l	48.03b-d	41.20e-n	35.37e-l
Kakaritusi	331.3a-o	31.60e-p	72.70a-j	36.43d-j
Tema ekibila	276.1e-p	26.57g-q	77.53a-g	29.47i-s
Kashoro	296.2d-p	27.93g-q	40.10e-n	32.90f-p
Kailaguju	260.9g-p	35.70c-j	56.20c-n	29.33i-s
Meru	271.8g-p	29.90e-q	51.97d-n	36.23d-j
Kyakuponza	400.7a-g	34.00d-m	56.63c-n	34.70e-n

Kamoshi	303.4d-p	34.70c-m	52.00d-n	33.47f-o
Bwana shamba	230.6j-p	26.33g-q	60.90b-n	31.03h-r
Rojo	260.0g-p	26.53g-q	75.00a-i	39.40i-s
Masusu	237.4h-p	21.60i-q	72.77a-j	25.57m-t
SUA 90	349.5a-o	43.60b-e	36.60g-n	23.43p-t
Mwanamwana	293.1d-p	25.77g-q	35.53h-n	29.93i-s
Kyakaragwe	289.2d-p	22.87i-q	48.27d-n	40.63b-g
Mshindi	267.9g-p	23.07i-q	59.57b-n	27.67j-t
Kinyobwa	266.4g-p	21.00j-q	68.43a-l	35.90d-k
Kachele	407.3a-g	24.67h-q	98.33ab	28.50i-s
Soya fupi	255.9g-p	20.77j-q	43.00d-n	25.17m-t
Mayoha	462.9abc	29.90e-q	50.67d-n	28.50i-s
Jesca	210.9m-p	19.13m-q	65.27b-m	29.67i-s
Roba 1	262.9g-p	21.77i-q	51.10d-n	24.90o-t
Zebra	233.2i-p	20.83j-q	43.47d-n	22.80rst
White	365.7a-m	24.03h-q	53.00d-n	29.70i-s
Uyole 94	216.3l-p	19.87k-q	57.47c-n	36.03d-k
Maini	280.2d-p	52.77b	45.73d-n	33.80e-o
Kablanketi	307.2c-p	38.87b-h	56.40c-n	33.67e-o
Uyole 96	358.3a-n	18.43m-q	57.20c-n	21.60g-r
Gofta	362.8a-n	78.27a	76.67a-h	31.53g-r
Kirundo	217.0l-p	20.53j-q	81.00a-e	31.77g-r
Kikamba	438.6a-d	20.67j-q	44.20d-n	33.40f-o
Matawa	396.3a-h	24.33h-q	64.00b-n	33.70e-o
Ayenew	339.1a-o	18.23o-q	58.07b-n	31.47g-r
Ranjonomby	301.9d-p	20.93j-q	55.03c-n	31.13h-r
NUA 43	289.9d-p	15.73q	54.87c-n	31.17h-r
Ngwakungwaku	273.6f-p	23.03i-q	47.63d-n	30.77h-r
Lingot blanc	373.6a-l	27.90g-q	64.43b-n	40.13b-h
NUA 30	261.0g-p	32.23e-p	94.67abc	29.30i-s
PVA 8	381.2a-k	25.90g-q	50.67d-n	20.50st
Main de Kyondo	222.4k-p	29.90e-q	45.27d-n	28.00i-t
MCM 2001	195.6o-p	20.97j-q	50.33d-n	29.00i-s
NUA35	344.7a-o	21.97i-q	65.67b-m	34.80e-n
OBA 1	235.5h-p	23.53h-q	61.60b-n	34.83e-n
Mwamafutala	485.6a	34.70c-m	79.27a-f	28.03i-t
K132	163.7p	17.17p-q	47.50d-n	30.63h-r
NUA4	396.7a-h	23.70h-q	50.33d-n	37.60c-i
MLB49-89A	302.0d-p	21.90i-q	36.73g-n	27.27j-t
NUA59	209.7m-p	44.70b-e	36.23g-n	26.13l-t
NUA 56	433.9a-f	28.00g-q	44.40d-n	28.40i-t
Kasukanywele	304.0c-p	22.50i-q	74.33a-i	30.07i-s
Mean	310.49	28.03	55.01	31.44
CV(%)	24.86	26.62	35.91	14.73
SE±	44.57	4.308	11.41	2.675



Appendix 3: Iron contents in parts per million (ppm) in leaves and seeds of 20 common bean varieties



Appendix 4: Zinc contents (ppm) in leaves and seeds of 20 common bean varieties