INFLUENCE OF SUPPLEMENTARY IRRIGATION AND ORGANIC MANURE APPLICATION ON MICRONUTRIENT DENSITY AND YIELD OF FIVE COMMON BEAN VARIETIES

BY

ANNA ITWARI FELIX

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DECLARATION

I declare that this thesis is a record of my own research findings and has never been presented for an academic award in any other university.

Signed ______________________ Date 18-08-2009

Anna Itwari Felix

This thesis is submitted for examination with our approval as supervisors:

Signed ______________________ Date 18/8/2009

Dr. George N. Chemining'wa

Department of Plant Science and Crop Protection

University of Nairobi

Signed ______________________ Date 18 August 2009

Prof. Paul M. Kimani

Department of Plant Science and Crop Protection

University of Nairobi

DEDICATION

This work is dedicated to my late father Felix Longumo Otole, a renowned educationist whose four decades of teaching improved many lives in Sub-Saharan Africa; and to little Clementina and Felix I hope this work inspires them to grow biofortified food to eradicate micronutrient malnutrition in the world.

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ABSTRACT

Micronutrients deficiency especially (Zinc and Iron) have been identified as limiting in most diets of people who depend on staple foods. Bean varieties high in Iron and Zinc have been developed in East and Central Africa to combat micronutrient deficiency. However, the benefits from these varieties depend on the environment where they are grown. Agronomic practices have been known to influence micronutrient concentration in beans. However, little work has been done to develop management practices that will enhance expression of the high mineral density trait.

Field experiments were conducted during 2006 short rain and 2007 long rain seasons to determine the effect of supplementary irrigation and organic manure application on micronutrient density of five bean varieties grown in Kabete and Mwea. The experiments were laid out in a split-split plot design with irrigation as the main plot factor, organic manure application as subplot and bean varieties as sub-sub plot factor. The treatments were replicated three times. The irrigation treatments were: rain-fed and supplementary irrigation. The organic manure treatments were control (0 t/ha), cattle manure (10 t/ha) and chicken manure (10 t/ha). Micronutrient (iron and zinc) dense bean varieties tested in the study were: Gofta, AND 620, Maharagi Soja, Nakaja and MLB 49/89A. Supplementary irrigation was provided by overhead sprinkler in Kabete and by flooding using furrows in Mwea three hours daily three times a week. Data collected included time to emergence, plant height, and time to 50% flowering, time to 50% podding, bean stem maggot severity, number of nodules per plant, pods per plant, seeds per pods, grain yield, and iron and zinc content in leaves and seeds.

Days to 50% flowering, 50% pod formation were longer in Kabete than in Mwea sites. Generally, higher yields were obtained under chicken manure treated plots than under cattle and control plots. Bean stem maggot severity was higher under chicken manure than under cattle and control. Under irrigated conditions, severity was lower by 18% than under rain fed conditions. Supplementary irrigation and organic manures improved number of nodules per plant, pods per plant and seeds per pod. On average, higher yields (39%) were recorded under irrigation than under rain fed conditions.

Supplementary irrigation increased plant height, number of nodules per plant, grain yield and leaf iron content of bean plants. However, seed iron content and zinc content in both leaves and seeds were not influenced by irrigation. Organic manures increased number of days to flowering and number of days to podding in some varieties. Chicken manure increased severity of bean stem maggot (BSM) in the short rains while cattle manure had no effect. Chicken and cattle manure increased grain yield and yield components in most varieties. Chicken manure however increased grain yield in more varieties than cattle manure. Ranking of varieties based on leaf iron content, from the highest to the lowest, was AND 620 347.7 ppm, MLB 49/89A 339.1 ppm, Maharagi Soja 329.4 ppm, Nakaja 322.6 ppm and Gofta 245.4.9 ppm. Variety MLB 49/89A had the highest seed iron content 78.13 ppm while all other varieties did not vary much in both leaf and seed zinc contents. Bean plants grown in Mwea site had higher leaf iron content (364.8.ppm) than bean plants grown in Kabete site (282.7 ppm) while the converse was the case with respect to leaf and seed zinc content. More iron was accumulated in leaves during the short rains (342.6 mean ppm) than during long rains (304.9 mean ppm) while more zinc was accumulated in leaves in the long rains than in the short rains. In conclusion, iron content of bean plants can be improved by supplementary irrigation. AND 620 variety had the highest iron in leaves and also the highest grain yield and therefore a suitable candidate for dual-purpose use (leaf and seed utilization). Leaf iron content of beans is influenced by location and soil iron content.

CHAPTER 1: INTRODUCTION

1.1 Background information

Micronutrient malnutrition known as 'Hidden hunger' is caused by lack of essential minerals and vitamins in the diets of people that feed mainly on starchy foods. Zinc and iron have been identified as limiting in most diets of people dependent on staple foods (WHO, 1995). Deficiencies in these micronutrients affect a third of the world's population causing stunting, birth defects, increased morbidity and mortality in children and mothers especially in vulnerable HIV/AIDS patients (Roy *et al.,* 1999; Brown and Wuehler, 2000; Umeta *et al*., 2000; WHO 2003). Micronutrient deficiencies also occur in communities due to attitudes and taboos on sources of rich foods (Timotewose *et al.,* 2000) and total lack of knowledge on prevalence of malnutrition. Bean varieties high in iron and zinc are being developed to improve diets of children and adults and act as a powerful means to combat micronutrient malnutrition (Kimani, 2005). Genetic selection for enhanced bioavailable levels of iron, zinc, calcium and protein in beans are being carried out through plant breeding (Robin *et al.,* 1999; Welch *et al.,* 2000).

Common bean is a major source of dietary protein and the third most important source of calories for over 100 million people in rural and poor urban Africa (Beebe, 2005). The Biofortification Challenge program intends to add value to staple foods by enhancing nutrient availability through breeding and development of agronomic practices (CGIAR, 2006). Beans selected for high iron and zinc concentration are being tested both on-station and on-farm as part of a regional effort in Kenya.

In Kenya, activities to develop micronutrient rich beans started at the regional program based at the University of Nairobi in 2001. Germplasm from several countries in East and Central Africa were screened for micronutrient density at Kabete (Kimani, 2003). Selected lines were evaluated for agronomic adaptability in farmers' fields in Marani and Suneka divisions in Kisii (Kenya highlands). Thirty-eight fast-track lines with high levels of iron (>70 ppm) and zinc (>35 ppm) were identified and constituted into regional nursery for further evaluation. Some of the outstanding lines included Nakaja, MLB 49/89A, Gofta, Maharagi Soja, and AND620 (Kimani *et al.*, 2005).

Although agronomic practices have been known to increase grain yield and influence the grain mineral concentration, little work has been done to develop management practices that would enhance expression of the high mineral density trait. Efforts to develop management practices to enhance mineral concentration in common bean lines were initiated recently at the Department of Plant Science and Crop Protection, University of Nairobi. Variations in iron and zinc content of bean varieties grown in different soils were observed suggesting that environment influenced the trait (Kimani *et al.*, 2005; 2006).

According to Rengel *et al.* (1999), amending soil with organic matter increases availability of micronutrients concentration in plants by increasing solubility of ferric oxides through redox potential. This strongly suggests that micronutrient density enhancement using organic manure fertilizers under different soil types can contribute to a better understanding of the performance of micronutrient dense bean varieties across environments and agro-ecological zones in eastern Africa (Kimani *et al.,* 2005). Little work on enhancing micronutrient density through agronomic practices and fertilization regimes under various soils has been reported (CIAT, 2005). Further more, it is not

Drought and poor soil fertility are common stresses that cause crop failures, especially in hilly terrains with no supplemental irrigation. Drought affects both the vegetative and reproductive stages of bean crops drastically reducing bean grain yields (Boutra, 2005). Associated with poor grain yield is the strong negative effect on the absorption of nutrients in general and on biological nitrogen fixation in beans in particular (Visser, 1994). However, the effects of low soil fertility and moisture stress on grain yield and iron and zinc concentrations are largely unknown.

About a half billion hectares of agricultural land is moderately or severely degraded (CIAT, 2006). This has greatly undermined the efforts of African farmers to improve their livelihoods through more intensive production. Growing of beans and other legume crops improves soil fertility and stabilizes the general cropping system (Hoshikawa, 1991). Rengel *et al.* (1999) reported that comprehensive agronomic approaches specific to fertilization strategies (timing and placement of fertilizer, crop rotations, soil types) aimed at enhancing seed nutrient concentration have yet to be pursued. Agronomic practices such as fertilization with organic manures has the potential to enhance the micronutrient density in beans. Variation of iron and zinc content in bean varieties planted under different soil types manifested that micronutrient density trait is influenced by the environment (Kimani *et al.*, 2005). Due to erratic and uneven distribution of rainfall, irrigation is carried out because common beans are sensitive to moisture shortage and this can affect yield.

Limited work has been conducted on enhancement of micronutrient density of beans through appropriate agronomic practices and fertilization regimes under different soil types. Hence, there is need to conduct studies on the effect of supplementary irrigation, organic manure application and soil type on micronutrient density and yield of beans

1.3 Objectives

Overall Objective

The overall objective of the study was to determine the effect of supplementary irrigation and organic manure application on micronutrient density and yield of five bean varieties in two sites (Kabete and Mwea) with different soil types.

Specific objectives

1. To determine the influence of supplementary irrigation on micronutrient density and yield of five bean varieties.
2. To determine the effect of organic manure (cattle and chicken manure) on micronutrient density and yield of five bean varieties.
3. To determine the micronutrient density and yield performance of five bean varieties grown in two different locations.

1.4 Hypotheses

1. Supplementary irrigation has no effect on iron and zinc concentration of leaves and grains of common bean genotypes
2. Fertilization with organic cattle and chicken manures has no effect on iron and zinc concentration of leaves and grains of common bean genotypes.
3. Locations and seasons have no effect on yield, leaf iron and grain concentration of five micronutrient dense bean varieties grown under different organic manures and watering regimes.

CHAPTER 2: LITERATURE REVIEW

2.1 Importance and ecology of beans

Grain legumes domesticated early in history of plant cultivation in America, India, and Africa are widely distributed in various regions of the world due to their nutritional values and unique capacity to fix nitrogen (Willey, 1975). Though originally native to Central America, beans were taken to Europe, Asia and brought to Africa by Portuguese explorers in the 16th Century. Currently more than 70 countries in Africa, America, Europe and Asia grow beans (FAO, 1999; Beebe *et al.,* 2001).

The characteristics and constraints of the African bean production environment (ABPE) were described by Wortmann *et al.,* (1994) and later published in the African Bean Atlas (Wortmann et al., 1999). The area under bean production has increased significantly on annual bases. It was estimated at 58,260 ha in 1985 (Summerfield and Roberts, 1989), to 400,000 ha in 2004 (Buruchara, 2005). Because of the wide cultivation in Sub-Saharan Africa (over four million ha annually), beans greatly contribute to food security as a source of income (Buruchara, 2005). Africa's cities are expanding and market demand for beans is rising rapidly and opportunity for farmers especially women to increase their income is eminent (Wortmann *et al.*, 1998**).** Common bean (*Phaseolus vulgaris* L.) is generally accepted because it provides relatively low cost protein, calcium, iron, zinc, thiamine and riboflavin. Major commercial bush types such as Rose coco have an average composition of 11% moisture, 1.6% fat, 3.6% ash, 37% protein (Buruchara, 2005).

In Kenya, beans are second in importance to maize grown in five provinces namely Eastern, Central, Nyanza, Western and Rift valley Provinces (Rono *et al.*, 1999). The quantity produced increased from 11,000 tons in 1980, to 34,400 tons in 1989 and 684,000 tons in 1995, (FAOSTAT, 2006). The area under bean production has risen from 600,000 ha in 1980 to over 1 million ha in 2008. Production of beans in Kenya like in other African countries is primarily by smallholder farmers especially women. Most of this production is for home consumption and is increasing for income generation that is why common bean is referred to as a woman's crop (CIAT, 2000)..

2.2 Ecology of the bean crop

Common bean is a warm season crop that matures between 80 and 120 days from planting to maturity depending on variety (Hardman *et al.*, 1990). Some early maturing varieties take 60-110 days (Aduol, 1993). Beans thrive well in fertile, well-drained sandy loams with adequate moisture during the growing season and dry weather towards harvest. They do not tolerate waterlogging and high soil pH>7.2 because it induces chlorosis as a result of iron and zinc deficiencies (Hardman *et al.*, 1990; Franzen and Moraghan, 1995).

Growth type of the bean crop takes various forms as bush, erect, 25-60cm, or climbing and twining which grows to a height of 25-60 cm or 2-3 m, respectively. Flowers appear at four weeks after sowing. They may be variegated white, purple, or pink. Pods that are 10-20 cm long and 1-1.5 cm wide develop rapidly after flowering and mature within two weeks in early maturing cultivars. White and navy grain types have small seeds weighing 10-30 g, while large seeded types may weigh 50 -70 g per 100 seeds (Nwokolo and Smartt, 1996).

In Eastern, Central and Southern Africa, bean production is mainly rain fed (Kimani *et al.*, 2001). The cropping systems of beans are sole cropping and intercropping with cereals, beverage crops like coffee, plantain and forage crops. Generally, compound inorganic fertilizers like diamonium phosphate (DAP), triple supper phosphate (TSP) and Calcium ammonium nitrate (CAN) are used in bean production. Fertilizer application rates of N 64 kg/ha, P 35 kg/ha, K 35 kg/ha, and trace elements Fe, Mg and Zn are used in soil or foliar sprayed, depending on spacing and cropping system (Ogola, 1991).

Bean is cultivated at elevations that vary from 900 m to 2100 m with a relative humidity of 50%. Temperature ranges of 16-24°C are optimal for flowering, growth and production (Aduol, 1993). With extreme temperatures below 10°C or over 30°C, frost and high radiations kill plants, blossoms drop and fruit failure occurs (Muturi, 2000).

2.3 Beans in human nutrition

Processed or unprocessed beans are storable and transportable concentrated protein food suitable for both rural and urban utilization (Willey, 1975). Beans contain dietary fiber (15.2 %) and 4.8% crude fiber which aid in the control of renal, colon cancer and coronary heart diseases. The carbohydrates in beans are of slow release and greatly reduce obesity by lowering blood cholesterol (Nwokolo and Smartt, 1996). Nutritionists characterize the common bean as a nearly perfect food because of its high protein content (27-40%), iron, zinc, vitamin A; a generous amount of fiber, complex carbohydrates, and other dietary necessities (GAIN, 2006). Foods containing more than one nutrient are wholly essential to the human body because of the nutrients they provide (Tull, 2002).Therefore, beans are essential in human nutrition as they provide calories, protein, essential minerals and vitamins. In tropical and sub-tropical countries, diets are deficient in protein, so communities readily accepted and consume beans (FAO, 2000). The Protein Advisory Group of Uganda (PAG) estimated average daily beans consumption as 55 g in Rwanda, 65 g in Kenya, and nearly 75 g in Uganda (PAG, 1993).

In rural areas, bean consumption is high due to shortage or affordability of other vegetables or protein sources as poor starchy diets dominate (60%- 80%). Hence, beans are used to replenish nutrients (IFPRI, 2002). Bean leaves are used as green vegetables, mashed in potatoes, maize and plantain to make a complete meal. Beans straw is burnt into ashes, distilled and added to soften vegetables in order to give alternative tastes (IPGRI, 2003).

In farming systems, common bean improves soil fertility by fixing atmospheric nitrogen into the soil (Allen, 1990). Managing soils by cycling of nutrients is a viable approach to sustainable soil fertility in Sub Saharan Africa (Amede *et al.*, 2004). Beans and other legumes fix 44 to 66 million tonnes of nitrogen into the soil annually. This provides nitrogen required for growth of other crops. The amount of nitrogen fixed varies with species (Hoshikawa, 1991; Mkandawire *et al.,* 1998).

2.4 Constraints to bean production

The major constraints limiting sustainable bean crop production are moisture shortage, declining soil fertility, pest and diseases and high fertilizer costs affecting smallholder farmers in Sub-Saharan Africa (Ojiem *et al.*, 2000; Cheruiyot *et al.* 2001; Chemining'wa *et al.,* 2004). Other stresses and socio-economic factors include migration from rural to urban and to marginal lands, low yielding varieties and inadequate supply of quality seeds (Okonda, 2007). Among the major nutrients, requirements for N and P exceed any nutrient because soils in the tropics do not have enough of these nutrients to produce high sustainable yields (Mkandawire, 1996).

2.4.1 Moisture shortage due to drought

Drought affects the vegetative and reproductive growth stages of beans greatly limiting growth. Boutra and Sandra (2005) evaluated the effect of water stress at flowering and pod filling in two bean genotypes of tropical and Mediterranean origins. The findings from the two phenological stages (flowering and pod filling) indicated that yield components (leaves, stem height, branches, nodes per stem) were greatly reduced due to drought; and time to maturity was prolonged.

There is a strong negative effect associated with drought because it limits absorption of nutrients by crops and causes a decline in biological nitrogen fixation of beans. Soil moisture shortage often limits phosphorus availability to plants and this reduces nitrogen fixation by bean plants in particular (Bert Visser, 1994).

2.4.2 Soil fertility decline

Most of the bean production areas in Sub-Saharan Africa are very low in soil fertility. Soil fertility is one of the major constraints in bean production in the region (Wortman *et al.*, 1998). It has a significant influence on plant growth and grain yield (Welch, 2004). There is a widespread decline in soil fertility in Africa with a half billion hectares of agricultural land considered moderately or severely degraded (CIAT, 2006). There is a higher rate of transported nutrients and minerals from cropped areas as a result of mining, crop removal, leaching and runoff. This depletes the soil nutrients especially when there is limited or no replenishment of nutrients into the soils (Katyal and Sharma, 1997; CIAT, 2000). Nutrient and mineral deficiencies notably N, P, Fe and Zn affect crop growth and seed formation (Howard, 1999).

Soil infertility is aggravated by overgrazing, population pressure, clearing trees and vegetation, migration and expansion into marginal and fragile areas, burning, insufficient decomposition of organic manures, and transport of nutrients out from the field and poor soil and water conservation (Visser, 1994). These factors increase nutrient deficiencies in soils and make micronutrients less available to crops particularly under moisture shortage (Runkulatile, 1991; Bouis, 1999).

2.4.3 Diseases

Diseases caused by viral, bacterial and fungal pathogens affect bean performance in the field resulting in partial or total crop failure. Diseases include angular leaf spot, anthracnose, common bacterial blight and bean common mosaic virus (Wortman and Allen, 1994). Most of the viral and fungal diseases cause stunting of crops, contamination of produce and reduced yields through infection of plant parts (Hill and Walker, 1991). Damping- off and root rot caused by *Rhizoctonia solani* and species of *Pythium* and *Phytophthora,* affect production of legumes in the greenhouse causing stunted growth and poor plant stands (Wheeler and Rush, 2001).

2.4.4 Insect pests

Insect pests affect crops in the field and in storage reducing crop yields and seed quality. The bean stem maggot (BSM), or bean fly, is the major field pest of economic importance that can result in total crop failures due to damages at seedling stage. It causes root rots most of which result in total crop failure. Wireworms, cutworms, bean thrips, leafhoppers and pod borers greatly affect beans while storage pests affect bean seed quality (Hardman *et al.*, 1990; Wortman *et al.*, 1998; Okonda, 2007). Storage losses from bruchids, common bacterial blight, aphids and bean common mosaic virus are other major constraints (Wortmann and Allen, 1994).

2.5 'The Hidden hunger challenge'

2.5.1 Micronutrient malnutrition

Micronutrient malnutrition referred to as 'hidden hunger' is the lack of essential nutrients in the diet and anaemia is the indirect indicator. Protein, vitamin A, zinc and iron are the most notable nutrients whose deficiencies cause severe morbidity and mortality rates worldwide affecting learning and productivity. Micronutrient malnutrition occurs in 30–40% children, 52% pregnant women, 30% adults and 45% elderly people (WHO, 2001).

The UN World Population prospects reported that by 1999, there were two billion anemic people worldwide. Of the 535 million Africans, 46% (244 million) are already affected by anemia (UNICEF, 2000). Malnourished and anemic people are four times more in Sub Saharan Africa than other parts of the world (WHO, 2000; FAO, 2001). The major cause of deficiencies is poor diets high in carbohydrates only (cereals, potatoes, cassava) and limited in proteins, minerals and vitamins. Rampant poverty makes it difficult for most families to afford animal-based products, which are the main sources of vitamins and minerals. Iron deficiency (anemia) incidence is reported as 8% in Ethiopia, 67% in Tanzania and 69% in Burundi (ASARECA, 2004; UNICEF, 2004). Seven out of ten children in rural areas die from deficiency conditions while those who survive suffer from improper brain and intelligent quotient (IQ) development. The national loss is rated at 1.5% loss of Gross Domestic Product (GDP) earnings (FAO, 2001). Sixty percent of pre-school children in Kenya are estimated to have iron deficiency and thus anemic. In Ethiopia, Mali, and Mozambique, the rates are up to 75 %, (UNICEF, 2004; GAIN, 2006).

In developing countries, close to 20 million pregnant women are vitamin A deficient and about one-third of them are also clinically night blind (Harvest Plus, 2003). Seventy percent (70%) of pre-school children in Kenya are estimated to be Vitamin A deficient. The Vitamin A deficiency rates in Nigeria and Mozambique are 25 and 26%, respectively (UNICEF/The Micronutrient Initiative, 2004). Approximately 30,000 people are estimated to be at risk of inadequate zinc intake in Kenya. The clearest indicator of zinc deficiency is stunting in children (Gibson, 1994; FAO, 2004). Hidden hunger poses a serious health challenge particularly among women and children, it has a high social and economic cost due to reduced work capacity and tragic loss of human potential (FAO, 1993). By 2012, hidden hunger will cause one million children less than five years of age to die from preventable diseases, 50,000 women to die from childbirth and 10,000 infants to be born with preventable defects; thus, the nutritionists described anaemia and iron deficiency as a national disaster (CGIAR, 2002; GAIN, 2006).

Iron and zinc deficiency prevalent in Sub Saharan Africa calls for Biofortification of beans to improve diet, prevent anemia, mitigate severity of HIV/AID and reduce morbidity and mortality rates (UNICEF, 2004; CGIAR, 2006). Common bean offers a unique opportunity to alleviate the situation because it is widely grown in Africa with over 4 million hectares annually (Buruchara, 2005). Varieties rich in iron and zinc offer a powerful means of combating malnutrition and disease in the region (Welch and Graham, 2000; Welch *et al.*, 2004; Kimani, 2005).

2.5.2 Key micronutrient deficiencies

Iron deficiency

Iron deficiency is the most common micronutrient deficiency in the world and the main cause of anemia, a condition in which the blood contains low levels of red blood cells. Two billion people worldwide are estimated to suffer from anemia, about 50% of all anemia's' can be attributed to iron deficiency (WHO/UNICEF, 2004). Iron deficiency weakens the immune system, causes fatigue and reduces work capacity. Severe anemia heightens the risk of women dying during childbirth. In children it impairs physical and mental growth, development and learning capability (WHO, 2005; Higdon, 2005).

Iron is a component of hemoglobin, the red pigment-carrying oxygen in the red blood cells of vertebrates. It is obtained from animals and plants. Meat, fish, liver, animal products, dark green leaves, tropical vegetables and finger millet are rich sources of iron. Lack of iron during childhood and adolescence impairs physical growth and mental development lowering learning capacity and the capacity for adults' physical labour (WHO, 1992; Fairbanks, 1994).

Vitamin A

Vitamin A is obtained from plants and animals. Beta-carotene is the precursor of Vitamin A in yellow maize, carrots, and yellow sweet potatoes while liver is main animal source. Vitamin A is essential for the functioning of the human immune system. It can help increase resistance to disease, protect against blindness, and improve chances for survival growth, and development (WHO, 2006).

Zinc

Zinc is an essential micronutrient for growth in both plants and animals, It is key in plant physiological functions of enzymes and carbohydrate metabolism occurring in photosynthesis and respiration. Zinc deficiency in plants causes short internodes stunted growth, chlorosis and reduced physiological functions. In humans, zinc is a constituent of insulin used in the treatment of diabetes and is documented to possess antiviral, anti-bacterial and anti-cancer properties. The human body relies on zinc to heal wounds, grow and repair body tissue, properly clot blood, and ensure sound fetal development (Brown and Wuehler, 2000). Chronic zinc deficiency enhances susceptibility to killer diseases especially in nutritionally vulnerable populations, severely malnourished children or those with persistent diarrhea or respiratory problems (GAIN, 2006).

2.6. The Biofortification challenge

2.6.1 Biofortification

Micronutrient malnutrition 'hidden hunger' poses a challenge to plant breeders to focus on production of quality food crops through biofortification. Biofortification is the breeding of food crops with high levels of bioavailable minerals and vitamins in their seeds and roots that are harvested and eaten (House *et al.*, 1999). This new paradigm shift from quantity to quality production of crops is a revolutionary process that nutritionally enhances staple crop varieties with higher levels of vitamins and minerals. It holds great potential to improve the health of poor people in rural developing countries (House *et al.*, 1999; Graham *et al.*, 1996). As an additional tool to fight and improve the nutritional status of the poor, biofortification complements existing nutrition interventions, vitamin and mineral supplements, commercial food fortification, and dietary diversity (Combs *et al.*, 1996).

Research reveals that high levels of minerals in seeds (shared by the poorest of farmers at no additional cost) contribute to stronger and hardier plants, leading to improved pest and drought resistance and increased productivity. Through biofortification, farmers get crop varieties that naturally reduce anemia, cognitive impairment, and other nutritionally related health problems potentially in millions of people (Harvest plus, 2006). Nutrient dense seeds are also associated with greater seedling vigor, higher plant yields, as deeper roots tap subsoil water and minerals more efficiently in low fertility soils reducing the need for fertilizers and irrigation (Bennett and Bunting, 1987; Marschner, 1995; Welch, 1995; Graham and Welch, 1996; Welch, 1999).

2.6.2 The Biofortification Bean Program

The Biofortification Challenge program intends to add value to staple foods by enhancing nutrients availability through breeding and development of agronomic practices (CGIAR; 2006). Beans selected for high iron and zinc concentration are being tested both on-station and on-farm as part of a regional effort.

Genetic selection of beans for enhanced bioavailable levels of iron, zinc, calcium and protein have been carried out through plant breeding (Robin *et al.,* 1999; Welch *et al.,* 2000). As such bean varieties high in iron and zinc are being developed to improve diets of children and adults and act as a powerful means to combat micronutrient malnutrition (Kimani, 2005).

In Kenya, activities to develop micronutrient rich beans started at the regional program based at the University of Nairobi. Germplasm from several countries in East and Central Africa were screened for micronutrient density at Kabete (Kimani, 2003). Selected lines were evaluated for agronomic adaptability in farmers' fields in Marani and Suneka divisions in Kisii (Kenya highlands). Thirty-eight fast track lines with high levels of iron (>70 ppm) and zinc (> 35 ppm) were identified and constituted into regional nursery. Some of the outstanding lines included Nakaja, MLB 49/89A, Gofta, Maharagi soja, and AND620 that were been used in this study (Kimani *et al*., 2005).

2.7 Effect of supplementary irrigation on common bean performance

In Africa, low yields of beans occur mostly due to fluctuating weather resulting from global warming. Dry spell limits moisture availability for optimum crop growth. Yields in Africa average 400-600 kg/ha compared to 800-1500 kg/ha in warm temperate regions where moisture is not limiting (HCDA, 2007). Therefore, irrigation is required for common bean production in dry areas because of erratic rainfall and uneven distribution. Moisture shortage during the early vegetative and reproductive phases can significantly affect crop yield (HCDA, 2007). There are various irrigation methods used for common bean production. These include furrow irrigation by flooding (practiced in Mwea) and overhead sprinkler system (used in Kabete). However the inadequate control of water quantity results to high water losses, low irrigation efficiency and may result to subsequent drainage and salinity problems (FAO, 2001).

Lack of water during the vegetative and reproductive stages is the most limiting factor in bean production. It results to poor stands, predisposes plants to fungal infections; at flowering stages flower abortion occurs and blossoms drop resulting to low pod set, poor seed quality and high yield losses (Aduol, 2000; Sezen *et al.*, 2006). As such bean crop requires supplementary irrigation to ensure vigorous growth, good stand establishment, hardy and resistant plants to diseases attacks. Supplementary irrigation also ensures effective pod filling and good quality seeds (Boutra and Sandra, 2001).

Generally, dry beans yield higher under irrigated than rain fed conditions. The average yields in arid areas with low rainfalls in Kenya were reported 3.6 times higher under irrigation than rainfed conditions (Jaetzold and Schmidt, 1983). Studies conducted on response of dry beans to irrigation indicated higher yields obtained with more irrigation frequency (Sezen *et al.*, 2006). As such, beans must be supplied with adequate water for higher yields and quality seeds. The effect of supplementary irrigation on micronutrient density of the micronutrient dense varieties recently developed by the CIAT program at the University of Nairobi has not been established.

2.7.2 Effect of organic manure on common bean performance

Most cultivated tropical soils are very low in fertility and these results in low unsustainable yields (Wrigley, 1982). Therefore, the addition of organic manure into the soil restores soil nitrogen and phosphorus as well as high level of organic matter. Organic manures are animal or plant residues added to the soil mainly to improve the physical condition and replenish humus status of the soil. Adequate manure maintains optimum conditions for the activities of soil microorganisms, and replenishes nutrients removed by plants or lost through leaching and soil erosion (Maerere *et al.*, 2001). Manures supply practically all the elements of fertility required by crops, though not in adequate proportions (Massomo, 1989). Food elements contained in manure are released and become available after manure is applied and decomposed by microorganisms in the soil. Farmyard manure (FYM), a mixture of cattle dung, bedding, and remnants of straw, hay and plant stalks fed to cattle, is the most commonly used organic matter, and has a value of increasing crop yield recognized from time immemorial (Nzuma *et al.*, 1998). Animal manure and compost are beneficial as they increase the water holding capacity and cation exchange capacity of the soil (Kimani *et al.*, 1998; Nzuma *et al.*, 1998). Therefore, organic nutrient sources are a logical alternative to expensive imported inorganic fertilizers because they avail inexpensive nutrients in farmers' fields (Woomer *et al.,* 1997; Chemining'wa *et al.*, 2004). In Kenya, farmers realize the need for soil amendments by using available resources such as farmyard manure, poultry wastes and piggery effluent in their fields though quantity and quality of these materials are limited (Ojiem *et al.*, 2000). Farmers also appreciate the use of

CHAPTER 3: MATERIALS AND METHODS

3.1. Experimental sites

The study was carried out at the University of Nairobi's Kabete Field Station and Mwea Irrigation Settlement Scheme in Kirinyaga district. Kabete soils are classified as humic Nitosols according to the FAO – UNESCO System (FAO, 1990). They are very deep well-drained, dark reddish brown, friable clays when moist, slightly acidic (pH 5.5) and deficient in available phosphorus and nitrogen (Siderus, 1976; Michieka, 1977). The climate in Kabete is semi-humid with a bimodal rainfall distribution. The mean minimum temperature is 12°C and mean maximum temperature is 23°C (Siderus, 1976). The long rains start in mid March to May, and the short rains start from mid October and taper off in December. According to the Kenya Soil Survey, the agro-ecological zone of the area is UM 3 i.e. Upper Midland Zone 3 (Jaetzold and Schmidt, 1983). The first field in Kabete was previously planted with sweet potatoes during the October-December 2006 short rains; while the second field was formerly planted with maize in 2007 long rains. Supplementary irrigation was provided using overhead sprinklers in Kabete Field Station.

The Mwea irrigation Scheme is located in Kirinyaga district of central Kenya about 120 km North West of Nairobi. This settlement scheme lies between latitude O° 37', and O° 45' West; and longitude 37° 17' and 37° 26' East; at an altitude of 1200 m above sea level. The topography is basin with free draining, reddish brown lateritic clay loams and heavy black cotton clays (Vertisols) mostly used for irrigated rice cultivation (Owido, 1981). The rainfall is about 950 mm with peak long rains mostly in April /May and June, while short rains start in October/November and end in December. Mean daily temperatures are relatively higher at 26-28° C with dry winds and cooler nights. In Mwea the experiments were located in a farmer's field previously planted with local maize (*Zea mays L.*) intercropped with pigeon pea (*Cajanus cajan L.)* in 2006, while the plot in 2007 was previously planted with watermelons. Supplementary irrigation was carried out through furrow irrigation.

3.2 Experimental design and treatments

The treatments laid out in a randomized complete block design with a split-split plot arrangement were replicated three times. Irrigation treatments were: rain fed plus supplementary irrigation and rain fed alone. Organic manure treatments were: cattle manure (10 tonnes/ha), chicken manure (10 tonnes/ha) and control (0 tonnes/ha). Five bean varieties namely Gofta, AND 620, Maharagi Soja, Nakaja, and MLB 49/89A were tested. Irrigation treatments were assigned to the main plots, organic manure treatments assigned to the subplots, while the bean varieties were assigned to the sub-sub plots. Cattle manure was obtained from the Biogas Engineering Unit while chicken manure was obtained from the Department of Animal Production Poultry Unit, College of Agriculture and Veterinary Sciences, Upper Kabete Campus, University of Nairobi.

The bio-fortified bean varieties were obtained from the Bean Unit of the College of Agriculture and Veterinary Sciences, University of Nairobi. The bean varieties consisted of bush types with determinate growth (AND620 and MLB49/89A), bush type with semi-determinate growth (Gofta), and climbing types of long maturity (Maharagi soja and Nakaja). Variety AND620 has red, mottled seeds; MLB49/89A has large, black seeds; Gofta has large, tan seeds; Maharagi soja has small, cream seeds; and Nakaja has large, yellow seeds.

3.3 Agronomic practices

The land was ploughed and harrowed by a tractor and experimental field laid out manually with the help of ropes and wooden pegs. A right-angled triangle base line (3 m X 4 m X 5 m) was erected to guide in making straight lines. Three blocks (replications) of size 12 m x 7.5 m were made with 2 m distance between them. The sizes of the plots, sub-plots and sub-sub plots were 36 x 7.5 m, 7.5 m X 4 m and 4 m X 1.5 m, respectively. Each block was separated with spacing of 4 m apart from the other while the subplots and sub-sub plots were each separated by 2 m and 1 m apart respectively. Supplementary irrigation was supplied three times a week between 8 am and 11 am i.e. three hours per irrigation day from the third week after planting. This timing was sufficient to provide plants with additional water to maintain field capacity for proper growth. Only one split plot was irrigated and the other depended entirely on rainfall. Sprinkler irrigation was used in Kabete and furrow irrigation was used in Mwea. Well-decomposed cattle and chicken manure obtained from the Poultry unit and Livestock unit in Kabete Campus were applied in the furrows mix in soil thoroughly before planting at the rate of 10 t/ha. Two kg of cattle or chicken manure was mixed in furrows of the target plots. One bean seed was planted per hill at a spacing of 50 cm between rows and 15 cm within plants. The spacing was the same for all the varieties. The plants were sprayed with Dimethoate (40%) at emergence, flowering and pod formation to reduce infestation by bean stem maggots, aphids and other pests. Hand weeding was carried out three times; at two, four, and seven weeks post emergence to keep the fields relatively weed free.

3.4 Field and laboratory data collection

3.4.1 Soil sampling and weather data collection

Soils were sampled at the experimental sites and analyzed in the laboratory for macro and micronutrients, pH and organic matter content. The sampling across the field was carried out with a hoe at a depth of 30 cm from topsoil; the samples were mixed to make one composite sample for each experimental site. Two samples (one kg) each of wet fresh soil were oven dried at 80° for 72 hours to determine the available soil water. Soil moisture content was determined using the following formula:

% soil moisture = [(Fresh soil weight - dry soil weight) / fresh soil weight (g)] x 100

Weather data for average daily rainfall and mean temperatures were collected from monthly records at the University of Nairobi's Kabete Field Station, Mwea National Irrigation Board and KARI-Mwea.

3.4.2 Growth and development, nodulation and pest severity, days to emergence, days to 50% flowering and days to 50% pod formation

Number of days to emergence was assessed by counting the number of days from the date of planting to 50% emergence. Number of days to flowering was assessed when 50% of plants in a plot had developed flowers from the time they first emerged. Number of days to podding was counted from the first date of plant emergence to the time when 50% of the plants in a plot had formed pods. Number of days to physiological maturity was determined when at least 80% of all the plants in a plot were fully mature i.e. not growing any longer and whole plants dried up.

Plant height and pest severity

Plant height was assessed at every two weeks' intervals. Five plants were randomly selected from the two middle rows of the four rows in a plot and measured. A ruler placed next to the live plant base and growing plant tip was used to measure heights and recorded in centimeters.

Pest and disease severity were observed based on absence or presence of visible symptoms on the bean crop. A rated scale of 1 to 9 was used to assess visible disease and pest attack, susceptibility and severity of symptoms (Schoonhoven and Pastor-Corrales, 1987). Severity was rated as 1-2 =

very severe, 3-5 = severe, 5-7 = moderate and 8-9 =mild. The most common pest observed was Bean Stem Maggot or the bean fly.

Nodulation

The average number of nodules per plant was determined at early flowering when the nodules were fully matured at about 30-35 days after planting. Three plants were randomly uprooted from the two middle rows in each plot. The plants were washed individually in water to remove soil and shoots were separated from roots. The nodules were carefully removed from the roots, placed in a container and counted.

3.4.3 Grain yield and yield components

Pod and seed count

At physiological maturity (just before harvest), five plants were randomly picked from the two middle rows of each plot. The pods on each plant were counted threshed, and the seeds were counted. The average number of pods per plant and seeds per pod were then determined.

A hundred seed weight

After harvest, 100 sun dried seeds were picked randomly from every plot and oven dried at 70^0C for 48 hours till moisture content declined to about 12%. Each sample was weighed using an electric balance model XL 6100, manufactured by Denver Instrument Company, USA. The weights of the seeds were recorded in grams

Grain yield

Grain yield was determined after harvest and sun drying of seeds from all the plants in each plot in grams per plant. The yield was then converted to kg/ha.

3.4.4 Micronutrient analysis in leaves and seeds

Micronutrient density in leaves was determined prior to flowering. The most recently matured leaves (MRML), i.e. fully opened were sampled from ten plants randomly selected from the middle rows. These leaves were washed under tap water to remove loose soil, dipped in weak hydrochloric acid (83 ml acid in 5L water) to remove contaminants, then double rinsed in distilled water to neutralize the acid. The clean leaves were then left to dry in the oven at 70°C for 48 hours and ground into fine powder using an electric iron free grinder Model RETSCH MM 2000 (Germany), (Okalebo *et al.*, 2001). After harvest, a handful of sun-dried seeds was picked washed and wiped dry with a clean cloth. The seeds were dried in the oven and crushed in a porcelain pestle and mortar into coarse particles. These were later ground into fine powder using the same electric grinder machine. The procedures followed in processing leaves and seeds ensured reduced contamination that can influence iron or zinc levels during the readings with Atomic Absorption Spectrophotometer (Robinson, 1993; Walls *et al.*, 2005).

One gram of the finely ground leaf or seed sample passed 2 mm sieve was weighed into digestion test tubes and 10 ml Nitric acid (70% HNO_3) added then left to digest overnight at room temperature. The digested solution was heated at 120°C for one hour then the temperature was raised to 175°C and heated at this level for twenty minutes. At this high temperature, smoke like red gas fumes appeared and cleared shortly. The mixture was then removed from the block digester and allowed to cool for half an hour. After the digest was cooled and cleared, 4 ml perchloric acid (70%

$HCLO_4$) was added and heated again at 120°C for one hour, then temperatures were raised to 175°C and heated at this level for twenty minutes. Thereafter, the digest was heated at 225°C for ten to fifteen minutes until the digested liquid was clear. The digested liquid was then removed from the block digester (Benton 1984; Zarcinas *et al.*, 1987).

After cooling, about 2-ml of the cleared digest was topped up to the mark with 1% nitric acid (10 ml acid in 1000 ml distilled water) into 50 ml volumetric flasks. The digest was then left to settle for about an hour and filtered through ash less filter paper into 100 ml bottles closed tightly ready for Fe and Zn readings according to respective wavelengths (AOAC 2000; Okalebo and Woomer, 2001).

Readings for iron and zinc were taken with the atomic absorption spectrophotometer AAS digital (Model 210 VGP Buck Scientific, Germany) in the Soil Science Laboratory of the University of Nairobi (UON). Absorption was measured at the wavelengths of 248.3 nm and 213.9 nm for Fe and Zn, respectively (Okalebo and Woomer, 2001). The following formula (Okalebo and Woomer, 2001; Okonda, 2007) was used to determine iron and zinc concentrations;

Fe or Zn (ppm) = $(a - b) \times V/W$

Where: a = concentration of Fe or Zn in the sample digest in parts per million

b = concentration of Fe, or Zn in the blank digest, parts per million

V= total volume in ml of digest at the end of the digestion procedure

W= weight of plant material sample digested in grams.

3.5. Data analyses

The data collected were subjected to analysis of variance (ANOVA) using GENSTAT 9^{th} edition (2006) software manufactured by Lawes Agricultural Trust (Rothamsted Experimental Station, United Kingdom). Mean separations were done using the least significant difference (LSD) test at 5% level of significance as described by Steel and Torie (1987).

CHAPTER 4: RESULTS

4.1 INFLUENCE OF SUPPLEMENTARY IRRIGATION ORGANIC MANURE AND LOCATION ON GROWTH, DEVELOPMENT, NODULATION AND PEST INFESTATION OF FIVE BEAN VARIETIES

4.1.1 Number of days to emergence

Supplementary irrigation and organic manure application had no significant influence on the number of days to emergence (Appendix 1). However, varieties responded differently across locations and seasons in days to emergence (Table 1a). All varieties emerged significantly earlier in Mwea than in Kabete in both seasons. In Mwea, MLB49/89A and Gofta took significantly shorter time than AND 620. In both sites and seasons, MLB49/89A took the shortest time to emerge while Nakaja took the longest time. In the long rains, Nakaja, Maharagi soja and AND620 emerged later than Gofta. Similar observations were made in the short rains except that AND620 was not significantly different from Maharagi Soja.

Table 1 a: Number of days to emergence of bean varieties grown in Kabete and Mwea

	Variety					
Location	**Gofta**	**AND 620**	**Maharagi Soja**	**Nakaja**	**MLB 49/89A**	**Means**
Kabete	9.2	10.1	10.3	10.4	8.6	9.7
Mwea	6.2	6.6	6.6	7.0	6.0	6.5
Seasons						
Long rains	8.3	8.6	8.9	8.9	7.9	8.6
Short rains	7.1	7.9	8.1	8.4	6.6	7.6
Mean	7.7	8.3	8.5	8.7	7.3	8.9

$LSD_{0.05}$ Location	0.1
$LSD_{0.05}$ Seasons	0.1
$LSD_{0.05}$ Variety	0.3
$LSD_{0.05}$ Location X Variety	0.3
$LSD_{0.05}$ Season X Variety	0.3
CV	2.5 %

4.1.2 Number of days to 50% flowering

Supplementary irrigation had no significant effect on number of days to flowering (Table 2a). Generally, varieties interacted significantly with organic manures and locations affecting number of days to 50% flowering of bean plants. Application of chicken manure increased the mean number of days to flowering in all the varieties except Nakaja and AND620; whereas cattle manure application reduced the number of days to flowering in Maharagi Soja and Nakaja.

Variety Nakaja was the latest to flower among all the varieties irrespective of the organic manure treatment. Variety MLB49/89A reached 50% flower the earliest in control and cattle manure plots but it was not different from Gofta and AND620 in chicken manure treated plots. Gofta and AND620 were not significantly different in time to 50% flowering in all the manure treatments except that Gofta flowered earlier in plots treated with cattle manure than control and chicken manure treated plots.

Varieties flowered significantly earlier in Mwea than in Kabete. Variety MLB49/89A flowered earlier while Nakaja flowered latest in both sites. Gofta and AND620 flowered earlier than Nakaja at Kabete but took similar time to flower at Mwea. In both seasons Nakaja took the longest time to flower while MLB49/89A took the least time to flower. All the varieties flowered earlier in short rains than the long rains.

Table 2 a: Influence of manure, location and season on number of days to flowering in five bean varieties

	Variety (V)					
	Gofta	**AND 620**	**Maharagi soja**	**Nakaja**	**MLB 49/89A**	**Means**
Manure						
Control	32.1	32.7	34.7	37.7	29.1	33.3
Cattle	31.0	33.0	32.7	36.0	27.9	32.1
Chicken	33.7	33.5	36.3	38.6	32.5	34.9
Location						
Kabete	33.9	34.4	36.3	39.6	31.6	35.2
Mwea	30.6	32.0	33.0	35.2	28.1	31.7
Season						
Long rains	33.1	33.4	35.1	38.2	31.7	34.8
Short rains	31.0	32.3	33.6	36.8	27.4	31.5
Means	32.1	33.2	34.9	37.8	30.2	33.7
LSD$_{0.05}$ Manures		0.4				
LSD$_{0.05}$ Location		0.2				
LSD$_{0.05}$ Season		0.2				
LSD$_{0.05}$ Variety		0.6				
LSD$_{0.05}$ Manure X V		0.6				
LSD$_{0.05}$ Location X V		0.5				
LSD$_{0.05}$ Season X V		0.5				
LSD$_{0.05}$ Irrigation		NS				
LSD$_{0.05}$ Irrigation X V		0.6				
CV		2.3%				

Location had a significant interactive effect on mean number of days to flowering with both irrigation and manure application (Table 2 b). Supplementary irrigation increased number of days to flowering in Mwea but decreased in Kabete. Chicken manure increased number of days to flowering while cattle manure tended to reduce days.

Table 2 b: Effect of interaction of location with irrigation and manure, respectively, on number of days to 50% flowering of beans.

	Location (L)		
Irrigation	Kabete	Mwea	**Means**
Rain fed	36.7	30.9	33.7
Irrigated	33.7	32.5	33.1
Manure			
Control	35.3	31.3	33.3
Cattle	33.8	30.4	32.1
Chicken	36.3	33.5	34.9
L means	35.2	31.7	33.4
$\mathbf{LSD_{0.05}}$ Irrigation	NS		
$\mathbf{LSD_{0.05}}$ Manure	0.6		
$\mathbf{LSD_{0.05}}$ Loc X Irrigation	1.1		
$\mathbf{LSD_{0.05}}$ Location X manure	1.5		
CV	2.7%		

4.1.3 Number of days to 50% podding

Supplementary irrigation had no significant effect on number of days to 50% podding. However, organic manure, location and season significantly affected days to 50% pod formation (Table 3a). Varieties interacted significantly with irrigation, manure application, location and seasons in affecting number of days to 50% podding. Supplementary irrigation reduced the number of days to 50% podding in Gofta only. Varieties MLB49/89A and Nakaja podded earliest and latest, respectively, in both rain fed and irrigated plots.

Cattle manure had no effect on 50% podding in all varieties except Nakaja where it delayed podding. Chicken manure increased the number of days to podding in Gofta, AND620 and Nakaja but no effect was observed in Maharagi soja and MLB49/89A.

Each variety podded earlier in Mwea than in Kabete by a range of about 6 days (Nakaja) to 8 days (Maharagi soja). Variety MLB49/89A was the earliest to pod while Variety Nakaja was the latest. The difference in number of days to 50% podding between MLB49/89A and Nakaja in was 14 days in Kabete and 12 days in Mwea, while for all the other varieties AND620, Gofta, the range was about 2-4 days in the two sites. All varieties podded earlier in the short rains than in the long rains except MLB49/89A which took similar time to pod in both seasons.

Plants podded earlier in the short rains than in the long rains (Table 3 b). Also, plants took shorter days to pod in Mwea than in Kabete. Season had no significant effect on number of days to 50% podding in Mwea, but number of days to 50% podding was higher in the long rains than in the short rains at Kabete.

Table 3 a: Number of days to 50% podding as influenced by interaction of variety with supplementary irrigation, manure application, location and season, respectively

	Variety (V)					
	Gofta	**AND 620**	**Maharagi soja**	**Nakaja**	**MLB 49/89A**	**Means**
Irrigation						
Rain fed	54.4	58.6	60.4	63.7	50.4	57.5
Irrigated	53.3	59.0	60.1	63.5	50.3	57.2
Manure						
Control	53.4	58.5	60.0	62.9	50.1	56.9
Cattle	53.7	58.1	60.3	63.9	50.6	57.3
Chicken	54.5	59.9	60.4	63.9	50.5	57.8
Location						
Kabete	57.6	61.9	63.9	67.8	53.6	60.9
Mwea	50.1	55.7	56.6	59.4	47.1	53.8
Season						
Long rains	54.1	59.6	60.7	64.3	50.5	57.8
Short rains	53.6	58.0	59.8	62.9	50.2	56.9
V Means	53.9	58.8	60.3	63.6	50.3	57.4
LSD$_{0.05}$ Irrigation		NS				
LSD$_{0.05}$ Manure		0.4				
LSD$_{0.05}$ Location		0.2				
LSD$_{0.05}$ Season		0.2				
LSD$_{0.05}$ Variety		0.6				
LSD$_{0.05}$ Var X Irrigation		0.6				
LSD$_{0.05}$ Var X Manure		0.5				
LSD$_{0.05}$ Var X Location		0.5				
LSD$_{0.05}$ Var X Season		0.5				
CV		3.8%				

Table 3 b: Number of days to 50% pod formation as influenced by interaction between location and season

	Location (L)		
Season	**Kabete**	**Mwea**	**S-Means**
Long rains	61.5	54.2	57.8
Short rains	60.4	53.4	56.9
L-Means	60.9	53.8	57.4

$LSD_{0.05}$ Season 0.6
$LSD_{0.05}$ Location 0.5
$LSD_{0.05}$ Location X Season 0.7
CV 1.7%

4.1.4 Number of nodules per plant

Supplementary irrigation, organic manure treatments and seasons had a significant effect on number of nodules per plants (Table 4). Irrigated plots had a higher number of nodules per plant than non-irrigated plots. Cattle manure treated plots produced significantly more nodules than chicken manure treated and control plots. All varieties produced more nodules in Kabete than in Mwea. More nodules were produced during the long rains in all sites than in the short rains.

locations. Performance in plant height was Nakaja>Maharagi soja>Gofta>AND620>MLB49/89A in descending order of height. The plants were generally taller during the long rains than in the short rains.

Table 5: Plant height (cm) at four weeks after emergence in Kabete and Mwea over two seasons

	Variety (V)					
	Gofta	**AND620**	**Mah Soja**	**Nakaja**	**MLB49/89A**	**Means**
Irrigation						
Rain fed	29.1	30.5	23.4	46.1	33.8	32.6
Irrigated	34.5	32.1	26.1	50.5	37.6	36.1
Manure						
Control	29.0	29.9	24.2	46.4	31.9	32.3
Cattle	29.1	27.6	22.9	45.7	52.8	32.3
Chicken	37.5	31.3	27.2	31.9	38.9	38.5
Location						
Kabete	24.4	28.7	23.9	44.3	29.4	30.2
Mwea	39.3	33.8	25.5	52.3	41.9	38.6
Season						
Long rains	40.9	35.8	24.0	64.9	43.7	41.9
Short rains	22.7	26.7	25.5	31.8	27.7	26.9
V-Means	31.8	31.3	24.8	48.3	35.5	34.4
LSD$_{0.05}$ Irrigation		2.7				
LSD$_{0.05}$ Manure		2.4				
LSD$_{0.05}$ Location		1.4				
LSD$_{0.05}$ Season		1.5				
LSD$_{0.05}$ Variety		2.0				
LSD$_{0.05}$ Variety X Irrigation		NS				
LSD$_{0.05}$ Var X Manure		NS				
LSD$_{0.05}$ Var X Location		1.3				
LSD$_{0.05}$ Var X Season		3.3				
CV		4.8%				

4.1.6 Pest severity

Supplementary irrigation, variety, location and season had significant effects on bean stem maggot (BSM) severity while manure had no effect. Interactions between variety and irrigation, location and seasons respectively had significant effects on BSM severity. Supplementary irrigation significantly reduced BSM severity in Gofta, Maharagi soja and Nakaja but had no effect on MLB49/89A (Table 6a). All varieties had higher disease severity in Kabete during the short rains than in long rains. In Mwea, Nakaja and Gofta were the most affected while AND620 was the least affected. The interaction between manure and season had a significant effect on BSM (Table 6 b). During the long rains, chicken and cattle manure applications had no effect on BSM severity; however, in the short rains, chicken manure significantly increased BSM severity while cattle manure had no effect.

Table 6 a: Effects of the interactions between bean variety with supplementary irrigation, location and season respectively on bean stem maggot severity based on a scale of 1-9.

	Variety (V)					
	Gofta	**AND620**	**Maharagi soja**	**Nakaja**	**MLB49/89**	**Means**
Irrigation						
Rain fed	2.4	1.3	2.0	2.1	1.4	1.8
Irrigated	1.8	1.4	1.6	1.7	1.4	1.6
Season						
Long rains	1.4	1.0	1.3	1.2	1.1	1.2
Short rain	2.8	1.6	2.3	2.6	1.7	2.2
Location						
Kabete	1.9	1.2	1.5	1.2	1.1	1.4
Mwea	1.3	1.2	1.8	1.9	1.7	1.5
V-Means	2.1	1.3	1.8	1.9	1.4	1.7
LSD$_{0.05}$ Irrigation		0.1				
LSD$_{0.05}$ Season		0.1				
LSD$_{0.05}$ Location		1.0				
LSD$_{0.05}$ Variety		0.2				
LSD$_{0.05}$ Var X Location		1.2				
CV		23%				

Table 6 b: Effect of the interaction between organic manure and season on bean stem maggot severity in bean varieties

	Manure (M)			
Seasons	**Control**	**Cattle**	**Chicken**	**Means**
Long rains	1.2	1.2	1.2	1.2
Short rains	2.1	2.0	2.5	2.2
M-Means	1.6	1.6	1.8	1.7

$LSD_{0.05}$ Manure	0.1
$LSD_{0.05}$ Season	0.1
$LSD_{0.05}$ Manure X Season	0.2
CV	23%

4.2. INFLUENCE OF SUPPLEMENTARY IRRIGATION, ORGANIC MANURE AND LOCATION ON GRAIN YIELD AND YIELD COMPONENTS OF FIVE BEAN VARIETIES

4.2.1 Number of pods per plant

Supplementary irrigation, season and organic manure treatment had no effect on number of pods per plant. However, variety and location had a significant effect on this plant attribute. The interactions between variety and location and between variety and organic manure application were significant (Table 7a). Maharagi soja registered a higher number of pods per plant in Kabete than in Mwea, whereas the rest of the varieties were not significantly different in the number of pods per plant in the two sites. At Kabete, Maharagi soja had significantly the highest number of pods per plant. Variety MLB49/89A had similar number of pods per plant as AND620 and Gofta. However, both AND620 and MLB49/89A had more pods per plant than Nakaja. Similar observations were made in Mwea, where Nakaja had the least number of pods per plant and there were no differences amongst the rest of the varieties. Chicken manure treated plots had higher number of pods per plant in Maharagi Soja than manure treated and control plots. There were no differences among chicken, manure and control treatments with respect to number of pods per plant in all varieties.

Table 7 a: Effect of the interactions between variety with location and manure, respectively, on number of pods per plant of common bean

	Variety (V)					
Location	**Gofta**	**AND 620**	**Maharagi soja**	**Nakaja**	**MLB49/89A**	**Means**
Kabete	13.3	15.8	21.6	12.5	15.3	15.7
Mwea	13.9	14.2	16.1	10.3	14.2	13.7
Manure						
Control	12.0	13.2	17.5	12.3	15.1	14.0
Cattle	14.5	16.5	16.4	10.7	13.9	14.4
Chicken	14.4	15.3	22.7	13.9	15.2	15.8
V-Means	13.6	14.9	18.9	12.6	14.7	
LSD$_{0.05}$ Location		0.8				
LSD$_{0.05}$ Manure		NS				
LSD$_{0.05}$ Variety		2.0				
LSD$_{0.05}$ Var X Location		2.3				
LSD$_{0.05}$ Var x Manure		3.4				
CV		12%				

4.2.2 Number of seeds per pod

Supplementary irrigation had no significant effect on the number of seeds per pod while variety, manure, location and season significantly affected this parameter (Table 8). The highest number of seeds per pod was recorded in variety Nakaja whereas all the other varieties were not significantly different in this parameter. Chicken manure treated plants had significantly higher number of seeds per pod than both the control and cattle treated plots. There was no difference between the latter two in numbers of seeds per pod. The variety by season interaction was significant. Higher numbers of seeds per pod were recorded during the long rains than in the short rains. Nakaja had significantly the highest number of seeds per pod during the long rains while all other varieties had a simila number of seeds per pod. Number of seeds per pod was higher in the long rains than in the sho rains for all varieties except AND620.

Table 8: Effect of the interaction of variety with manure, location and season respectively on number of seeds per pod

			Variety (V)			
Manure	**Gofta**	**AND 620**	**Maharagi soja**	**Nakaja**	**MLB49/89A**	**Means**
Control	4.0	4.3	4.5	5.2	4.5	4.5
Cattle	4.5	4.2	4.2	5.3	4.2	4.5
Chicken	4.7	4.5	4.5	5.4	4.3	4.5
Location						
Kabete	4.2	4.3	4.4	5.2	4.4	4.5
Mwea	4.1	4.3	4.4	5.2	4.3	4.5
Seasons						
Long rains	4.8	4.4	5.0	5.9	4.7	4.9
Short rains	4.1	4.3	3.8	4.6	4.0	4.2
V-Means	4.4	4.4	4.4	5.3	4.3	4.5

LSD$_{0.05}$ Manure	0.1
LSD$_{0.05}$ Location	0.1
LSD$_{0.05}$ Season	0.1
LSD$_{0.05}$ Variety	0.3
LSD$_{0.05}$ Var X Manure	NS
LSD$_{0.05}$ Var X Season	0.3
LSD$_{0.05}$ Var X Location	NS
CV	5.5 %

4.2.3 A hundred seed weight

Variety, manure application, location and season had significant effects on 100 seed weight of bean plants while irrigation had no effect (Table 9). Variety AND620 had the highest seed weight while Maharagi Soja had the lowest seed weight. Chicken manure application produced a higher seed weight than cattle manure application. However, none of these treatments were significantly different from the control in 100-seed weight. Beans grown in Kabete had significantly higher 100-seed weight than beans grown in Mwea. The interaction of variety and season had a significant effect on 100-seed weight. No seasonal effect was noted in 100 seed weight of Gofta, AND 620 and MLB49/89A. However, Maharagi soja and Nakaja had higher 100 seed weight in the long rains than in the short rains.

Table 9: Effect of variety, manure, location and season on 100 seed weight (g) of beans

	Variety (V)					
	Gofta	**AND 620**	**Maharagi soja**	**Nakaja**	**MLB 499/89A**	**Means**
Irrigation						
Rain fed	31.1	38.1	23.7	28.7	30.3	30.4
Irrigated	32.7	37.4	28.9	32.8	33.8	33.1
Manure						
Control	30.9	37.1	27.4	30.1	32.3	31.6
Cattle	31.7	36.3	24.8	30.3	31.6	30.9
Chicken	33.1	39.4	26.7	31.6	32.5	32.6
Location						
Kabete	32.6	38.8	26.7	32.2	33.5	32.8
Mwea	31.2	36.7	25.9	29.3	30.7	30.8
Season						
Long rains	32.9	38.3	28.4	32.2	32.7	32.9
Short rains	30.9	37.2	24.2	29.3	31.5	30.6
V Means	31.9	37.7	26.3	30.7	32.1	
$LSD_{0.05}$ Variety		1.9				
$LSD_{0.05}$ Irrigation		NS				
$LSD_{0.05}$ Manure		1.2				
$LSD_{0.05}$ Location		0.7				
$LSD_{0.05}$ Season		0.7				
$LSD_{0.05}$ Variety X Irrigation		NS				
$LSD_{0.05}$ Var X Location		NS				
$LSD_{0.05}$ Var X Manure		NS				
$LSD_{0.05}$ Var X Season		2.2				
CV		4.8%				

4.2.4 Grain yield

Supplementary irrigation, organic manure application, location and season had significant effects on grain yield of bean varieties. Varieties interacted significantly with location, manure application, supplementary irrigation and season in terms of grain yield (Table 10). Supplementary irrigation improved grain yield in all the varieties except in Nakaja. AND620 and Gofta had higher yields than both Maharagi Soja and Nakaja. The latter two were not significantly different in grain yield, MLB49/89A had higher yield than Maharagi soja but not different from Nakaja. Under rain fed conditions, Maharagi Soja had significantly the lowest grain yield while the rest of the varieties were not significantly different. Chicken manure significantly increased yield in all the varieties except AND 620 and Maharagi Soja. Cattle manure increased grain yield in Gofta and Nakaja but had no effect on the rest of the varieties. Chicken manure produced higher grain yield than cattle manure in AND620 and MLB49/89A. Higher grain yields were observed in Kabete than in Mwea for all the varieties. On average, the long rains produced higher grain yield than short rains.

Table 10: Effect of interactions of variety with irrigation, location, manure and season respectively on grain yield (kg/ha) of beans

	Variety (V)					
	Gofta	**AND620**	**Maharagi**	**Nakaja**	**MLB**	**Means**
Irrigations						
Rain fed	971	947	715	1033	1078	949
Irrigated	1505	1513	1096	1163	1320	1319
Locations						
Mwea	909	1063	1022	902	1028	985
Kabete	1567	1397	789	1294	1370	1283
Manures						
Control	867	1244	927	840	949	965
Cattle	1316	996	788	1123	1134	1071
Chicken	1530	1451	1002	1331	1514	1365
Seasons						
Long rains	1611	1409	1147	1430	766	1273
Short rains	865	1052	664	1434	963	996
V Means	1238	1230	905	1098	1199	1130

LSD$_{0.05}$ Irrigation	193
LSD$_{0.05}$ Location	101
LSD$_{0.05}$ Manure	194
LSD$_{0.05}$ Season	101
LSD$_{0.05}$ Variety	159
LSD$_{0.05}$ Variety X Irrigation	223
LSD$_{0.05}$ Var X Location	224
LSD$_{0.05}$ Var X Manure	314
LSD$_{0.05}$ Var X Season	NS
CV	20.9%

4.3 INFLUENCE OF SUPPLEMENTARY IRRIGATION ORGANIC MANURE APPLICATION AND LOCATION ON MICRONUTREINT DENSITY IN LEAVES AND SEEDS OF FIVE BEAN VARIEITIES

4.3.1 Leaf iron content

Supplementary irrigation, season, location and variety had significant effects on leaf iron content of the bean plants (Table 11a). The interaction between variety and irrigation had a significant effect on leaf iron content. Supplementary irrigation increased leaf iron content in AND620, Maharagi soja and MLB49/89A but had no effect on this attribute in Gofta and Nakaja. Under rain fed plots, Gofta had lower iron content than AND620, Nakaja and MLB49/89A, but it was not significantly different from Maharagi soja. Similar observations were made under irrigated plots; however, Maharagi Soja had significantly higher iron content than Gofta. Significantly higher leaf iron content was recorded when varieties were grown in Mwea than when grown in Kabete. The ranking of the varieties based on leaf iron content was in the order: AND620>, MLB> Maharagi Soja> Nakaja >Gofta. Leaf iron content was higher in the short rains than in the long rains.

Interaction of irrigation with manure and location respectively significantly affected leaf iron content of bean plants (Table 11b). Irrigation significantly improved leaf iron content in all the manure treatments. In Kabete, irrigation significantly increased leaf iron content but had no effect in Mwea.

Table 11 a: Influence of supplementary irrigation, manure, location and season on leaf iron content (ppm) of beans.

	Variety (V)					
	Gofta	**AND 620**	**Maharagi soja**	**Nakaja**	**MLB49/89A**	**Means**
Irrigation						
Rain fed	260.1	323.5	272.8	308.5	306.7	294.3
Irrigated	299.6	372.0	386.1	336.7	371.5	353.2
Manure						
Control	311.9	350.7	327.7	300.6	361.7	330.5
Cattle	252.5	352.1	365.4	346.0	324.8	328.2
Chicken	275.2	340.4	295.2	321.0	330.8	312.5
Location						
Kabete	232.8	299.4	309.7	275.0	296.5	282.7
Mwea	326.9	396.0	349.2	370.1	381.7	364.8
Season						
Long rains	270.7	327.1	306.8	300.3	319.6	304.9
Short rains	289.0	368.3	352.1	344.9	358.6	342.6
V Means	245.2	347.7	329.4	322.6	339.1	323.7
LSD Irrigation		50.8				
LSD Manure		NS				
LSD Location		NS				
LSD Season		14.6				
LSD Variety		20.1				
LSD Variety X Irrigation		29.5				
LSD Variety X Manure		NS				
LSD Variety X Location		NS				
LSD Variety X Season		NS				
CV		13.5%				

Table 11 b: Effects of interaction of supplementary irrigation with organic manure and location respectively on leaf iron content (ppm) of beans

	Irrigation (I)		
Manure	**Rain fed**	**Irrigated**	**Means**
Control	307.5	353.5	330.5
Cattle manure	291.9	364.4	328.2
Chicken manure	283.5	341.6	312.5
Location			
Kabete	207.4	381.2	282.7
Mwea	358.0	348.3	364.8
I-Means	294.3	353.2	323.7
LSD Irrigation X manure		39.41	
LSD Irrigation X Location		39.16	
CV		13.5%	

4.3.2 Leaf zinc content

Leaf zinc was significantly affected by location and season, while irrigation and manure had no effect. Interaction of location with manure application and season respectively was also significant (Appendix 14a). There were also no significant variety interactions with other treatments with respect to leaf zinc content. Higher zinc content was recorded in Kabete than in Mwea. Leaf zinc content was significantly higher in the long rains than in the short rains.

Table 12: Effect of interactions of manure with locations and season, respectively, on leaf zinc content (ppm) of beans

	Location (L)		
	Kabete	**Mwea**	**Means**
Manure			
Control	38.8	27.5	33.1
Cattle	38.8	25.3	32.1
Chicken	37.6	28.7	33.1
Season			
Long rains	38.1	29.4	33.8
Short rains	38.7	24.9	31.8
L Means	36.7	28.9	
LSD manure		NS	
LSD Season		1.38	
LSD Location		1.38	
LSD Location X Season		1.95	
LSD Location X Manure		1.35	
CV		22.9%	

4.3.3 Seed iron content.

Seed iron content varied significantly with variety, manure, location and season but not irrigation (Tables 13 a and b). The interactive effects of locations with irrigation and irrigation with season significantly affected seed iron content (Appendix 15.) On average MLB49/89A had the highest seed iron content whereas iron contents of the rest of the varieties were not significantly different. Chicken and cattle manure treated bean plants had lower seed iron content than bean plants in the control plots (Table 13a).

Table 13 a: Influence of supplementary irrigation and organic manure application on seed iron content of bean varieties in parts per million (ppm)

	Manure			
	Control	**Cattle manure**	**Chicken manure**	**Means**
Variety				
Gofta	78.1	71.3	68.8	72.7
AND 620	73.5	69.6	72.7	71.9
Maharagi soja	76.0	69.4	70.4	71.9
Nakaja	70.6	67.5	71.7	69.9
MLB 49/89A	84.4	71.7	78.3	78.1
M-Means	76.5	72.5	72.4	72.9
LSD Variety	4.5			
LSD Manure	3.7			
LSD Variety X manure	NS			

Table 13 b: Effect of the interaction of manure with irrigation, location and season, respectively, on seed iron content (ppm)

	Organic manures			
	Control	**Cattle**	**Chicken**	**Means**
Irrigation				
Rain fed	77.9	73.3	73.2	74.8
Irrigation	75.2	70.2	71.6	72.3
Season				
Long rains	86.7	81.1	80.7	82.8
Short rains	66.4	62.3	64.1	64.3
Location				
Kabete	70.2	69.0	71.8	70.3
Mwea	83.0	74.4	73.0	76.8
M-Means	76.5	71.7	72.4	73.6
LSD Irrigation	NS			
LSD Manure	3.7			
LSD Season	2.9			
LSD Location	2.9			
LSD Manure X Irrigation	7.1			
LSD Manure X season	6.5			
LSD Manure X location	6.5			
LSD Locations X season	4.2			

4.3.4 Seed zinc content

Seed zinc content was only significantly different in locations and seasons. Variety and its interaction with other treatments had no effect on seed zinc content (Table 14). More zinc was accumulated in Kabete than in Mwea. Zinc was more available to plants under cattle manure and control than chicken manure.

Table 14: Influence of supplementary irrigation, organic manure treatment and soil type on seed zinc content (ppm)

	Location (L)		
Irrigation	**Kabete**	**Mwea**	**Means**
Rain fed	35.1	30.2	32.7
Irrigated	33.7	31.7	32.7
Manure			
Control	33.2	30.6	31.9
Cattle	34.3	31.1	32.7
Chicken	35.8	31.2	33.5
Season			
Long rains	32.8	29.3	31.0
Short rains	35.9	32.6	34.3
L-Means	34.4	30.9	32.7
LSD Irrigation		NS	
LSD Manure		NS	
LSD Season		1.0	
LSD Location		1.0	
LSD Location X Irrigation		1.7	
LSD Location X manure		2.7	
LSD Location X season		1.4	

CHAPTER 5: DISCUSSION

5.1 Effect of supplementary irrigation

Supplementary irrigation increased plant height, number of nodules per plant, grain yield and leaf iron content. Moisture stress has been reported to limit vegetative and reproductive growth of beans, resulting in poor crop stand and yield losses (Aduol, 2000; Boutra and Sandra, 2001;Sezen *et al.*, 2006). Studies by Boutra and Sandra (2001) and Sezen *et al.* (2006) also demonstrated that supplementary irrigation improved the yield and quality of dry beans. The increase in leaf iron content of irrigated plants could be attributed to improved iron uptake by crops. Moisture stress limits absorption of nutrients by crops (Bert Visser, 1994). These results clearly indicate that supplementary irrigation is a good option to produce high yields and higher leaf iron content in beans (Kimani, 2005; Okonda, 2007). Moisture stress is associated with decline in nodulation and nitrogen fixation by beans (Giller, 2001). This may explain the increase in nodule numbers under irrigated conditions. Supplementary irrigation reduced bean stem maggot severity. This is in agreement with the finding that moisture stress predisposes plants to insect attack and fungal infections (Sezen et al., 2006). The varieties responded differently to supplementary irrigation with respect to grain yield and leaf iron content among others. This indicates genotype by environment interaction with respect to these traits. Evaluation of bean germplasm for leaf iron content should be conducted under different moisture conditions. It is worth noting that supplementary irrigation did not affect seed and leaf zinc contents as well as seed iron content in beans.

5.2 Effect of organic manure application

Chicken manure increased number of days to flowering and number of days to podding in most varieties while cattle manure also delayed flowering and podding in a few varieties. This could be attributed to high N especially in chicken manure that may have extended vegetative growth at the expense of reproductive growth.

Cattle manure improved nodulation but chicken manure had no effect. Otieno (2006) reported similar findings in grain legumes. This could be attributed to the moderate dose of N that was present in cattle manure (2.04%N) compared to chicken manure (3.05%N). Application of moderate dose of N to legumes under N-limiting soil conditions has been reported to improve nodulation and nitrogen fixation. The legumes may require starter-N during the first 3 weeks "N-hunger" period when the nodules are not fully developed or not actively fixing atmospheric N_2 (Giller, 2001; Otieno 2006). High doses on the other hand reduce nodulation and nitrogen fixation (Giller, 2001).

Chicken manure increased severity of bean stem maggot (BSM) in the short rains. This finding is in agreement with reports that high soil fertility tends to attract pests and diseases. Byabagambi et al., (1997) and Nderitu et al., (1997) reported that BSM was associated with organic fertilizers and high soil fertility. Therefore, the high nutrient levels in chicken manure used in the experiment may have increased the attack by BSM.

Chicken and cattle manure increased number of seeds per pod, 100 seed weight and grain yield of beans in some varieties and not others. Chicken manure however increased grain yield in more varieties than cattle manure. Similar findings have been reported for grain legumes (Otieno, 2006).

Animal manure can provide nitrogen, phosphorous and micronutrients required by legumes and also increase water holding capacity and cation exchange capacity of the soil (Kimani *et al.*, 1998; Nzuma *et al.*, 1998; Otieno, 2006). Chicken manure may have increased grain yield in more varieties than cattle manure because it had higher nutrient levels as per the soil analysis results (Appendix 1). The varying grain yield response of the varieties to manure application indicates genotype by environment interaction for this trait.

Generally, chicken manure and cattle manure treatments had no effect on iron and zinc content in leaves and seeds of bean varieties. According to Rengel *et al.* (1999), amending soil with organic matter increases availability of micronutrients in plants by increasing solubility of ferric oxides through reduction to forms available to plants. Nutrient mobility from soil to plant and plant parts is effectively accomplished under high organic matter content because moisture is highly retained and plants easily obtain soil nutrients (Grusak *et al.*, 1999). The lack of clear micronutrient response to manure treatments in the present study may suggest that iron and zinc delivered by the manures were not adequate.

5.3 Effect of location

Plants emerged, flowered, podded and reached physiological maturity earlier in Mwea vertisols than in Kabete Nitosols. This could be attributed to distinctly warmer temperatures in Mwea (28°C) than in Kabete (24°C). Beans require warmer temperatures and sufficient moisture for faster germination, quick flowering and early maturity (Runkunlatile, 1997; Muturi, 2000; Aduol, 2001; Buruchara, 2005).

Nodulation levels were higher in Kabete than Mwea. This may suggest that indigenous rhizobia nodulating beans may have been higher in Kabete than in Mwea. Chemining'wa et al. (2004) reported high levels of indigenous rhizobia nodulating common bean in Kabete soils. Wangechi (2009) reported very low nodulation levels in snap bean in the study area in Mwea.

Beans grown in Kabete site had higher number of seeds per pod, 100 seed weight and grain yield than those grown in Mwea. On the other hand, bean plants grown in Mwea had higher leaf and seed iron contents than those grown in Kabete. In contrast, bean plants in Kabete had higher leaf and seed contents than bean plants in Mwea. This could be attributed to the differences in iron and zinc contents of the two sites. Mwea site was deficient in zinc (0.1 ppm) compared to Kabete (0.4 ppm) that in turn had higher iron content than Mwea site (Appendix 1). This suggests that the inherent soil iron and zinc contents play an important role in determining micronutrient density in bean varieties. Bean plants grown in soils low in iron and zinc may require to be supplemented with these nutrients. Zinc and iron fertilizer additions increase micronutrients iron and zinc levels in the plants (Marshner, 1995).

Varieties responded differently to the two locations in terms of micronutrient density, suggesting genotype by environment interactions. This finding is in agreement with observations made by Kimani (2005) and Okonda (2007).

5.4 Effect of variety

Bush and determinate varieties matured earlier than climbing variety types. The significant difference in days to emergence, days to flowering, days to podding and days to physiological maturity is attributed to genotypes of early maturity and late maturing. Varieties Gofta, MLB49/89A and AND620 emerged earlier than Maharagi soja and Nakaja in both sites. Similar results were obtained and recorded in farmers' fields and research stations in central and western Kenya (Kimani, 2005; Okonda, 2007).

Gofta had the least leaf and seed iron content probably due to its genetic make up. Variety MLB49/89A had the highest seed iron content 78.13 ppm, Gofta 72.71 ppm, AND 620 and Maharagi soja 71.94 ppm each, and Nakaja 69.93 ppm. In contrast, AND620 had the highest leaf iron content followed by MLB49/89A. Varieties did not vary in both leaf and seed zinc contents. Variation in iron content among the varieties suggests genotypic variation in ability to mobilize iron into leaves and reproductive parts. Concentrations of elements in edible portions of crops is a complex and dynamic process, it involves interaction of genotype, soil properties, environmental conditions and nutrients (Welch and Graham, 1999).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results clearly indicate that supplementary irrigation increased leaf iron content but had no effect on seed iron, and leaf and seed zinc contents. Supplementary irrigation also improved nodulation, grain yield and yield components. Response to supplementary irrigation was dependent on variety. Selection of micronutrient dense bean varieties should therefore be carried over a broad range of agro-ecological conditions due to genotype by environment interactions.

Chicken and cattle manures increased grain yield and yield components of beans. They however did not improve iron and zinc content in leaves and seeds of bean varieties as would have been expected. The amount of zinc and iron in manure may not have been adequate. Genotype by environment interaction was evident in grain yield trait as varieties responded differently to manure application.

Mwea site had more bean seed and leaf iron contents than Kabete location and the converse was the case with respect to bean seed and leaf zinc content. Since Mwea and Kabete had higher iron and zinc, respectively, it can be concluded that inherent soil micronutrient status is key in determining micronutrient density of bean varieties. Micronutrient response to location is dependent on variety due to genotype by environment interactions.

Seed iron concentration and grain yield were higher in variety AND 620 than most of the other varieties. The ranking of bean varieties based on these attributes and others is AND 620 >MLB 49-98A> >Gofta, >Nakaja and Maharagi Soja. Variety AND620 stands out as the most promising variety for farmer adoption. Its leaves (of AND620) are good source of iron if used as a vegetable. MLB49/98A is second in vigor, hardiness, high yielder with the highest zinc and quickest maturing. The seeds of MLB49/89A can make good component in a high iron concentrate supplement for humans and livestock.

6.2 Recommendations for further research

1. Evaluate a broader range of bean varieties for grain yield and micronutrient density in many agro-ecological zones and soil types
2. Evaluate bean varieties for micronutrient density using a broader range of organic manures, including compost and green manures
3. Conduct studies to establish the appropriate rates of chicken manure, cattle manure and other organic manure for improved seed and leaf micronutrient density in bean varieties
4. Conduct studies to determine appropriate irrigation regimes and soil and water conservation strategies that would enhance iron and zinc concentrations in leaves and seeds

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APPENDICES

Appendix 1 Chemical properties of soils of the experimental sites and organic manures used and soils type during the experiments in Kabete and Mwea sites

	Soils types		**Organic manures**	
Parameter	**Kabete Nitosols**	**Mwea Vertisols**	**Chicken manure**	**Cattle manure**
Soil pH	6.11	6.21	8.33	8.39
Nitrogen (%)	0.45	0.2	3.56	2.04
Phosphorous (ppm)	6.4	170.5	1133.3	500
Calcium (Cmol/kg)	8.25	7.6	26.67	28.76
Magnesium (Cmol/kg)	2.4	5.9	5.4	5.6
Organic carbon (%)	1.23	2.86	43.18	50
Potassium (meq %)	1.9	1.5	43	43
Manganese (meq %)	1.3	0.8	0.30	0.60
Sodium (Cmol/kg%)	0.42	0.42	22	3
Zinc (ppm)	0.4	0.1	1.40	0.20
Iron (ppm)	0.4	0.3	3.4	3.7
Copper (ppm)	0.09	0.04	0.64	0.08

Appendix 2 Weather data during experimental period

Month	**Temperature (^{o} C) Kabete**		**Rainfall Kabete**	**Temperature (^{o} C) Mwea**		**Rainfall Mwea**
	Mini	Max	(mm)	Min	Max	(mm)
September 2006	12.4	22.7	25.9	14.2	24.4	20.7
October 2006	14.0	24.8	87.4	15.3	25.9	25.8
November 2006	14.7	22.	348	15.4	24.2	105.9
December 2006	14.6	22.9	246	14.1	24.3	237.2
January 2007	14.3	23.6	30.2	15.2	25.8	38.9
February 2007	13.9	25.4	90.0	15.6	26.9	29.3
March 2007	14.4	25.0	52.6	16.9	27.3	23.7
April 2007	14.9	24.2	348.8	16.0	25.1	98.7
May 2007	15.2	25.0	234.9	15.4	24.3	279.3
June 2007	14.8	24.8	189.6	13.8	23.6	74.0
July 2007	13.1	22.7	89.6	17.1	26.8	41.7
August 2007	12.6	22.1	46.8	17	26.9	31.5

Sources; UON Kabete Field Station, Kenya Agricultural Research Institute and National Irrigation Board at MIAD.

Appendix 3 ANOVA days to emergence

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	0.7389	0.3694	1.46
Locations	1	912.025	912.025	<.001
Error (a)	2	0.5056	0.2528	0.5
Seasons	1	79.3361	79.3361	<.001
Seasons x locations	1	8.4028	8.4028	<.001
Error (b)	8	2.4222	0.3028	0.69
Irrigation	1	0.0694	0.0694	0.652
Irrigation x Locations	1	0.8028	0.8028	0.077
Irrigation x seasons	1	0.0694	0.0694	0.602
Irrigation x locations x seasons	1	0.1361	0.1361	0.466
Error (c)	48	21.1667	0.441	1.73
Manures	2	1.2056	0.6028	0.224
Manures x locations	2	0.2167	0.1083	0.654
Manures x Seasons	2	0.0056	0.0028	0.989
Manures x irrigations	2	1.1056	0.5528	0.252
Manures x seasons x irrigations	2	0.9722	0.4861	0.151
Manures x locs x Seasons x Irrigation	2	0.0056	0.0028	0.989
Error (d)	180	45.8333	0.2546	
Varieties	4	100.2778	25.0694	<.001
Varieties x Locations	4	12.8778	3.2194	<.001
Varieties x seasons	4	4.8444	1.2111	0.001
Varieties x irrigations	4	0.8333	0.2083	0.797
Varieties x manures	8	3.4889	0.4361	0.368
3-way interactions	8	3.0333	0.3792	0.467
4-way interactions	8	1.3111	0.1639	0.74
5-way interaction	8	4.8556	0.6069	0.018

Appendix 4 ANOVA Plant height four weeks

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	243.42	121.71	3.47
Locations	1	6384.04	6384.04	<.001
Error (a)	2	70.05	35.03	1.08
Seasons	1	20205.02	20205.02	<.001
Seasons x locations	1	11764.9	11764.9	<.001
Error (b)	8	533.71	66.71	0.94
Irrigation	1	1137.78	1137.78	0.029
Irrigation x Locations	1	605.8	605.8	<.001
Irrigation x seasons	1	17.07	17.07	0.565
Irrigation x loc x seasons	1	21.61	21.61	0.517
Error (c)	48	3408.37	71.01	1.38
Manures	2	3128.34	1564.17	<.001
Manures x locations	2	220.1	110.05	0.12
Manures x Seasons	2	1716.6	858.3	<.001
Manures x irrigations	2	58.86	29.43	0.711
Manures x seasons x irrigations	2	286.01	143	0.064
Manures x locs x Seasons x Irrigation	2	250.39	125.2	0.09
Error (d)	180	9235.9	51.31	
Varieties	4	21901.41	5475.35	<.001
Varieties x Locations	4	2110.62	527.66	<.001
Varieties x seasons	4	11667.13	2916.78	<.001
Varieties x irrigations	4	154.43	38.61	0.353
Varieties x manures	8	550.64	68.83	0.602
3-way interactions	8	1120.73	140.09	0.145
4-way interactions	8	411.11	51.39	0.437
5-way interaction	8	95.01	11.88	0.985

Appendix 5 ANOVA Nodules per plant

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	23.8	11.9	2.43
Locations	1	0	0	0.988
Error (a)	2	9.81	4.9	0.23
Seasons	1	193.31	193.31	<.001
Seasons x locations	1	1146.33	1146.33	<.001
Error (b)	8	50.98	6.37	0.33
Irrigation	1	161.07	161.07	0.029
Irrigation x Locations	1	0.06	0.06	0.952
Irrigation x seasons	1	153.66	153.66	0.003
Irrigation x loc x seasons	1	72	72	0.038
Error (c)	48	937.09	19.52	1.19
Manures	2	163	81.5	0.011
Manures x locations	2	28.46	14.23	0.423
Manures x Seasons	2	52.35	26.17	0.207
Manures x irrigations	2	88.51	44.25	0.075
Manures x seasons x irrigations	2	31.59	15.79	0.385
Manures x locs x Seasons x Irrigation	2	59.22	29.61	0.168
Error (d)	180	2961.82	16.45	
Varieties	4	68.31	17.08	0.552
Varieties x Locations	4	113.4	28.35	0.147
Varieties x seasons	4	72.32	18.08	0.359
Varieties x irrigations	4	142.38	35.59	0.214
Varieties x manures	8	118.79	14.85	0.504
3-way interactions	8	149.73	18.72	0.34
4-way interactions	8	80.98	10.12	0.764
5-way interaction	8	268.41	33.55	0.044

Appendix 6 ANOVA Pest incidence

Source	Df	SS	MS	F
Replicates	2	1.6056	0.8028	15.21
Locations	1	51.3778	51.3778	<.001
Error (a)	2	0.1056	0.0528	0.23
Seasons	1	92.0111	92.0111	<.001
Seasons x locations	1	49.8778	49.8778	<.001
Error (b)	8	1.1222	0.1403	0.57
Irrigation	1	6.9444	6.9444	0.008
Irrigation x Locations	1	92.0111	92.0111	<.001
Irrigation x seasons	1	2.1778	2.1778	0.003
Irrigation x loc x seasons	1	0.1778	0.1778	0.395
Error (c)	48	11.8333	0.2465	1.01
Manures	2	4.2056	2.1028	<.001
Manures x locations	2	0.6056	0.3028	0.292
Manures x Seasons	2	2.6722	1.3361	0.005
Manures x irrigations	2	1.0722	0.5361	0.112
Manures x seasons x irrigations	2	1.2056	0.6028	0.088
Manures x locs x Seasons x Irrigation	2	0.4056	0.2028	0.438
Error (d)	180	44	0.2444	
Varieties	4	29.6833	7.4208	<.001
Varieties x Locations	4	6.2056	1.5514	<.001
Varieties x seasons	4	9.7944	2.4486	<.001
Varieties x irrigations	4	4.9722	1.2431	0.006
Varieties x manures	8	1.9333	0.2417	0.422
3-way interactions	8	1.6778	0.2097	0.523
4-way interactions	8	1.4778	0.1847	0.642
5-way interaction	8	0.8444	0.1056	0.901

Appendix 7 ANOVA Days to flowering

Source	Df	SS	MS	F
Replicates	2	10.839	5.419	0.89
Locations	1	1047.211	1047.211	<.001
Error (a)	2	12.172	6.086	0.62
Seasons	1	364.011	364.011	<.001
Seasons x locations	1	10	10	0.038
Error (b)	8	12.922	1.615	0.22
Irrigation	1	33.611	33.611	0.143
Irrigation x Locations	1	444.444	444.444	<.001
Irrigation x seasons	1	16.044	16.044	0.009
Irrigation x loc x seasons	1	88.011	88.011	<.001
Error (c)	48	360.233	7.505	3.27
Manures	2	468.572	234.286	<.001
Manures x locations	2	21.006	10.503	0.012
Manures x Seasons	2	10.239	5.119	0.11
Manures x irrigations	2	18.472	9.236	0.196
Manures x seasons x irrigations	2	4.006	2.003	0.42
Manures x locs x Seasons x Irrigation	2	3.172	1.586	0.502
Error (d)	180	413.167	2.295	
Varieties	4	2292.194	573.049	<.001
Varieties x Locations	4	37.372	9.343	0.003
Varieties x seasons	4	4.85	1.212	0.715
Varieties x irrigations	4	7.583	1.896	0.938
Varieties x manures	8	127.289	15.911	0.011
3-way interactions	8	25.5	3.187	0.783
4-way interactions	8	21.167	2.646	0.33
5-way interaction	8	5.022	0.628	0.974

Appendix 8 ANOVA Days to pod formation

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	4.6167	2.3083	0.6
Locations	1	4629.669	4629.669	<.001
Error (a)	2	3.5389	1.7694	1.26
Seasons	1	79.3361	79.3361	<.001
Seasons x locations	1	3.8028	3.8028	0.042
Error (b)	8	5.8111	0.7264	0.73
Irrigation	1	8.4028	8.4028	0.161
Irrigation x Locations	1	6.6694	6.6694	0.007
Irrigation x seasons	1	2.6694	2.6694	0.087
Irrigation x loc x seasons	1	1.4694	1.4694	0.204
Error (c)	48	47.5333	0.9903	1.1
Manures	2	42.4667	21.2333	<.001
Manures x locations	2	2.2889	1.1444	0.284
Manures x Seasons	2	21.6222	10.8111	<.001
Manures x irrigations	2	4.2889	2.1444	0.075
Manures x seasons x irrigations	2	4.6222	2.3111	0.08
Manures x locs x Seasons x Irrigation	2	8.0222	4.0111	0.013
Error (d)	180	162.5	0.9028	
Varieties	4	7987.556	1996.889	<.001
Varieties x Locations	4	51.1222	12.7806	<.001
Varieties x seasons	4	21.8444	5.4611	<.001
Varieties x irrigations	4	23.9444	5.9861	0.015
Varieties x manures	8	34.9778	4.3722	<.001
3-way interactions	8	22.9889	2.8736	0.002
4-way interactions	8	27.7556	3.4694	<.001
5-way interaction	8	7.9222	0.9903	0.367

Appendix 9 ANOVA Pods per plant

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	51.63	25.82	1.17
Locations	1	345.94	345.94	<.001
Error (a)	2	44.3	22.15	0.59
Seasons	1	38.35	38.35	0.114
Seasons x locations	1	7.25	7.25	0.491
Error (b)	8	218.99	27.37	0.79
Irrigation	1	126.14	126.14	0.14
Irrigation x Locations	1	872.04	872.04	<.001
Irrigation x seasons	1	10.03	10.03	0.418
Irrigation x loc x seasons	1	32.7	32.7	0.144
Error (c)	48	1672.01	34.83	2.29
Manures	2	201.33	100.67	0.055
Manures x locations	2	273.6	136.8	<.001
Manures x Seasons	2	297.96	148.98	<.001
Manures x irrigations	2	235.88	117.94	0.035
Manures x seasons x irrigations	2	297.96	148.98	<.001
Manures x locs x Seasons x Irrigation	2	285.36	142.68	<.001
Error (d)	180	2736.38	15.2	
Varieties	4	2140.69	535.17	<.001
Varieties x Locations	4	363.65	90.91	<.001
Varieties x seasons	4	19.59	4.9	0.863
Varieties x irrigations	4	147.99	37	0.443
Varieties x manures	8	630.16	78.77	0.03
3-way interactions	8	94.94	11.87	0.931
4-way interactions	8	119.9	14.99	0.449
5-way interaction	8	83.84	10.48	0.701

Appendix 10 ANOVA Seeds per pod

Source	Df	SS	MS	F
Replicates	2	0.2667	0.1333	0.05
Locations	1	6.6694	6.6694	<.001
Error (a)	2	5.4889	2.7444	3.62
Seasons	1	53.6694	53.6694	<.001
Seasons x locations	1	24.025	24.025	<.001
Error (b)	8	1.7111	0.2139	0.38
Irrigation	1	4.6694	4.6694	0.322
Irrigation x Locations	1	3.8028	3.8028	0.005
Irrigation x seasons	1	0.225	0.225	0.492
Irrigation x loc x seasons	1	0.1361	0.1361	0.593
Error (c)	48	27.2	0.5667	1.2
Manures	2	3.6167	1.8083	0.02
Manures x locations	2	0.9722	0.4861	0.361
Manures x Seasons	2	0.4056	0.2028	0.653
Manures x irrigations	2	0.2722	0.1361	0.725
Manures x seasons x irrigations	2	1.55	0.775	0.198
Manures x locs x Seasons x Irrigation	2	2.3722	1.1861	0.085
Error (d)	180	85.3333	0.4741	
Varieties	2	3.6167	1.8083	0.02
Varieties x Locations	4	3.4278	0.8569	0.129
Varieties x seasons	4	18.0389	4.5097	<.001
Varieties x irrigations	4	2.0944	0.5236	0.609
Varieties x manures	8	6.8278	0.8535	0.067
3-way interactions	8	5.3389	0.6674	0.158
4-way interactions	8	1.7833	0.2229	0.876
5-way interaction	8	1.4056	0.1757	0.935

Appendix 11 ANOVA Hundred seed weight

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	40.67	20.34	0.43
Locations	1	356.61	356.61	<.001
Error (a)	2	95.58	47.79	3.9
Seasons	1	478.63	478.63	<.001
Seasons x locations	1	709.24	709.24	<.001
Error (b)	8	98.11	12.26	0.49
Irrigation	1	683.1	683.1	0.063
Irrigation x Locations	1	380.48	380.48	<.001
Irrigation x seasons	1	15.33	15.33	0.251
Irrigation x loc x seasons	1	11.7	11.7	0.316
Error (c)	48	1206.87	25.14	2.17
Manures	2	158.95	79.47	0.021
Manures x locations	2	62.28	31.14	0.071
Manures x Seasons	2	1.62	0.81	0.933
Manures x irrigations	2	128.08	64.04	0.035
Manures x seasons x irrigations	2	3.9	1.95	0.845
Manures x locs x Seasons x Irrigation	2	17.75	8.87	0.466
Error (d)	180	2084.75	11.58	
Varieties	4	4808.76	1202.19	<.001
Varieties x Locations	4	54.86	13.71	0.319
Varieties x seasons	4	120.85	30.21	0.037
Varieties x irrigations	4	54.86	13.71	0.319
Varieties x manures	8	113.71	14.21	0.801
3-way interactions	8	384.51	48.06	0.08
4-way interactions	8	60.16	7.52	0.735
5-way interaction	8	47.61	5.95	0.845

Appendix 12 ANOVA yield in kg/ha

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	30640	15320	0.08
Locations	1	8001808	8001808	<.001
Error (a)	2	362113	181057	0.68
Seasons	1	26669204	26669204	<.001
Seasons x locations	1	11861419	11861419	<.001
Error (b)	8	2128101	266013	1.18
Irrigation	1	12354043	12354043	0.014
Irrigation x Locations	1	2036292	2036292	0.004
Irrigation x seasons	1	2.1778	2.1778	0.003
Irrigation x loc x seasons	1	4281806	4281806	<.001
Error (c)	48	10818648	225389	0.95
Manures	2	10312538	5156269	<.001
Manures x locations	2	4159339	2079669	<.001
Manures x Seasons	2	877885	438943	0.16
Manures x irrigations	2	128437	64218	0.791
Manures x seasons x irrigations	2	405064	202532	0.428
Manures x locs x Seasons x Irrigation	4	1649442	412360	0.144
Error (d)	180	42722523	237347	
Varieties	4	5598419	1399605	<.001
Varieties x Locations	4	7631804	1907951	<.001
Varieties x seasons	4	1786409	446602	0.116
Varieties x irrigations	4	2510221	627555	0.037
Varieties x manures	8	5133729	641716	0.011
3-way interactions	8	3292462	411558	0.095
4-way interactions	8	2617124	327140	0.209
5-way interaction	8	3892403	486550	0.043

Appendix 13 ANOVA Leaf iron content

Source	Df	SS	MS	F
Replicates	2	28201	14100	1.12
Locations	1	606473	606473	<.001
Error (a)	2	25068	12534	3.88
Seasons	1	127841	127841	<.001
Seasons x locations	1	238909	238909	<.001
Error (b)	8	22995	2874	0.41
Irrigation	1	311876	311876	0.038
Irrigation x Locations	1	757534	757534	<.001
Irrigation x seasons	1	1932	1932	0.533
Irrigation x loc x seasons	1	44090	44090	0.003
Error (c)	48	335596	6992	1.42
Manures	2	22897	11449	0.063
Manures x locations	2	198400	99200	<.001
Manures x Seasons	2	1332	666	0.874
Manures x irrigations	2	10548	5274	0.509
Manures x seasons x irrigations	2	8839	4420	0.411
Manures x locs x Seasons x Irrigation	2	22588	11294	0.105
Error (d)	180	889368	4941	
Varieties	4	199459	49865	<.001
Varieties x Locations	4	42360	10590	0.077
Varieties x seasons	4	8893	2223	0.772
Varieties x irrigations	4	79704	19926	0.003
Varieties x manures	8	125003	15625	0.067
3-way interactions	8	136337	17042	0.046
4-way interactions	8	7049	881	0.994
5-way interaction	8	27516	3439	0.695

Appendix 14 ANOVA Leaf zinc content

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	3687.64	1843.82	7.25
Locations	1	11334.44	11334.44	<.001
Error (a)	2	508.47	254.24	4.9
Seasons	1	360	360	0.005
Seasons x locations	1	587.78	587.78	<.001
Error (b)	8	563.89	70.49	1.65
Irrigation	1	54.44	54.44	0.689
Irrigation x Locations	1	71.11	71.11	0.204
Irrigation x seasons	1	1.11	1.11	0.874
Irrigation x loc x seasons	1	40	40	0.341
Error (c)	48	2056.67	42.85	0.98
Manures	2	86.81	43.4	0.564
Manures x locations	2	315.14	157.57	0.029
Manures x Seasons	2	451.25	225.63	0.007
Manures x irrigations	2	33.47	16.74	0.69
Manures x seasons x irrigations	2	41.81	20.9	0.621
Manures x locs x Seasons x Irrigation	2	11.25	5.62	0.88
Error (d)	180	7883.33	43.8	
Varieties	4	150.69	37.67	0.587
Varieties x Locations	4	363.47	90.87	0.086
Varieties x seasons	4	37.92	9.48	0.929
Varieties x irrigations	4	82.36	20.59	0.808
Varieties x manures	8	445.14	55.64	0.3
3-way interactions	8	502.64	62.83	0.225
4-way interactions	8	360.14	45.02	0.417
5-way interaction	8	463.75	57.97	0.234

Appendix 15 ANOVA Seed iron content

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	130.4	65.2	0.38
Locations	1	3835.1	3835.1	<.001
Error (a)	2	340.1	170.1	1.47
Seasons	1	30895.1	30895.1	<.001
Seasons x locations	1	18705.6	18705.6	<.001
Error (b)	8	2978.6	372.3	2.06
Irrigation	1	550.1	550.1	0.214
Irrigation x Locations	1	15275.1	15275.1	<.001
Irrigation x seasons	1	17430.6	17430.6	<.001
Irrigation x loc x seasons	1	4876.7	4876.7	<.001
Error (c)	48	8696.7	181.2	0.91
Manures	2	1646.7	823.3	0.045
Manures x locations	2	2097.2	1048.6	0.006
Manures x Seasons	2	203.9	101.9	0.601
Manures x irrigations	2	37.2	18.6	0.927
Manures x seasons x irrigations	2	31.7	15.8	0.924
Manures x locs x Seasons x Irrigation	2	123.9	61.9	0.734
Error (d)	180	35920.8	199.6	
Varieties	4	5557.6	1389.4	<.001
Varieties x Locations	4	147.9	37	0.946
Varieties x seasons	4	1076.8	269.2	0.254
Varieties x irrigations	4	144	36	0.866
Varieties x manures	8	979	122.4	0.85
3-way interactions	8	892.6	111.6	0.88
4-way interactions	8	4124.9	515.6	0.011
5-way interaction	8	2815.7	352	0.087

Appendix 16 ANOVA Seed zinc content

Source	**Df**	**SS**	**MS**	**F**
Replicates	2	96.34	48.17	1.9
Locations	1	1064.34	1064.34	<.001
Error (a)	2	50.67	25.34	2.48
Seasons	1	963.67	963.67	<.001
Seasons x locations	1	0.34	0.34	0.903
Error (b)	8	577.52	72.19	5.05
Irrigation	1	0	0	0.993
Irrigation x Locations	1	186.34	186.34	0.004
Irrigation x seasons	1	14	14	0.431
Irrigation x loc x seasons	1	664.23	664.23	<.001
Error (c)	48	685.63	14.28	0.64
Manures	2	152.01	76	0.393
Manures x locations	2	64.34	32.17	0.241
Manures x Seasons	2	136.17	68.09	0.051
Manures x irrigations	2	142.34	71.17	0.088
Manures x seasons x irrigations	2	22.17	11.09	0.611
Manures x locs x Seasons x Irrigation	2	42.62	21.31	0.389
Error (d)	180	4040.5	22.45	
Varieties	4	89.37	22.34	0.117
Varieties x Locations	4	40.82	10.2	0.769
Varieties x seasons	4	65.09	16.27	0.576
Varieties x irrigations	4	49.59	12.4	0.344
Varieties x manures	8	406.91	50.86	0.096
3-way interactions	8	112.69	14.09	0.84
4-way interactions	8	535.13	66.89	0.004
5-way interaction	8	171.58	21.45	0.473