EFFECTS OF PHOSPHORUS AND SOIL MOISTURE ON GRAIN YIELD, LEAF AND GRAIN TISSUE CONCENTRATION OF IRON AND ZINC IN THREE BEAN (*PHASEOLUS VULGARIS L.)* GENOTYPES

BY

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DECLARATION

Declaration by the Candidate

I HARRISON KATWIKILA MUNUVE declare that this thesis is my original work and has not been presented for the award of a degree in any university or any other award.

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We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors.

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DEDICATION

This research project is dedicated to my wife Peninah Mutethya and children, my late brother James Mutuku for their encouragementduring my study period.

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ABSTRACT

Common bean (*Phaseolus vulgaris L.*) is an important source of protein, minerals and food for the majority of the poor population in sub-Saharan Africa. However, its contribution to grain yield and micronutrient level is constrained by moisture stress and low available soil phosphorus. A study was carried out to determine the effects of bean genotypes, P fertilizer and moisture regimes on bean (*P. vulgaris L.*) grain yield and tissue concentration of Zn and Fe in three bean genotypes at KARI, Katumani Research Centre in Machakos, located at 1560 masl. The study was laid out in a randomized complete block design with five P fertilizer treatments in split-split plot arrangement with three replications. The main plot was moisture regimes, subplots were genotypes and the sub-sub plots were P rates. Data on concentration of Fe and Zn at flowering, grain yield and grain concentration of Fe and Zn at bean maturity were recorded. Data for grain yield, grain and leaf tissue concentration of Fe and Zn were analysed using ANOVA, Means were separated using LSD at significant ($P < 0.05$) level and correlation between grain concentration of Fe and Zn, as well as between grain yield and leaf concentration of Fe and Zn were determined.(SAS 8.2; SAS institute, 1999). The results of grain yield showed highly significant ($P = 0.0006$) interaction between moisture regimes, genotypes and P application rates. Grain yield of the three genotypes increased with P application rate up to 60 kg ha^{-1} regardless of the moisture regimes. The leaf and grain concentration of Fe and Zn showed highly significant ($P < 0.01$)interaction between moisture regimes, genotypes and P fertilizer application rate. Similarly, percentage of leaf Fe and Zn accumulated to grain showed highly significant ($P < 0.0001$)interactions between moisture regimes, genotypes and P fertilizer application rate. All genotypes had significantly ($P < 0.05$) higher concentration of Fe and Zn in leaves compared to the grain. Grain Fe concentration was higher in beans grown under adequate moisture conditions than in moisture stressed condition. A highly significant ($n = 90$; $r = 0.79736$; $P < 0.0001$) positive correlation between grain Fe and Zn concentration and highly significant ($n = 90$; $r = 0.53662$; $P < 0.0001$) positive correlation between leaf Fe and Zn concentration were observed. It follows that an increase in grain Fe concentration correlates with an increase in grain Zn concentration, also an increase in leaf Fe concentration correlates with an increase in leaf Zn concentration. A significant ($n = 90$; $r = -0.34860$; $P = 0.0008$) negative correlation between the grain yield and leaf Fe concentration and highly significant ($n = 90$; $r = -0.58292$ $P = 0.0001$) negative correlation between grain yield and leaf Zn concentration were observed. Hence an increase in grain yield correlates with a decrease in leaf concentration of Fe and Zn. In order to increase the grain yield and grain concentration of Fe and Zn, application of P fertilizer, maintaining adequate soil moisture and planting superior bean genotype is required.

Key Words: Phosphorus; Soil Moisture; Yields; Bean; Iron; Zinc.

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LIST OF ABBREVIATION AND ACRONYMS

AEZ - Agro Ecological Zone

AGRA - Alliance for Green Revolution in Africa

AIDS - Acquired Immune Deficiency Syndrome

ANOVA - Analysis of Variance

CGIAR - Consortium of International Agricultural Research Centers

CCRP - Collaborative Crop Research Program

CIAT - International Centre for Tropical Agriculture

FAO - Food and Agricultural Organisation of the United Nation.

HIV - Human Immunodeficiency Virus

ILRI - International Livestock Research Institute

KARI - Kenya Agricultural Research Institute

LSD - Least Significant Difference

Masl - Meters above Sea Level

PABRA - Pan Africa Bean Research Alliance

TSP - Triple Super Phosphate

UK - United Kingdom

USA - United States of America

RCBD	-	Randomized Complete Block Design
SAS	-	Statistical Analysis System
SE	-	Standard Error
SEKU	-	South Eastern Kenya University
SIDA	-	Swedish International Development Cooperation Agency
V_1	-	Awash 1
V_2	-	Awash Melka
V_3	-	Mexican 142

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Common bean (*Phaseolus vulgaris L.)* is one of the five cultivated species from the genus *Phaseolus* and is a major grain legume crop, third in importance after soya bean and peanut (Broughton *et al*., 2003). It is one of the principal food and cash crop legume grown in the tropics. It is the primary and least expensive source of calories, protein, dietary fiber, minerals (Fe and Zn) and vitamins for the population in the tropical countries although its intake does not satisfy their mineral requirement (Pachico, 1993; Welch and Graham *et al*., 1999). Common bean is a small-scale farmer crop in Eastern Africa where it is often cultivated in unfavourable conditions with minimal inputsand is mainly consumed as dried or green beans, the dried beans are sold while raw or as canned beans for local or export market(Katungi *et al*., 2009). Some of the characteristics that are considered for canning beans include mineral composition, nutrient levels, effective moisture content, percent volume increase, cooking time after soaking, seed dimensions, crude protein, crude fiber, crude fat, carbohydrates and seed colour which is popular in different regions of the world with different cultures (Schoonhovern and Voysest, 1991; Voysest *et al*., 1994; Gathu, 2012). The growth in demand for pre-cooked beans in the market has necessitated the need to diversify the number of bean varieties on offer thereby creating greater stability of production, increase in nutritional value and food securityespecially with the looming climate change (Buruchara, 2006).

Bean farming in Kenya is also constrained by decreasing rain due to climatic change leading to reduced grain yields (Remenyik and Nemeske, 2010) and low soil P status accentuated by soil erosion and fixation by oxides in acidic soils (Sixbert and George, 2012).Low soil N and

P levels and acid soil conditions are important constraints for bean production in most of the areas where this crop is grown (Graham *et al*., 2003). However, in the semi-arid areas where beans are grown the soils are not acidic and are able to fix appreciable amounts of N, if P is supplied (Abdul and Saudi, 2012). This leaves P as a constraint of major concern in the semi-arid areas (Karanja *et al*., 2011).The decision to fertilizers use in soils with low P levels must be made against the economic background of the cost of fertilizers and the extra crop to be produced (Cooke, 1982). Fertilizer usage has been known to increase grain yield and increase bean nutritional value in terms of mineral contents (McKenzie and Middleton, 1997; Georgina *et al*., 2007; Sixbert and George, 2012).

Ongoing bean researches addressing the current climate changes have developed heat tolerant beans in Mexico, Nicaragua and Rwanda whose genes are yet to be transferred to bean varieties for breeders in dry land areas (Flora *et al*., 2011).Studies have shown that an application of P fertilizer led to an increased legume grain yields, particularly with velvet bean, and soya bean (Kamanga *et al*., 2010). Other studies have shown that bean crop require more P because it is important for nodulation to take place effectively (Ssali and Keya, 1986; Vladimir, 2010). Studies carried out in Columbia showed significant genotype variability on P fertilizer application rates, indicating an increase in Fe concentration in the seeds, but a reduced Zn concentration in seeds (Astudillo *et al*., 2008).

Studies show varying response of genotypes to shoot biomass, P uptake and yield in a treatment without P which can be utilized in selecting superior genotypes for use in such low soil P environment as breeding material (Sixbert and George, 2012).Selecting improved bean genotypes with high grain yield, high P uptake, moisture stress tolerant and high Zn and Fe concentration for breeding will contribute in addressing the problem of low yields and low dietary mineral intake such as Fe and Zn (Jerome, 2007). The improved breeds can be selected among the genotypes available from various studies that show significant genotype variability in the tissue concentration of Zn and Fe due to moisture stress reported (Priscila*et al*., 2008).The aim of this study was to determine the effect of P fertilizer application on grain yield and grain and leaf tissue concentration of Zn and Fe in three bean genotypes grown under different moisture regimes.

1.2 Statement of the Problem

In recent years, bean crop production trend has experienced low yield and has not kept in pace with the annual population growth rate in some countries (Kambewa, 1997; Xavery *et al*., 2007). Currently Kenya consumes approximately 450,000 tons of beans against a local production level of between 150,000 and 200,000 tons produced annually and imports the deficit mainly from Uganda, Tanzania and Central Africa (MOA, 2011).

Though factors such as low N and acidic soils are important constraints in some parts of Kenya, in the semi-arid areas the soils are less acidic and beans improve soil fertility through biological N-fixation (Giller, 2001) leaving P as the most important constraint for bean production in the dry land areas (Graham *et al*., 2003; Fabricio *et al*., 2013). The low soil P and water stress affect bean grain yields and tissue concentration of both Fe and Zn (Astudillo *et al*., 2008). Studies have shown that the concentration of Fe and Zn in grains and leaves differ (George and Susan, 2010). Decrease in grain and leaf concentration of Fe and Zn has been associated with P toxicity (Gianquinto *et al*., 2000) or due to dilution effect as a result of P stimulation of growth (Fan *et al*., 2008; Murphy *et al*., 2008).

The current study involved three bean genotypes that were developed for canning, theses are Awash 1, Awash Melka and Mexican 142. Awash 1 and Awash Melka is a first generation of improved bean genotypeswhich are more resistant to diseases whileMexican 142 has dominated production as canning beans for over fifty years (Karanja *et al*., 2011).The response of the three bean genotypes to P application rate and soil moisture on grain yield and tissue concentration of Fe and Zn is unknown.

1.3 Justification of the Study

The three bean genotypes represents some of the promising lines of beans for dry areas and have not been tested on their response to P application rate and soil moisture which is known to affect grain yield and tissue concentration of Fe and Zn (Astudillo *et al.*,2008). The need for beans with high micronutrients is high as either dry beans, green beans or as canning beans due to changing eating habits, preference for fast cooking off-shelf products and high cost of cooking fuel (Karanja *et al*., 2011). Beans have been grown in Eastern Africa since early 1950s with little work having been done to develop improved bean varieties that combine tolerance to biotic and abiotic stresses with high micronutrient quality. Consequently, bean variety such as Mexican 142 has dominated production despite its susceptibility to Common Bacterial Blight (Tedele, 2006).

The current study contributed in determining the response in grain yield and tissue concentration of Fe and Zn due to P fertilizer application rate and soil moisture in the three beans. Their responses will contribute to the development of agronomic management practices such as P fertilizer application rate and soil moisture requirement which is known to influence productivity of beans and micronutrient quality (Loggerenberg, 2004). In addition, the study contributesin the development ofimproved bean genotypes that have high grain yield and high micronutrients such as Fe and Zn hence creatingan increasedstability of beans production (Katungi *et al*., 2009).

1.4 Objectives

1.4.1 Main Objective

To contribute in determining the effect of P fertilizer application rate on grain yield and tissue concentration of Zn and Fe in three bean genotypes grown under different moisture regimes.

1.4.2 Specific Objectives

1. To determine the effect of P rate on grain yields in three bean genotypes grown under adequate and stressed soil moisture conditions.

2. To evaluate the effect of P rate on grain and leaf tissue concentration of Zn and Fe in three bean genotypes under adequate and stressed soil moisture conditions.

3. To investigate the effect of soil moisture condition on grain and leaf tissue concentration of Zn and Fe in three bean genotypes.

1.4.3 Hypothesis

H_0: Phosphorus rate has no difference on grain yield in three bean genotypes under adequate and stressed soil moisture conditions.

H_0: Phosphorus application rates have no difference on grain and leaf tissue concentration of Fe and Zn in the three bean genotypes.

H_0: Soil moisture regimes have no effect on the grain and leaf tissue concentration of Zn and Fe in three bean genotypes.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Information

2.1.1 Major Producing areas

Common beans originated in Latin America and have two primary centers of origin in the Mesoamerican and Andean regions that are easily distinguished by molecular means (Blair *et al*., 2006).Major producing countries for national consumption are Brazil and Mexico while the United States, Canada, Argentina and China are all exporting countries. The crop is also important in a range of developing countries of Central America, South America and of Eastern and Southern Africa (Singh, 1999). Beans are grown both for subsistence agriculture and for regionalmarketswhere they play an important role in food security and income generation; much of the world's bean production is on small farms ranging from 1-10 acres in size (Katungi *et al*., 2009)

2.1.2 Common Bean Distribution in Kenya

Common bean production in Kenya is mainly in highland and the midland, about 75 percent of the annual cultivation occurs in three regions namelyRift Valley, Nyanza and Eastern Province (Katungi *et al*., 2009). In terms of output, the Rift valley contributes the biggest share, accounting for 33 percent of the national output followed by Nyanza (Katungi *et al*., 2009). Output from eastern parts of the country and the coast is constrained by adverse climatic conditions (Katungi*et al*., 2009). An impressive high diversity of common bean exists in Kenyahaving about 80 different cultivars distinguished in different places of the country in late 1970s (Njunguna *et al*., 1980). The varieties have been losing area because of increased problem of soil infertility such as P deficiency and associated diseases (Ronno *et al*., 2001).

2.1.3 Bean Farming Requirements

Common bean is a warm season crop that does not tolerate frost or long periods of exposure to near-freezing temperatures at any stage of growth, high temperatures do not affect it if adequate soil moisture is present, although high nocturnal temperatures will inhibit pollination(Katungi *et al*., 2009). The crop requires moderate amounts of rainfall (300 – 600 mm) but adequate amounts are essential during and immediately after the flowering stage (Gomez, 2004). Generally, common bean is considered a short-season crop with most varieties maturing in a range of 65 to 110 days from emergence to physiological maturity (Buruchara, 2007). Maturity period can continue up to 200 days after planting amongst climbers that are used in cooler upland elevations (Graham and Ranalli, 1997; Gomez, 2004).

2.2 The Role of Fertilizer in Bean Production

Half of the huge yield gaps existing between sub-Saharan African countries and the developed world must be closed through improved soil nutrient management and accompanying field practices while the remainder resolved through widespread adoption of improved crop varieties (Huang *et al.*, 2009). African farmers need better technology that includes more sustainable practices, improved seeds and fertilizer to increase and sustain crops productivity and prevent further degradation of the agricultural lands (Don, 2007). Over exploitation of the soil nutrient has led to P and N deficient soils that are unable to produce a good yield, hence use of fertilizers have become mandatory in farms (Odum, 1989). In order to increase food production in a sustainable manner, farmers will need to use the right fertilizer at the right rate, right time and right place (Norman, 2008).

2.2.1 Role of P on Bean Growth

Beans need P for growth, utilization of sugar and starch, photosynthesis, nucleus formation and cell division, fat and albumen formation, transfer and storage of energy within plants.

Energy from photosynthesis and the metabolism of carbohydrates is stored in phosphate compounds for later use in growth and reproduction(McKenzieand Middleton, 1997; Georgina *et al*., 2007;Sixbert and George, 2012).Adequate P results in rapid growth, earlier maturity and increased root growth which means plant can explore soil for nutrients and moisture and its deficiency slow overall plant growth, (McKenzieand Middleton, 1997; Sixbert and George, 2012).Crop plants show genotypic variation in P uptake efficiency (i.e. total P uptake), breeding for P-efficient crop cultivars has been recognized as one approach to the management of P-deficient soils (Graham, 1984;Caradus, 1994).Phosphorus and Zn deficiencies are widespread nutritional constraints in bean crop production, and P and Zn interactions have been widely investigated (Marschner, 1995).The genetic improvement of legume P efficiency, deployed in consideration of the social and economic context of local cropping systems, has great potential to address a principal constraint to food security in Africa (Xiaolong *et al*., 2008).

The currentstudy contributed to the understanding of the responses of the three bean genotypes to varying P application rates and soil moisture regimes on grain yield and tissue concentration of Fe and Zn, which can be utilized in optimizing bean productivity in low soil P farmland (Ann, 2010; Joe *et al*., 2013).

2.2.2 Root Architecture and P Uptake

Genetic differences exist in the root architecture traits of different bean genotypes that are key adaptations to P stress in low-input agro-ecosystems (Lynch and Brown, 2008). Root traits that enhance topsoil foraging are advantageous in low P soil since P bio-availability is typically greatest in surface horizons (Lynch and Brown, 2001). Genotypes with shallow root architecture have greater growth and yield in low-P soil than related genotypes with deep architectures (Rubio *et al*., 2003; Zhu *et al*., 2005).Adventitious roots may improve crop adaptation to low-P soils by enhancing topsoil foraging (Zhu *et al*., 2005). In a tropical field study, P stress stimulated adventitious rooting in P-efficient genotypes of common bean (*P. vulgaris*) but not in P-inefficient genotypes. Choosing adventitious rooting is a useful adaptation to low P availability, because adventitious roots explore topsoil horizons more efficiently than other root types (Miller *et al*.,2003)

Root architectural plasticity traits of *P. vulgaris*that increase topsoil foraging are advantageous for P acquisition but may incur tradeoffs for the acquisition of deep soil resources such as moisture (Lynch and Brown, 2001). In a combined moisture and P stress the genotype that have a dimorphic root system that permit vigorous rooting throughout the soil profile are more advantageous for multiple resource acquisition particularly when resources are differentially localised in the soils (Ho *et al*., 2005).The roots of plant genotypes that are efficient in mobilizing nutrients from surrounding soil are better able to penetrate and make use of the moisture and minerals contained in subsoil (Susan and George, 2010). These qualities are also associated with greater seedling vigor resulting in increased crop yields (Rengel and Graham, 1995).

2.2.3 The Role of Soil Moisture in P Availability

Inadequate soil moisture lead to poor seed filling resulting into physiological maturity to happen earlier than expected (Brevedan and Egli, 2003; Muasya and Auma, 2003)consequently leading to reduction in grain yield (Szilagyi, 2003). Adequate soil moisture will enhance fertilizer solution and reaction in the soilenhancing plant growth and leading to increased P and other nutrient requirements especially in crops grown under irrigation or in higher rainfall areas(Mckenzieand Middleton, 1997; Georgina *et al*., 2007).Plants grown in wet soil produced twice as much as those grown on dry soils, nodule weight and activity were five to ten times greater than those from dry soils (Siu*et al*., 1984;

Vladimir, 2010).The mineral contents in the seeds are influenced by the soil type and chemical composition and by interaction between genotypes and environment (Moraghan *et al*., 2002; Cichy *et al*., 2005). The genotypes by environments interaction has been a large complicating factor in breeding studies with a view toward improvement, principally in bean grain yields, among other traits (Allard and Bradshaw, 1964; Ramalho *et al*., 1998; Carbonell *et al*., 2004; Pereira *et al*., 2009).

2.2.4 Effect of P on Tissue Concentration of Fe and Zn

Studies have shown that increasing the availability of P in the growth medium can induce Zn deficiency in plants by altering soil and plant factors(Robson and Pitman, 1983), but little is known about specific mechanisms. Studies carried out on barley roots observed that Zn deficiency (low tissue Zn concentrations) causes an increase in the expression of P transporter genes in barley roots and also an enhanced P uptake efficiency may cause a decrease in plant uptake of Zn, leading to potentially low Zn concentrations (densities) in food (Huang *et al*., 2000). A study on grain and straw of winter wheat reported a depressed Zn concentration on increasing P application (Yue *et al*., 2012). Other studies have shown that an increase in P supply depressed Zn concentration in *P.Vulgaris*, which was attributed to a dilution effect of plant growth (Singh *et al*., 1988; Gianquinto *et al*., 2000). Therefore, it remains unclear whether an increase in P availability in the growth medium can reduce Zn uptake by plant roots (Zhu *et al*., 2001). Many studies have shown that a low Zn supply but a high P supply markedly enhance P concentration in plant tissues, which may cause P toxicity and contribute to symptoms resembling Zn deficiency (Loneragan *et al*., 1979, 1982; Cakmak and Marschner, 1986; Webb and Loneragan, 1988, 1990).

Study on grain wheat showed that P application significantly decrease grain Zn by 17 to 56 % while the grain levels of Fe, manganese and copper either remained the same or decreased, also P application increased grain yield but it restricted the accumulation of shoot Zn, but enhanced the accumulation of shoot Fe, Cu and especially manganese (Yue *et al*., 2012).

2.3 NutritionalValue of Common Beans

Common beans are important for nutritional well-being as well as poverty alleviation among consumers and farmers with few other food or crop options(Broughton *et al*., 2003). Bean is widely used in the country to make recipe like *Githeri* (cooked mixture of beans and maize) due to increased demand among low income population in the urban areas (Katungi *et al*., 2009). Common beans provide the crucial proteins (20%), energy (32%) and generous amounts of micro-nutrients especially Fe and Zn, and vitamins A and B complex to over 50 million resource poor rural and urban consumers in eastern Africa (Karanja *et al*., 2011).

The protein consumption by individual people in the world is estimated at about 77gm of protein per day (FAO, 2010). Recent research also indicates that consumption of grain legumes slows the onset of AIDS in HIV positive people and therefore, an improved bean production would directly address several critical health issues for African communities (Xiaolong *et al*., 2008).Bean leaves can be used in much the same way that other plant leaves are, as a base for salad or a topping for sandwiches, hamburgers and other foods, they are relatively low in calories and fat, so they may be appropriate choices for dieting individual (Brian, 2011).

2.3.1 Role of Zn and Fe in Human and Plants

Iron is essential for preventing anaemia in human being and for the proper functioning of many metabolic processes while Zn is essential for adequate growth and sexual maturation and for resistance to gastro-enteric and respiratory infections, especially in children (Bouis, 2003).Fe deficiency causes anaemia whose consequences arenumerous and grave. Zn deficiency leads to poor child growth, delayed maturation, poor appetite and impaired immune function(Bouis, 2003). Micronutrient rich common bean cultivars offer unique opportunities for alleviating these disorders in eastern Africa (Karanja *et al.*, 2011).

Plants require the proper balance of Zn for normal growth and optimum yield, Zinc availability in plants depends on soil factors such as the concentration of Zn in solution, ion speciation and the complex interaction of Zn with other macronutrients and micronutrients (Li*et al.,*2003). Selection of cultivars which are rich in Zn can be used in plant breeding to developing high Zn concentration of edible parts without negatively impacting yield(Sadeghzadeh, B**.** 2013).Zinc deficiency may cause large reductions in crop quality and yield without any visible sign (Alloway, 2004; McDonald*et al.,*2001). Zn deficiency also decreases the amount of Zn in cereal grain and diminishes its nutritional quality **(**Sadeghzadeh, B.2013). Iron is an essential nutrient for plants whose functions are to accept and donate electrons and also plays important roles in the electron-transport chains of photosynthesis and respiration. Iron is toxic when it accumulates to high levels and act catalytically via the Fenton reaction to generate hydroxyl radicals which can damage lipids, proteins and DNA, hence plants respond to Fe stress in terms of both Fe deficiency and Fe overload (Erin and Connolly, 2002; Goff *et al*., 2002).

2.3.2 Source and Sink Strength in Plant Growth

Studies have shown that the sink strength which depend of sink age rather than sink size may determine the partitioning of plants assimilates in the plant organ(Marcelis, 1996). Information on the effect of source strength on the partitioning of assimilates among the plant organs is limited (Ho, 1988, 1992). However, studies show hierarchy among sink in different cultivars that is some (e.g. fruits, seeds or underground storage organs) have priority and suffer less from a reduction in assimilate supply than other organs e.g. flowers. In relation to in assimilate supply, moisture condition affects the net photosynthesis, hence the growth and the supply of assimilates and photosynthates (Wardlaw, 1990).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was carried out between August and November 2012 at KARI, Katumani in Machakos located at1º35'S: 37º14'E, and 1560 masl. The Centre experiences a semi-arid tropical climate described as AEZ 3 (Jaetzold and Schimidt, 1983), with a bimodal pattern of rainfall. The long rains are received between March and June, with the peak in April, followed by a dry period that extends to mid-October. The short rains begin in mid-October, peak in November and taper off towards mid-December.

3.2 BeanGenotypes

Three genotypes used in the study were Awash 1, Awash Melka and Mexican 142. Awash 1 and Awash Melka is a first generation of improved bean genotypes, a promising line of micronutrient dense and disease resistant beans for canning that originated from Ethiopia. Mexican 142has low grain yield and susceptible to diseases such as Common Bacterial Blight and has dominated production as canning beans for over 50 years (Karanja*et al.*, 2011). The three bean genotypes were assigned letters as follows Awash 1 (V_1), Awash Melka (V_2) and Mexican 142 (V_3).

3.3 Experimental Design Layout

The experiment was laid out in a RCBDwith treatments in a split–split plot arrangement with three replicates, comprising moisture regimes as main plots, the three bean genotypes as the sub plots and fiveP fertilizer application ratesas sub-sub plots. The five rates of P fertilizer application were 0 kg ha^{-1}, 20 kg ha^{-1}, 40 kg ha^{-1}, 60 kg ha^{-1} and 80kg ha^{-1}. Each sub plot measured 1.5 m x 1.5 m. Soil analysis was done before the start of the experiment and indicated that it was sandy loam of Ferric Luvisol origin as described by Kibe *et al*., (1981), it wasalso slightly acidic with low total N, organic carbon and P.

Two bean seeds were sown per hill with 20 cm spacing within rows and 50 cm between rows and thinned to one plant per hill two weeks after germination. Main plot was separated by a one meter foot path for easy access during data collection and harvesting. The plots were kept weed free by hand weeding. To prevent cutworms, Sulban©48ec (Chlorpyrisos 480g/l) insecticide was applied after germination. Dimethoate©40ec(Dimethoate 400g/l)insecticide was applied to control white flies on emergence. Soil samples were randomly obtained prior to sowing from the top soil at increments of 10cm upto a depth of 30cm for chemical analysisas described (Hinga *et al*., 1980).

The amount of moisture that the soil can hold at field capacity was determined using gravimetric method prior to starting the experiment. After flowering, moisture stress was applied to one of the main plot selected randomly at 70% of field capacity by watering once in two weeks.Watering was done using drip irrigation by laying drip lines along each bean rows (Eachsub–subplot served by four drip lines). A 5000 liter water tank was placed near the farm to act as water reservoir throughout the growing season. A main pipe connected to the water tank and fitted with valves to regulate flow of water supplied water to the drip lines. To prevent blockage of the drip lines, care was taken to prevent dust and soil particles from falling on the drip holes during laying and subsequent weeding.

3.4 Data Collection

3.4.1 Temperatures and Days to 50% Flowering

Minimum and maximum temperatures were recorded from the meteorological station using a wet and dry bulb thermometer.The number of days to 50% flowering was recorded for each P application rate to determine the period for taking the leaf sample for Fe and Zn test.

3.4.2 Leaf Sampling for Zn and FeAnalysis

At 50% flowering, three fully expanded leaves were picked from six plant grown in the two middlerows (except those grown on the end of each) for Fe and Zn test samples making a total of 12 plants sampled per each sub-sub plot (each sub-plot had a total of 32 plants). The leaves picked were initially washed in distilled water, then hydrochloric acidand distilled water to remove any impurities that may have longed on the leaves and finally rinsed in distilled water and sun dried for two hours toremove dripping water which can damage Khaki paper envelope where it was later stored. The sample was then oven dried for 48 hours at a temperature of 60° C and stored in khaki paper for analysis.

3.4.3 Harvesting at Bean Maturity

At harvest time, pods were picked from each plant in the two middle rows, in each sub-sub plot, leaving one plant at the two ends of each row to avoid the border effects. The harvested beans were threshed and the grains weight taken, this was followed by drying the beans at 60 °C for 48 hours. The weight of the dried beans was recorded for each sample. In order to obtain a 5 gram representative sample for micronutrient analysis, the dried grains from each plot were evenly placed on clean acid (Hydrochloric acid 0.1%) washed trayand spread in a circleof 15cm diameter then divided into four quarters of equal size. A sample of 5 grams was collected randomly from each quarter making a total of 20 grams. This was followed by a quartering procedure repeated until a total of 5gram representative sample was obtained.

The 5 gram samples obtained for both leaf and grain sample were grounded using a non-contaminating grinding Retsch mill with Teflon chambers and Zirconium balls to avoid Fe and Zn contamination. A sample of 0.5–0.8 grams was collected randomly for analysis from each of the 5gram sample through a quartering procedure. The grinded samples were packed and labeled in clean plastic screw-top tubes and stored in a clean, dry and insect-free location. Analysis was done using Inductively Coupled Plasma–Optical Emission Spectroscopic (ICP-OES).

3.5 Data Analysis

The data on mean grain weight, grain and leaf tissue concentration Fe and Zn were analysed using ANOVA(SAS 8.2) to isolate treatment effect of P and moisture. The mean values were separated using LSD at $P<0.05$ significant level. Correlationanalysis between grain and leaf tissue concentration of Fe and Zn, as well asgrain weight to leaf and grain tissue concentration of Fe and Zn were determined using Pearson Correlation (SAS 8.2).

CHAPTER FOUR

RESULTS

4.1.1. Effect of P rate on Grain Yield of Three Bean Genotypes under Adequate and Stressed Moisture Conditions

Bean grain yields showed a significant (df = 16; F = 3.19; P = 0.0006)interactionbetween P application rates, moisture regimes and genotypes(Table 2). Under both adequate and stressed moisture conditions, bean grain yields of the three bean genotypesincreased significantly (df = 28; t = 2.05; P< 0.05)with increased P fertilizer application rateup to 60 kg ha^{-1}, above which there was noincrease(Table 1).Genotype V_2 produced significantly (df=28, t = 2.05; P<0.05) higher grain yields than all the others under adequate moisture but under moisture stress it was similar to V_3 but higher than V_1.The three bean genotypes produced significantly (df = 58; t = 2; P< 0.05)higher grain yield under adequate soil moisture than under stressed soil moisture.

Table 1: Grain Yields (Kg Ha^{-1}) of Three Bean Genotypes (V_1, V_2, V_3) Under Varying P Rate in Adequate and Stressed Moisture Conditions

		P rate (kg ha^{-1})					
Moisture regimes	**Genotypes**	**0**	**20**	**40**	**60**	**80**	**Mean**
Adequate	V_1	2242.33Dc	2868.11Cb	3536.53Ba	4036.67Aa	4065.13Aa	**3349.8b**
	V_2	2774.70Da	3155.40Ca	3574.46Ba	3873.13Ab	3953.70Ab	**3466.4a**
	V_3	2550.46Db	2743.40Cc	2932.10Bb	3318.47Ac	3306.60Ac	**2970.4c**
	Mean	2522.7D	2922C	3348B	3743A	3775.3A	**3262.2**^{1}
	LSD	Rows = 180.25					
	SE	Rows ±62.22					
Stressed	V_1	1171.86De	1477.67Ce	1602.83Bd	1787.30Ae	1825.60Ae	**1573.0e**
	V_2	1320.77Dd	1531.23Cd	1764.50Bc	1883.46Ad	1931.83Ad	**1686.60d**
	V_3	1254.37Dd	1576.27Cd	1735.10Bc	1877.30Ad	1952.70Ad	**1679.00d**
	Mean	1249.00Dd	1528.30C	1701.00B	1849.30A	1903.70A	**1646.20**^{2}
	LSD	Rows = 122.42					68.20
	SE	Rows ±42.2586					
	Grand Mean	1885.80D	2225.2C	2524.50B	2796.20A	2839.50A	
	LSD	Row 107.81, Across all columns 83.51					
	SE	last row±38.09; Across all columns ±29.5					

Grain yield in the same row followed by the same upper case letters(A,B...D) or in the same column followed by the same lower case letters(a, b, c…e)or superscript numerical letter(1,2) are not significantly ($P<0.05$) different.

4.1.2The Effect of P Rate on Grain Fe(Mg Kg^{-1}) of Three Bean Genotypes under Adequate and Stressed Moisture Conditions

Bean grain Fe concentration showed a significant (df = 16; F = 5.47; $P<$ 0.0001) interactionbetween P application rates, moisture regimes and genotypes(Table 2).

Grain Fe concentration significantly (*df* = 28; *t* = 2.05; $P < 0.05$) increased with P applicationrate up to 40kg ha^{-1} in all the three bean genotypesirrespective of the soil moisture condition. An increase in P fertilizer rate above 40kg ha^{-1} led to a decrease in grain Fe in all the three genotypes undermoisture adequate condition. Under the moisture stressed condition a decrease in grain Fe in V_1 and V_2at rate above 40kg ha^{-1} was noted while V_3 showed a decrease at P application above 60kg ha^{-1}(Table 2). Genotype V_2 had highest grain Fe concentration irrespective of moisture conditions followed by V_3 whilst genotype V_1 showed the lowest grain Fe concentration.

Table 2: Grain Fe (Mg Kg^{-1}) Concentration of Three Bean Genotypes in Varying P Application Rate under Adequate and Stressed Moisture Condition

		P rate (kg ha^{-1})					
Moisture regimes	**Genotypes**	**0**	**20**	**40**	**60**	**80**	**Mean**
Adequate	V_1	61.2Ed	68.8Be	71.7Ae	66.0Ce	63.0De	66.0e
	V_2	80.7Ea	84.8Da	92.4Aa	90.0Ba	83.1Ca	86.0a
	V_3	71.4Dc	75.0Cc	82.6Ac	81.4Bc	72.1Dd	77.0c
	Mean	71.0E	76.0C	82.0A	79.0B	72.0D	76.3^{1}
	LSD	Rows = 1.5408					
	SE	Rows ±0.5319					
Stressed	V_1	57.1Ce	63.8Bf	66.2Af	64.5Bf	58.3Cf	62f
	V_2	77.6Db	78.8Db	87.1Ab	82.6Bb	80.7Cb	81b
	V_3	70.4Ec	73.8Dd	78.6Ad	78.8Ad	76.7Cc	76d
	Mean	68E	72C	77A	75B	72C	73^{2}
	LSD	Rows = 1.2251					0.6063
	SE	Rows ±0.4229					
	Grand Mean	**69.7E**	**74.2C**	**79.8A**	**77.2B**	**72.3D**	**74.64**
	LSD	Row 0.9586, Across all columns 0.7425					
	SE	last row ±0.3386; Across all columns ±0.2623					

Grain Feconcentration in the same row followed by the same upper case letters(A,B...D) or in the same column followed by the same lower case letters (a, b, c…e)or superscript numerical letter(1,2)are not significantly (*P* < 0.05) different.

4.1.3The Effect of P Application rates on Bean Grain Zn (Mg Kg^{-1}) Concentration in Three Bean Genotypes under Adequate and Stressed Moisture Conditions

The grain Zn concentration had a significant (*df* = 16; *F* = 6.67; *P*< 0.0001) interaction between soil moisture, P application rate and genotypes. Under moisture adequate condition, genotype V_1 and V_2 showed significant(*df* = 28; *t* = 2.05; $P < 0.05$) increase in grain Zn concentration up to 40 kg ha^{-1} P(Table 3), but P fertilizer application rate above 40 kg ha^{-1} led to a decrease in grain Zn concentration in genotypesV_2 and $V_{3.}$

Similarly, under moisture stressed condition, P fertilizer application significantly(*df* = 28; $P < 0.05$) increased grain Znconcentration up to 40 kg ha^{-1} in genotype V_1, but in genotype V_2 it increased up to 60kg ha^{-1}.GenotypesV_2 and V_3 had the highest grain Zn concentration compared to V_1 under moisture stressed condition (Table 4).The grain Zn concentration showed no significant(*df* = 58; *t* = 2.0; $P < 0.05$) difference.

Table 3: Grain Zn (Mg Kg^{-1}) Concentration of Three Bean Genotypes in Varying P Application rate under Adequate and Stressed Moisture Condition

		P rate (kg ha^{-1})					
Moisture regimes	**Genotypes**	**0**	**20**	**40**	**60**	**80**	**Mean**
Adequate	V_1	18.9Cd	19.6Be	19.8Ae	20.3Ac	19.6Bd	19.5b
	V_2	22.2Db	24.3Bc	25.5Aa	24.4Ba	23Cb	23.9a
	V_3	20.2Dc	24.7Ab	25.2Ab	24.3Ba	23.9Ca	23.7a
	Mean	20.4C	22.9A	23.5A	23A	22.2B	**22.4**^{1}
	LSD	Rows = 0.616					
	SE	Rows ±0.2126					
Stressed	V_1	19.1Cd	19.6BCe	20.9Ad	19.9Bd	19.3BCd	19.8b
	V_2	23Da	23.8Cd	24.2BCc	24.4Aa	23Db	23.7a
	V_3	22.1Cb	25.7Aa	25.9Aa	23.4Bb	21.5Dc	23.7a
	Mean	21.4D	23B	23.7A	22.6B	21.3C	**22.4**^{1}
	LSD	Rows = 0.5545					0.2578
	SE	Rows ±0.1914					
	Grand Mean	**20.9D**	**23B**	**23.6A**	**22.8B**	**21.7C**	**22.4**
	LSD	Row 0.4077, Across all columns 0.3158					
	SE	Last row ±0.1440; Across all columns ±0.1115					

Grain Znconcentration in the same row followed by the same upper case letters(A,B...D) or in the same column followed by the same lower case letters (a, b, c…e)or superscript numerical letter(1,2) are not significantly (*P*<0.05) different.

4.2.1 The Effect of P Application rate on Leaf Fe Concentration of Three Bean Genotypes under Adequate and Stressed Moisture Conditions

Bean leaf Fe concentration showed highly significant (*df* = 16; *F* =82.76; $P<$ 0.0001)interaction between P fertilizer application rate, genotypes and moisture regimes. Under adequate moisture conditions, leaf Fe concentration in genotype V_2 and V_3significantly(*df* = 28; *t* = 2.05; $P<$ 0.05)decreased with increasing rate of P application up to 60 kg ha^{-1} above which there was no significant (*df* = 28; *t* = 2.05; $P<$ 0.05) decrease in leaf Fe concentration in all the three bean genotypes (Table 5). Under adequate moisture conditions, genotype V_2 had highest (overall) leaf Fe concentration while genotype V_3 had the highest leaf Fe concentration under stressed moisture condition. Under stressed moisture condition, leaf Fe concentrationsignificantly (*df* = 28; *t* = 2.05; $P<0.05$) decreased with increasing rate of P application up to 40kg ha^{-1} of P application(Table 4).There was a significantly (*df* = 58; *t* = 2.0; $P<0.05$) higher leaf Fe concentration in bean grown under stressed water condition thanin those grown under adequate water condition.

Table 4: Leaf Fe Concentration of Three Bean Genotypes in Varying P Application Rate under Adequate and Stressed Moisture Conditions

		P rate (kg ha^{-1})					
Moisture regimes	**Genotypes**	**0**	**20**	**40**	**60**	**80**	**Mean**
Adequate	**V_1**	291Ae	278Bd	239Dd	242Cd	241Cd	**258e**
	V_2	316Ad	285Bc	267Cb	262Db	261Db	**278b**
	V_3	332Ac	305Bb	244Cc	233De	232De	**269d**
	Mean	313A	289B	250C	246D	245D	**268^{2}**
	LSD	Rows = 1.5384					
	SE	Rows ±0.5310					
Stressed	**V_1**	341Ab	288Bc	243Ec	258Cc	250Dc	**276c**
	V_2	256Af	242Be	227Ce	230Cf	230Ce	**237f**
	V_3	370Aa	352Ba	294Da	302Ca	285Ea	**321a**
	Mean	322A	294B	255C	263D	255C	**278^{1}**
	LSD	Rows = 3.7741					1.2431
	SE	Rows ± 1.3028					
	Grand Mean	**318.3A**	**292B**	**252.7C**	**255.7D**	**250.6E**	**273.9**
	LSD	Row 1.9655, Across all columns 1.5224					
	SE	Last rows ±0.6943; Across all columns ±0.5378					

Leaf Feconcentration in the same row followed by the same upper case letters(A,B...D) or in the same column followed by the same lower case letters (a, b, c…e)or superscript numerical letter(1,2)are not significantly different (*P*<0.05).

4.2.2 The Effect of P Application rate on Leaf Zn Concentration of Three Bean Genotypes under Adequate and Stressed Moisture Conditions

Leaf Zn concentration showed a significant (*df* = 16; *F* =2.95; *P* = 0.0013)interaction between P rate, moisture conditions and genotypes. Under adequate moisture condition, genotypeV_1 and V_2 had a significant(*df* = 28; *t* = 2.05; $P< 0.05$) decrease in leaf Zn at P application up to 40kg $ha^{-1}$and there was no significant(*df* = 28; *t* = 2.05; $P< 0.05$) differencein P application above 60 kg ha^{-1}in the three bean genotypes (Table 5).

Under moisture stressed condition, leaf Zn concentration significantly (*df* = 28; *t* = 2.05; $P< 0.05$)decreased up to 40 kg $ha^{-1}$of P application in genotype V_1 and V_2. Genotype V_3 had the highest leaf Zn concentrationunder moisture stressed condition(Table 5).The three bean genotypes had significantly (*df* = 58; *t* = 2.0; $P< 0.05$) higher leaf Zn concentration when grown under stressed moisture condition than under adequate moisture condition.

Table 5: Leaf Zn Concentration of Three Bean Genotypes in Varying P Application rate under Adequate and Stressed Moisture Conditions

		P rate (kg ha^{-1})					
Moisture regimes	**Genotypes**	**0**	**20**	**40**	**60**	**80**	**Mean**
Adequate	V_1	31.5Ab	29.2Bb	27.2Cc	28.3Bb	28.0Ba	29.1b
	V_2	30.4Ac	28.3Bc	26.5Cd	23.5Cd	23.3Cd	26.4e
	V_3	29.7Ad	29.3Ab	26.7Bc	26Bc	26.3Bb	27.6d
	Mean	30.5A	28.9B	26.8C	25.9D	25.9D	**27.7^{2}**
	LSD	Rows = 0.9316					
	Std Error	Rows ±0.3216					
Stressed	V_1	30Acd	29Bb	28.9Cb	28.3Cb	26.2Dc	28.5c
	V_2	30.5Ac	29.7Ba	28.7Cb	28.3Cb	28Ca	29bc
	V_3	33.3Aa	30Ba	29.6Ba	29.2Ca	26.8Db	29.8a
	Mean	31.3A	29.6B	29.1BC	28.6C	27D	29.1^{1}
	LSD	Rows =0.9474					0.4159
	SE	Rows ± 0.3270					
	Grand Mean	**30.9A**	**29.25B**	**27.9C**	**27.3C**	**26.4D**	**28.4**
	LSD	Row 0.6575, Across all columns 0.5093					
	SE	Last row 0.2323; Across all columns ±0.1799					

Leaf Zn concentration in the same row followed by the same upper case letters(A,B...D) or in the same column followed by the same lower case letters (a, b, c…e) or superscript numerical letter(1,2)are not significantly (*P*<0.05) different.

4.2.3.Effect of P Application rate on Percentage of Leaf Fe Accumulated in Grain under Adequate and Stressed Moisture Condition in Three Bean Genotypes

The results for the percentage of leaf Fe accumulated in grain indicated a highly significant (*df* = 16; F =13.36; $P< 0.0001$) interaction between P fertilizer application rates, genotypes and moisture regimes. Under adequate moisture conditions, an increase in P application up to 40 kg ha^{-1}led to a significant (*df* = 28; $t = 2.05$; $P<0.05$) increase in percentage leaf Fe accumulated in grain in genotypes V_1 and V_2 whilst in genotypes$V_{3,}$ percentage accumulation of leafFein grain increased significantly (*df* = 28; $t = 2.05$; $P<0.05$) with increasingrate of P application up to 60 kg $ha^{-1}$and declined (Table 6). Under stressed moisture condition, percentage of leaf Fe accumulated in grain increased significantly (*df* = 28; $t = 2.05$; $P<0.05$) with increasing P application up to 40 kg ha^{-1}in all genotypes. Genotype V_2 had the highest percentage of leaf Fe accumulated in grain under stressed moisture condition (Table 6). Beans grown under moisture adequate condition had significantly (*df* = 58; $t = 2.0$; $P < 0.05$)higher percentage of leaf Fe accumulated in grain Fe than those grown under moisture stressed conditions (Table 7).

Table 6: Percentage (%)of Leaf Fe Accumulated to Grain of Three Bean Genotypes in Varying P Application Rate under Adequate and Stressed Moisture Conditions

		P rate (kg ha^{-1})					
Moisture regimes	**Genotypes**	**0**	**20**	**40**	**60**	**80**	**Mean**
Adequate	V_1	21Dc	25Cc	30Ad	26Bd	26Bd	26d
	V_2	26Eb	30Db	35Ab	34Bc	32Ca	31b
	V_3	21Ec	25Dc	34Bc	35Ab	32Ca	29c
	Mean	23E	27D	33A	32B	30C	29^1
	LSD	Rows = 0.9265					
	SE	Rows ±0.3198					
Stressed	V_1	17E	22Dd	27Ae	25Be	23Ce	23f
	V_2	31Da	33Ca	38Aa	36Ba	31Db	35a
	V_3	19D	21Ce	27Ae	26Bd	27Ac	24e
	Mean	22D	25C	31A	29B	28.5B	27^2
	LSD	Rows = = 0.5727					_0.4159_
	SE	Rows ±0.1977					
	Grand Mean	**23E**	**26D**	**32A**	**30B**	**29C**	**28**
	LSD	Row 0.5267, Across all columns 0.408					
	SE	Last row ± 0.1861; Across all columns ±0.1441					

Percentage (%)of leaf Fe accumulated to grain in the same row followed by the same upper case letters(A,B...D) or in the same column followed by the same lower case letters (a, b, c…e) or superscript numerical letter(1,2)are not significantly (*P*<0.05) different.

4.2.4Effect of P Application Rate on Percentage of Leaf Zn Accumulated to Grain under Adequate and Stressed Moisture Condition in Three Bean Genotypes

A highly significant($df = 16$; $F = 4.16$; $P < 0.0001$) interactionbetween P application rates, genotypes and moisture regimes on percentage of Leaf Zn accumulated to grain was observed. Under adequate moisture condition, percentage of leaf Zn accumulated to grain increased significantly($df = 28$; $t = 2.05$; $P < 0.05$)with increasing rateof P application up to 40 kg ha^{-1}except genotype V_2 which increased up to 60kg ha^{-1}(Table 7).

Under moisture stress condition, percentage of leaf Zn accumulated to grain in genotype V_1 and V_2increased significantly ($df = 28$; $t = 2.05$; $P < 0.05$)with increasing rate of application up to 40 kg ha^{-1}. Under moisture stress condition, genotype V_2 and V_3 had the highest percentage of leaf Zn accumulation to grain(Table 7). Beans grown under moisture adequate condition had significantly ($df = 58$; $t = 2.0$; $P < 0.05$) higher percentage of leaf Zn accumulated to grain than those grown under moisture stressed conditions (Table 7).

Table 7: Percentage(%) of Leaf Zn Accumulated in Grain of Three Bean Genotypes in Varying P Application rate under Adequate and Stressed Moisture Conditions

		P rate (kg ha^{-1})					
Moisture regimes	**Genotypes**	**0**	**20**	**40**	**60**	**80**	**Mean**
Adequate	V_1	60Cf	67.3Bc	72.3Ae	71.6Ae	71Af	68.4d
	V_2	73.2Db	85.7Ca	96.2Ba	100Aa	98Aa	90.6a
	V_3	68.2Dc	84.7Ca	94.2Ab	93.6Ab	90.8Bb	86.3b
	Mean	67.1C	79.2B	87.6A	88.4A	86.6A	81.8^1
	LSD	Rows = 2.856					
	SE	Rows ±0.9859					
Stressed	V_1	63.7Ce	67.7Bc	72.3Ae	70.5Be	73.9Ae	69.6d
	V_2	75.4Ca	80.1Bb	84.3Ad	86.5Ac	82.3Bc	81.7c
	V_3	66.4Cd	85.7Aa	87.6Ac	80.2Bd	80.3Bd	80c
	Mean	68.5C	77.8B	81.4A	79.1A	78.8A	77.1^2
	LSD	Rows = 3.2141					_1.3278_
	SE	Rows ± 1.1095					
	Grand Mean	**67.8C**	**78.5B**	**84.5A**	**83.7A**	**82.4A**	**79.4**
	LSD	Row 2.0994, Across all columns 1.6262					
	SE	Last row ±0.7416; Across all columns ±0.5745					

Percentage (%) of leaf Zn accumulated to grain in the same row followed by the same upper case letters(A,B...D) or in the same column followed by the same lower case letters (a, b, c…e) or superscript numerical letter(1,2)are not significantly (*P*<0.05) different.

4.2.5.Comparison of Leaf and Grain Concentration of Fe in Three Bean Genotypes under Adequate and Stressed Moisture Conditions

All genotypes had higher mean leaf Fe concentration than grain Feconcentration under adequate moisture condition. Leaf Fe concentration followed the order $V_2>V_3>V_1$, while the grain Fe concentration followed $V_2>V_3>V_1$ (Fig.1) All genotypes had higher mean leaf Fe concentration than grain Fe under stressed moisture condition. Leaf Fe concentration followed the order $V_3>V_1>V_2$, while the grain Fe concentration followed $V_2>V_3>V_1$ (Fig. 2)

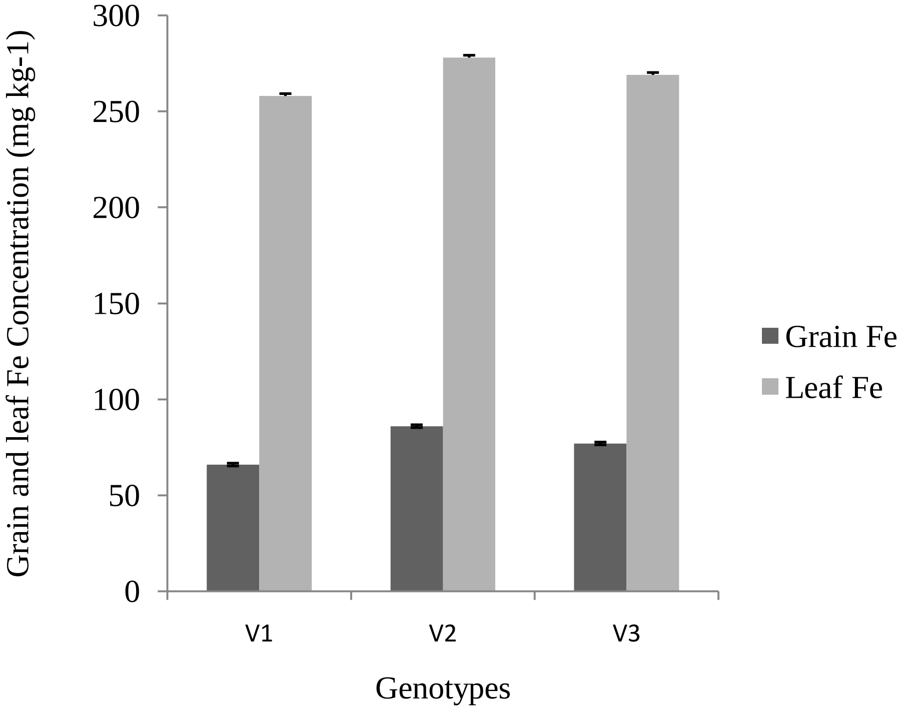

Figure 1: Comparison of leaf and Grain Concentration of Fe in Three Bean Genotypes under Adequate Moisture Condition.

NB: Error bar represent LSD ($P < 0.05$) for the comparison of genotypes at each P rates.

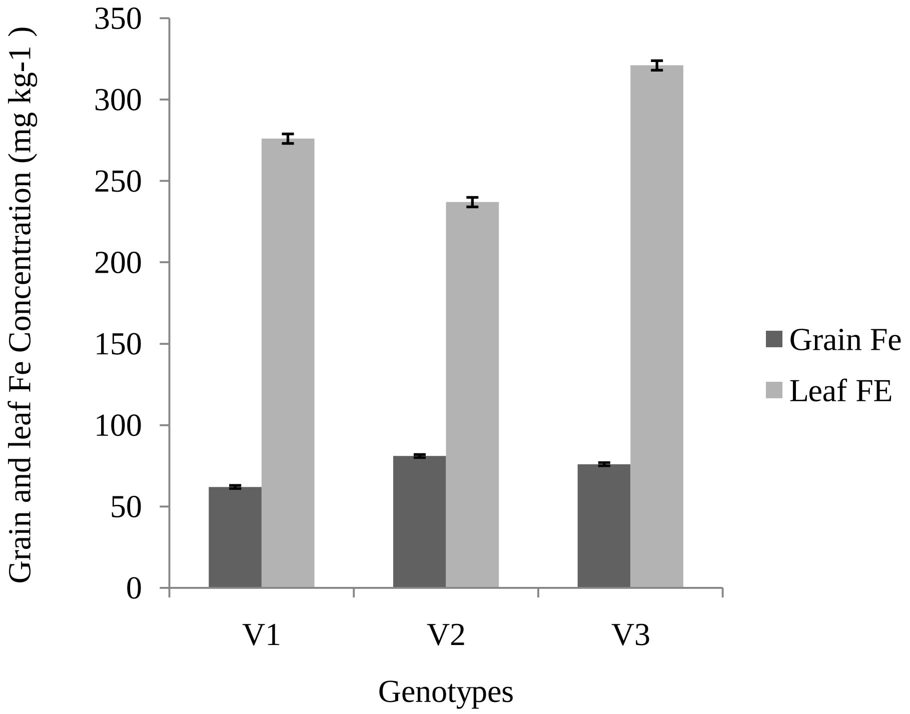

Figure 2: Comparison of Leaf and Grain Concentration of Fe in Three Bean Genotypes under Stressed Moisture Condition

NB: Error bar represent LSD (*P* < 0.05) for the comparison of genotypes at each P rates.

4.2.6.Comparison of Leaf and Grain Concentration of Zn in Three Bean Genotypes under Adequate and Stressed Moisture Conditions

Zinc concentration was higher in leaf than in grain in all genotypes. Leaf Zn concentration followed the order $V_1>V_3>V_2$, while the grain Zn concentration followed the order $V_2=V_3>V_1$(Fig.3) under adequate moisture condition. Zinc concentration was higher in leaf than in grain in all genotypes. Leaf Zn concentration followed the order $V_3=V_2$, $V_2=V_1$ while the grain Zn concentration followed the order $V_2=V_3>V_1$ under stressed moisture conditions (fig 4).

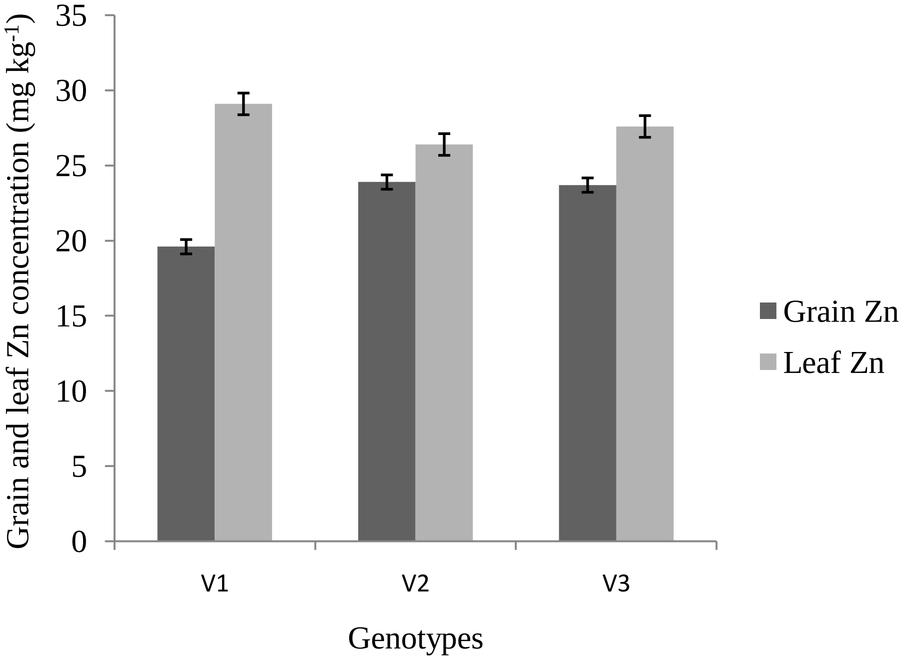

Figure3: Comparison of Leaf and Grain Concentration of Zn in Three Bean Genotypes under Adequate Moisture Condition.

NB: Error bar represent LSD ($P < 0.05$) for the comparison of genotypes at each P rates.

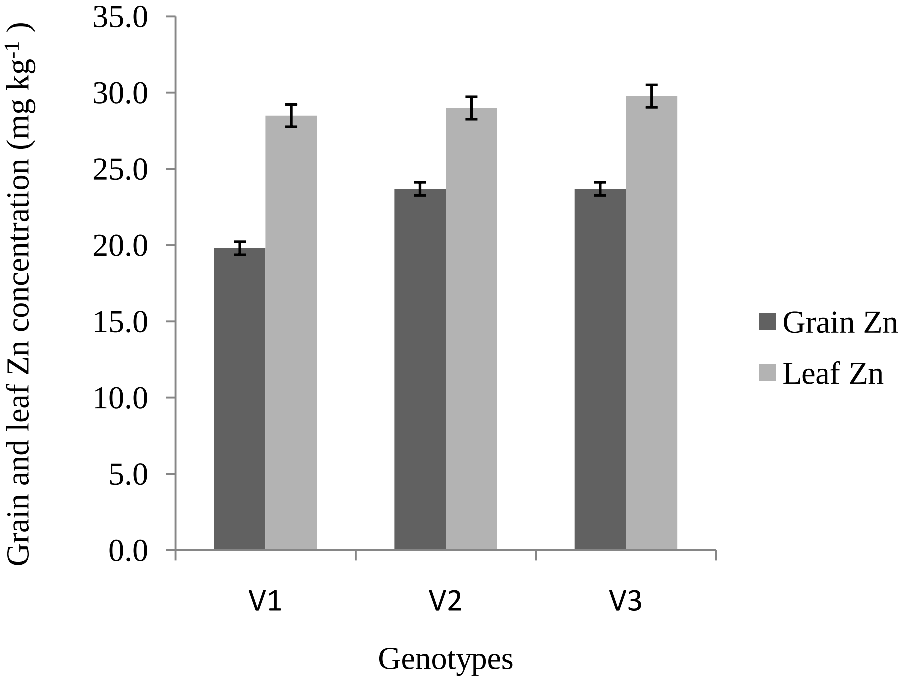

Figure 4: Comparison of Leaf and Grain Concentration of Zn in Three Bean Genotypes under Stressed Moisture Condition

NB: Error bar represent LSD ($P < 0.05$) for the comparison of genotypes at each P rates.

4.2.7. Correlation between Grain Yield and Leaf and Grain Tissue Concentration of Fe and Zn

A highly significant ($n = 90$;$r = 0.79736$;$P< 0.0001$) positive correlation between the grain Fe and Zn concentration and a highly significant($n = 90$; $r = 0.53662$; $P< 0.0001$) positive correlation between leaf Fe and Zn concentration were observed.A highly significant ($n = 90$; $r = - 0.34860$; $P = 0.0008$) negative correlation between the grain yield and leaf Fe concentration and highly significant ($n =90$; $r = - 0.58292$ $P = 0.0001$) negative correlation between grain yield and leaf Zn concentration were observed (Table 8)

Table 8: Correlation between Grain Yield, Leaf and Grain Concentration (Mg kg^{-1}) of Fe and Zn

Concentration	**Grain yield**	**grain Fe**	**grain Zn**	**leaf Fe**
Grain Fe	0.24686*			
Grain Zn	0.06638ns	0.79736**		
Leaf Fe	-0.34860*	-0.20375ns	-0.06295ns	
Leaf Zn	-0.58292**	-0.32753*	-0.16428ns	0.53662**

Significant (P< 0.05);** highly significant (P< 0.01); ns - not significant (P > 0.05).
.|r| Pearson Correlation Coefficients

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Effect of P Application on Grain Yields in Three Bean Genotypes under Adequate and Stressed Moisture Conditions

The current study showed that increase in P application rate led to an increase in grain yield up to the optimal level of 60kg ha^{-1} under adequate and stressed soil moisture conditions in all the bean genotypes. This shows that grain yield increase in the three beans is a combined effect of P application, soil moisture regime and genotypes. The significant grain yield increase with increasing P fertilizer in beans grown under adequate moisture regime in the current study implies that so long as soil conditions are favorable, grain yield will significantly increase with increasing P application up to an optimal level. Other studies indicated a significant increase in grain yield with increasing P application up to optimal levels (Al- Farha and Al-Rawi, 2002; Kamanga *et al.*, 2010; Davood, 2013).

Adequate P results in rapid growth, earlier maturity and increased root growth which means plant can explore soil for nutrients and moisture and its deficiency slow overall plant growth, (McKenzieand Middleton, 1997; Georgina *et al.*, 2007). This process of nutrient uptake by plants has been associated with increased root growth enabling plants to explore more soil nutrients leading to an increased grain yield (Ho *et al.*, 2005; Fageria and Stone, 2006; Fageria *et al.*, 2006).The increase in the grain yield with increasing rate of P application in the current study may be attributed to increased root growth due to increasing P application rate leading to increase nutrient uptake hence increased grain yield.

In the current study bean grown in soil moisture adequate conditions produced more grain yield than those grown in soil moisture stressed conditions irrespective of the P application rate and genotypes. This corroborates other studies that showed that regardless of the P rate of application and superiority of the bean genotype, inadequate soil moisture led to poor seed filling resulting into physiological maturity to happen earlier than expected (Brevedan and Egli, 2003; Muasya and Auma, 2003)eventually leading to reduction in grain yield (Szilagyi, 2003). Crops have marked moisture sensitive stages of which if moisture stress occurs during this stage, crop growth and development is hindered leading to reduced yield, hence bean crop growth needs to be synchronized to soil moisture and P rate to get maximum seed yield (Shah *et al*., 2001; Cassman and Waters, 2002).

The current study shows genotype variation in the grain yield responses regardless P rate of application and moisture regime. The genotype variations imply that choosing superior bean genotype should be considered in order to increase grain yield irrespective of rate of P application and moisture regime. Thecurrent study corroborate other finding that emphasized the importance of choosing bean genotypes that have high grain yield and had superiority in P acquisition efficiency (Zhang, 2007; Fageria *et al*., 2008; Hammond *et al*., 2008).Overall, the current studyemphasizes the importance of not only correction of P deficiency through P fertilizer application rate of 60kg ha-^{1} , ensuring adequate soil moisture is maintained as well as growing high yielding superior genotypesAwash Melka (V_2) in order to have increased bean grain yield.

5.1.2 Effect of P Application and Moisture Stress on Grain Concentration of Zn and Fe in Three Bean Genotypes

Grain Fe and Zn concentration in thethree bean genotypes significantly increased due to a combined effect of moisture regime, P rates and genotypes in the current study. The process of P acquisition in plants has been associated with root architecture development(Miller *et al*., 2003; Liao *et al*., 2004; Beebe *et al*., 2006). A favorable moisture condition improves root development leading to increasedscavenging of soil mineral resources such as Fe and Zn in P efficient genotypes (Ho*et al*., 2005). An increase in P application in bean genotype under favorable moisture condition led to increase in grain Fe and Zn concentration up to P optimal level, such increase was characterized by genotype variations.The genotype variability observed in the current study corroborates a study finding by Astudillo *et al*., (2008) that showed genotype variability in beans response to P rate of application on grain and leaf concentration of Fe and Zn.

Under adequate moisture condition, P application above the optimal level led to a decrease in Fe and Zn concentration irrespective of the genotype. This is associated with dilution effect as a result of P stimulation of growth leading to increased grain yield with increased P application(Fan *et al*., 2008; Murphy *et al*., 2008). Other studies have attributed such decrease in grain Fe and Zn to toxicity effect (Gianquinto*et al*., 2000)or due to complex interaction of P with other minerals such as Mn, Fe whichis known to occur under different cultural systems (Barben *et al*., 2011).The optimal P level at which the grain concentration of Fe and Zn begins to depress in the current study may be attributed to the low native soil P level of 15 mg kg^{-1}at Katumani field unlike soils with high P especially in countries like China with soil P level of 24 mg kg^{-1}(Li *et al*., 2011), East Asia and West Europe (MacDonald *et al*., 2011) which has experienced continuous P fertilizer applications from recent decades, resulting in cumulative surplus of P in croplands. In areas with high soil P levels a depressed grain concentration of Fe and Zn can be attained at a low soil P levels (Yue *et al*., 2012; Ryan *et al*. 2008; Astudillo *et al*., 2008).Irrespective of P application, beans grown under adequate moisture condition produced an overall higher grain Fe concentration than those grown under stressed moisture condition; this is attributed to the role of moisture in uptake of soil nutrients (Priscilao *et al*., 2008).

5.1.3 Effect of P Fertilizer Application and Moisture regime on Bean Leaf Fe and Zn Concentration

A significant decrease in leaf Fe and Zn concentration inthe current study is as a result of a combined effect of P application, moisture regimes and genotypes. A study by Yue *et al*, (2012) found that under favorable moisture condition, leaf concentration of Fe and Zn decrease with increasingP application in bean. The leaves of plants act as the source of assimilates which is accumulated in the grains as the sink, hence decreasing the leaf concentrationdue to the translocation of assimilates from the leaves to the grain (Li *et al*., 2006). Moisture enhances the translocation of assimilates (Fe and Zn) from the leaves to the sink such as flowers, pods and grains(Uauy, 2006).

The process leading to decreased concentration of Fe and Zn in the leaves is attributed to P stimulation of growth and subsequently diluting Fe and Zn concentration or partly due to P induced Zn deficiency (Amin *et al*., 2014). Irrespective of P application beansgrown in moisture stressed conditions had higher overall leaf concentration of Fe and Zn than those grown under adequate moisture condition in the current study. This is attributed to decreased translocation of minerals such as Fe and Zn due to moisture stress and hence retention in the leaves(Uauy, 2006).Inaddition to retention,low yield biomass in beans grown under moisture stress conditions lead to a decreased dilution effect(Fan *et al*., 2008; Murphy *et al*., 2008).

5.1.4 Comparison of leaf and grain concentration of Zn and Fe in three bean genotypes.

Higher concentration of Fe and Zn in the in leaves than in grains in the current study may be attributed to the role of leaves as the source of assimilates which is accumulated in grains as the sink (Li *et al*., 2006).It follows that in a fully grown leaf, concentration of Fe and Zn is maintained naturally higher in leaves than in grain in order for sink and source relation to exist (Visperas *et al*., 1976).

A significant positive correlation betweengrain Fe and Zn concentration and leaf Fe and Zn concentration suggests that genetic factors for increasing Fe and Zn are co-segregating with genetic factors for increasing Zn (Susan and George, 2010). While a significant negative correlation between the grain yield and leaf Fe concentration and grain yield and leaf Zn concentration implies that an increase in grain yield correlates with a decrease in leaf concentration of Fe and Zn suggesting that increase in grain yield will incur a tradeoffs in leaf Fe and Zn harvest. Based on this study, improvement of soil moisture is important in order to increase the grain concentration of Fe and Zn especially in communities where human consumption of bean grain is more preferred than leaf recipe (Brian *et al*., 2011). The current study implies that P application rate should be in consideration of grain yield as well as grain Fe and Zn harvest since the optimal P application rate for grain harvest (60kg ha^{-1}) which is higher than the optimal requirement for grain harvest of Fe and Zn under (40kg ha^{-1}).

5.2 CONCLUSIONS

- Application of P fertilizer up to 60kg ha^{-1}, maintaining adequate soil moisture throughout the growing period coupled with growing high yield bean genotypes led to increase in grain yield.
- Application of P fertilizerup to 40kg ha^{-1}, maintaining adequate soil moisturethought the growing period and growing superior bean genotypesAwash Melka (V_2) led to an increased grain concentration of Fe and Zn.
- Beans that experienced soil moisture stress during and after flowering had higher leaf Fe and Zn concentration than those that never experienced soil moisture stress.
- Beans grown under adequate soil moisture conditions had higher grain Fe concentration than those that experienced soil moisture stress during and after flowering, however, moisture stress experienced during and after flowering had no effect on the overall grain Zn concentration.

5.3 RECOMMENDATIONS

- Famers should be encouraged apply P fertilizer at a rate of 60kg ha^{-1}, maintain adequate soil moisture throughout the bean growing period in addition to planting superior Awash Melka (V_2) bean genotype.
- In order to have highbean grain concentration of Fe and Zn, farmers should apply P fertilizer up to 40kg ha^{-1}, maintained adequate soil moisture throughout the growing period and growAwash Melka (V_2) bean genotype which has higher grain concentration of Fe and Zn.
- Future study should be carried out on the effect of P application and soil moisture stress experienced before the flowering period in the three bean genotypes ongrain yield and leaf and grain tissue concentration of Fe and Zn in the three bean genotypes.

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APPENDICES

Appendix 1: ANOVA Table for Interaction of P Application rates, Moisture regimesand Genotypes on Bean Grain Yield (Kg Ha^{-1}).

```
Dependent Variable: Grain Yield.
         Sum of
    Source              DF      Squares         Mean Square           F Value    Pr> F

    Model                       31       75244118.71    2427229.64              92.97     <.0001

    Error                       58       1514291.47     26108.47

 Corrected Total        89      76758410.18

                R-Square        CoeffVar      Root MSE   Mean grain yield

                0.980272        6.584039      161.5812     2454.134

    Source                 DF       Anova SS     Mean Square    F Value    Pr> F

  Stress                  1     58750165.05     58750165.05    2250.23    <.0001
  P rate                             4     11619881.84      2904970.46     111.27   <.0001
    Genotypes                2       952245.34       476122.67      18.24    <.0001
    Stress x P rate                  4      1394745.89      348686.47      13.36    <.0001
    Stress X Genotype                2      1187117.77      593558.89      22.73    <.0001
    Stress x P rate X Genotypes  16      1330971.00      83185.69       3.19    0.0006
```

Appendix 2: ANOVA Table for Interaction of P Application rates, Moisture regimes and Genotypes on Bean Grain Fe Concentration (Mg Kg^{-1})

```
Dependent Variable: Grain Fe concentration.

                              Sum of
   Source              DF        Squares     Mean Square    F Value    Pr> F

   Model                31     7789.544444      251.275627     125.37    <.0001

   Error               58      116.244444       2.004215

  Corrected Total      89     7905.788889

           R-Square     CoeffVar      Root MSE   Mean grain Fe

           0.985296      1.898008      1.415703      74.58889

   Source               DF       Anova SS     Mean Square    F Value    Pr> F

   Stress               1      208.544444      208.544444     104.05    <.0001
   P rate              4       1118.511111    279.627778     139.52    <.0001
   Genotypes                2        6102.755556   3051.377778    1522.48    <.0001
   Stress X P rate            4       45.844444       11.461111       5.72    .0006
   Stress X Genotypes          2       64.355556       32.177778      16.06    <.0001
   Stress X P rate X Genotypes 16       243.777778     15.236111       7.60    <.0001
```

Appendix 3: ANOVA Table for the Interaction of P Application rates, Moisture regimes and Genotypes on Bean Grain Zn Concentration (Mg Kg^{-1})

Dependent Variable: Grain Zn concentration.

```
                         Sum of
Source             DF     Squares     Mean Square   F Value   Pr> F

Model              31   454.2217778   14.6523154    39.25    <.0001

Error              58    21.6511111    0.3732950

Corrected Total    89   475.8728889

          R-Square   CoeffVarRootMS      Mean grain Zn

          0.954502   2.728666   0.610979   22.39111
```

Source	**DF**	**Anova SS**	**Mean Square**	**F Value**	**Pr> F**
Stress	1	0.0017778	0.0017778	0.00	0.9452
P rate	4	80.2240000	20.0560000	53.73	<.0001
Genotypes	2	324.4115556	162.2057778	434.52	<.0001
Stress X P rate	4	8.5582222	2.1395556	5.73	0.0006
Stress X Genotype	2	0.3795556	0.1897778	0.51	0.6041
Stress X P rate X Genotype	16	39.8244444	2.4890278	6.67	<.0001

Appendix 4: ANOVA Table for Interaction of P Application rates, Moisture regimes and Genotypes on Bean Leaf Fe Concentration (Mg Kg^{-1})

```
Dependent Variable: Leaf Fe


Sum of
   Source                DF       Squares     Mean Square    F Value    Pr> F

   Model                  31     135281.1333       4363.9075     502.93    <.0001

   Error                58       503.2667         8.6770

 Corrected Total       89     135784.4000

             R-Square     CoeffVar      Root MSE      Mean leaf Fe

             0.996294      1.078213      2.945677      273.2000

   Source                 DF       Anova SS     Mean Square    F Value    Pr> F

   Stress                 1      1960.00000      1960.00000     225.88    <.0001
   P rate                4     65820.17778     16455.04444    1896.40   <.0001
   Genotype                  2     22786.06667     11393.03333    1313.01    <.0001
   Stress X P rate                 4      494.22222       123.55556      14.24    <.0001
   Stress X Genotype           2     32718.06667     16359.03333    1885.33    <.0001
   Stress X P rate X Genotypes 16   11489.20000     718.07500      82.76    <.0001
```

Appendix 5: ANOVA Table for Interaction of P Application rates, Moisture regimes and Genotypes on Bean Leaf Zn Concentration (Mg Kg^{-1}).

```
Dependent Variable: Leaf Zn Concentration.
Sum of
    Source                DF       Squares     Mean Square    F Value    Pr> F

    Model                 31     394.9344444     12.7398208      13.12    <.0001

    Error                58      56.3251111      0.9711226

    Corrected Total           89     451.2595556

               R-Square     CoeffVar     Root MSE   Mean Leaf Zn

               0.875182      3.476988      0.985456      28.34222

    Source                DF       Anova SS     Mean Square    F Value    Pr> F

    Stress                 1      51.0760000      51.0760000     52.59    <.0001
    P rate                 4      224.3984444     56.0996111      57.77    <.0001
    Genotype                 2      17.4648889      8.7324444       8.99    0.0004
    Stress X P rate            4      14.7428889      3.6857222       3.80    0.0083
    Stress X Genotype           2      37.8560000      18.9280000      19.49    <.0001
    Stress X P rate X Genotype  16      45.7946667      2.8621667       2.95    0.0013
```

Appendix 6: ANOVA Table for Interaction of P Application rates, Moisture regimes and Genotypes on Percentage (%) of Bean Leaf Fe Accumulated to Grain.

```
Dependent Variable: Percentage Leaf Fe Accumulated to Grain.


                     Sum of
   Source                DF       Squares     Mean Square    F Value    Pr> F

   Model                 31    2732.979227      88.160620    141.49    <.0001

   Error                58     36.139813      0.623100

 Corrected Total        89    2769.119040

              R-Square    CoeffVar     Root MSE     mean %leaf Fe:grain

              0.986949     2.824009     0.789367     27.95200

   Source                DF       Anova SS     Mean Square    F Value    Pr> F

   Stress               1       63.369671       63.369671     101.70    <.0001
   P rate               4     1055.847884      263.961971     423.63   <.0001
   Genotype                2     1167.462587      583.731293     936.82    <.0001
   Stress X P rate          4       10.493129        2.623282       4.21    0.0046
   Stress X Genotype          2      302.059742        151.029871     242.38    <.0001
   Stress X P rate X Genotype  16     133.227093        8.326693      13.36    <.0001
```

Appendix7: ANOVA Table for Interaction of P Application rates, Moisture regimes and Genotypes on Percentage (%) of Bean Leaf Zn Accumulated to Grain.

```
Dependent Variable: Percentage Leaf Zn Accumulated to Grain.

                           Sum of
  Source                   DF        Squares     Mean Square    F Value    Pr> F

  Model                    31      10454.63744   337.24637      34.06    <.0001

  Error                    58      574.20978     9.90017

    Corrected Total        89      11028.84722

             R-Square    CoeffVar     Root MSE     Mean % leaf Zn: grain

             0.947936     3.958080     3.146453     79.49444

    Source                DF       Anova SS     Mean Square    F Value    Pr> F

    Stress                 1       503.626778    503.626778     50.87    <.0001
    P rate                4        3461.136667   865.284167     87.40    <.0001
    Genotype                 2     5005.953556   2502.976778   252.82   <.0001
    Stress X P rate          4     369.343778    92.335944      9.33    <.0001
    Stress X Genotype          2        401.113556    200.556778    20.26      <.0001
    Stress X P rate X Genotype  16     658.566222    41.160389       4.16    <.0001
```

Appendix 8: Soil Analysis Results at KARI - Katumani Experimental Plot at the Beginning of the Experiment.

Soil Properties	**Content**
Soil pH (water 1 : 2.5)	6.36
Total N (%)	0.11
Organic Carbon (%)	0.89
P (mg kg^{-1})	15
Potassium (mg kg^{-1})	1.14
Magnesium (mg kg^{-1})	2.87
Calcium (mg kg^{-1})	3.40
Sodium (mg kg^{-1})	0.16
Copper (µgg^{-1})	4.63
Iron (µgg^{-1})	15.10
Zinc (µgg^{-1})	6.91
Manganese (µgg^{-1})	0.87

Appendix 9: Field Data.

moisture stress	Repeat Rep	P rate (Kg ha^{-1})	genotype v\$	Grain weight (Kg ha^{-1})	Grain Fe (mg kg^{-1})	Grain Zn (mg kg^{-1})	Leaf Fe (mg kg^{-1})	Leaf Zn (mg kg^{-1})	Grain/leaf Fe (%)	Grain/leaf Zn %
1	1	0	V_1	2005.3	60	19.6	291	31.5	21	62.2
1	2	0	V_1	2488.9	63	18.9	291	31.5	22	60.0
1	3	0	V_1	2232.9	61	18.2	291	31.5	21	57.8
1	1	20	V_1	2631.1	69	19.6	278	29.2	24.8	67.1
1	2	20	V_1	3100.4	69	19.3	278	29.3	24.8	65.9
1	3	20	V_1	2872.8	69	20.0	278	29.0	24.8	69
1	1	40	V_1	3527.1	70	20.4	240	27.2	29.2	75
1	2	40	V_1	3640.8	74	19.0	237	27.2	31.2	69.9
1	3	40	V_1	3441.7	72	20.1	239	27.2	30.1	73.9
1	1	60	V_1	3868.4	67	20.4	242	28.3	26	72.1
1	2	60	V_1	4159.9	66	20.1	242	28.3	25.6	71
1	3	60	V_1	4081.7	64	20.3	242	28.3	26.4	71.7
1	1	80	V_1	4088.8	64	19.7	241	25.2	26.6	78.2
1	2	80	V_1	4181.3	62	20.0	241	28.7	25.7	69.7
1	3	80	V_1	3925.3	63	19.1	241	29.3	26.1	65.2
1	1	0	V_2	2719.2	81	21.0	316	30.4	25.6	69.1
1	2	0	V_2	2717.8	80	22.1	316	29.0	25.3	76.1
1	3	0	V_2	2887.1	81	23.6	316	31.7	25.6	74.4
1	1	20	V_2	3393.4	85	24.6	285	28.4	29.8	86.6
1	2	20	V_2	3143.1	85	23.6	285	28.2	29.8	83.7
1	3	20	V_2	2929.7	85	24.6	285	28.3	29.8	86.9
1	1	40	V_2	3413.28	92	25.6	265	26.5	34.7	96.6
1	2	40	V_2	3697.7	90	25.5	270	26.5	33.3	96.6

1	3	40	V_2	3612.4	95	25.3	265	26.5	35.8	95.5
1	1	60	V_2	3868.4	90	24.7	262	23.5	34.4	100
1	2	60	V_2	3896.8	92	25.1	262	24.8	35.1	100
1	3	60	V_2	3854.2	88	23.4	262	22.2	33.6	100
1	1	80	V_2	4081.7	83	23.0	261	23.3	31.8	100
1	2	80	V_2	3839.9	83	23.8	260	23.3	31.9	100
1	3	80	V_2	3939.5	83	22.2	261	23.3	31.8	95
1	1	0	V_3	2545.7	70	20.2	335	30.7	20.9	65.8
1	2	0	V_3	2446.2	73	19.7	332	29.7	22	66.3
1	3	0	V_3	2659.5	71	20.8	330	28.7	21.5	72.5
1	1	20	V_3	2531.5	76	24.7	309	30.0	24.6	82.3
1	2	20	V_3	2897	74	24.9	305	30.3	24.3	82.2
1	3	20	V_3	2801.7	75	24.6	301	27.5	24.9	89.5
1	1	40	V_3	2659.5	80	25.5	244	26.8	31.9	95.1
1	2	40	V_3	2986.6	83	24.8	244	26.5	35.2	93.6
1	3	40	V_3	3150.2	85	25.2	245	26.8	34.7	94
1	1	60	V_3	3527.1	81	24.6	236	27.0	34.3	91
1	2	60	V_3	3100.4	79	23.7	231	24.8	34.2	95.6
1	3	60	V_3	3327.9	84	24.7	233	26.2	36.1	94.3
1	1	80	V_3	3640.8	71	24.4	232	26.3	34.9	92.8
1	2	80	V_3	3128.8	72	23.2	232	26.3	31	88.2
1	3	80	V_3	3150.2	73	24.0	232	26.3	31.5	91.3
2	1	0	V_1	1224.5	57	18.9	341	30.0	16.7	63
2	2	0	V_1	1167.6	56	19.4	341	30.0	16.4	64.7
2	3	0	V_1	1123.5	58	19.0	341	30.0	17	63.3
2	1	20	V_1	1499	64	19.6	288	29.0	22.2	67.6
2	2	20	V_1	1483.4	64	19.8	288	29.0	22.2	68.3
2	3	20	V_1	1450.6	63	19.5	288	29.0	21.8	67.2

2	1	40	V_1	1523.2	67	21.0	246	29.0	27.2	72.4
2	2	40	V_1	1792	66	20.7	243	28.9	27.2	71.6
2	3	40	V_1	1493.3	66	20.9	239	28.7	27.6	72.8
2	1	60	V_1	1848.9	64	19.6	269	28.0	23.58	70
2	2	60	V_1	1593	65	19.9	252	28.5	25.8	69.8
2	3	60	V_1	1920	65	20.3	252	28.3	25.8	71.7
2	1	80	V_1	1962.6	59	19.4	250	24.2	23.6	80.2
2	2	80	V_1	1807.6	59	19.3	250	28.5	23.6	67.7
2	3	80	V_1	1706.6	57	19.2	250	26.0	22.8	73.8
2	1	0	V_2	1564.4	77	24.4	256	30.5	30.1	80
2	2	0	V_2	1331.2	78	23.0	256	30.5	30.5	75.4
2	3	0	V_2	1066.7	79	21.6	256	30.5	30.9	70.8
2	1	20	V_2	1578.6	77	24.0	242	29.7	31.8	80.8
2	2	20	V_2	1479.1	80	23.6	242	29.7	33.1	79.5
2	3	20	V_2	1536	79	23.8	242	29.7	32.6	80.1
2	1	40	V_2	1720.9	85	24.2	224	28.7	37.9	84.3
2	2	40	V_2	1806.2	90	24.2	229	28.7	39.3	84.3
2	3	40	V_2	1766.4	87	24.2	229	28.7	38	84.3
2	1	60	V_2	1784.9	83	24.4	230	25.7	36.1	94.9
2	2	60	V_2	1910	84	24.4	230	30.2	36.5	80.8
2	3	60	V_2	1955.5	81	24.4	230	29.1	35.2	83.8
2	1	80	V_2	1863.1	81	23.8	265	28.0	30.6	85
2	2	80	V_2	1976.9	82	22.4	256	28.0	32	80
2	3	80	V_2	1955.5	79	22.9	261	28.0	30.3	81.8
2	1	0	V_3	1365.3	70	22.1	370	33.3	18.9	66.4
2	2	0	V_3	995.5	70	23.2	370	33.3	18.9	69.7
2	3	0	V_3	1402.3	70	21.0	370	33.3	18.9	63.1

2	1	20	V_3	1742.2	73	25.7	352	30.0	20.7	85.7
2	2	20	V_3	1507.5	74	26.0	352	30.8	21	84.4
2	3	20	V_3	1479.1	75	25.4	352	29.2	21.3	87
2	1	40	V_3	1848.9	79	24.9	284	29.5	27.8	84.4
2	2	40	V_3	1664	81	26.8	304	31.5	26.6	85.1
2	3	40	V_3	1692.4	77	25.9	294	27.7	26.2	93.5
2	1	60	V_3	1991.1	79	23.4	302	27.5	26.2	85.1
2	2	60	V_3	1706.6	77	23.5	302	29.6	25.5	79.4
2	3	60	V_3	1934.2	80	23.2	302	30.5	26.5	76.1
2	1	80	V_3	1991.1	77	22.0	286	26.7	26.9	82.4
2	2	80	V_3	1937	77	21.5	286	26.8	26.9	80.2
2	3	80	V_3	1930	77	21.0	284	26.8	27.1	78.4