BREEDING COMMON BEANS (*Phaseolus vulgaris* L.) FOR HIGHER GRAIN YIELD, IRON AND ZINC CONCENTRATION IN KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN PLANT BREEDING AND BIOTECHNOLOGY

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

2023

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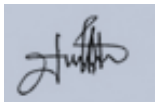
Signature  Date …05/12/2023

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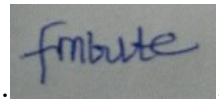
Dr. Felister Mbute Nzuve ……  …… Date …05/12/2023…

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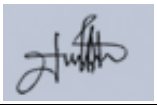
Signature:  **Date:** 4th December 2023

DEDICATION

I dedicate this thesis to Jehovah my refuge who made it possible for me in every way.

I also wish to dedicate this thesis to my parents and all mentors who supported me through life and throughout the process of putting this work together. You have been a blessing sent to me by Jehovah.

I also would wish to dedicate this work to my brothers Ocaya Michael and Okii James to thank them in a special way, for guidance and support they gave me throughout my struggle with life. To my mentors in this field, Dr. Luka Atwok and Susan Ayot for their unwavering support and guidance throughout this study, and may your efforts be reworded abundantly by God the almighty.

ACKNOWLEDGEMENTS

I would like to thank Jehovah our creator for pushing me to success through all academic barriers and challenges.

I wish to express my happiness and respect to all the supervisors, Dr. Felister Nzuve, Prof. James W. Muthomi from the Department of Plant Science and Crop Protection University of Nairobi, and Dr. Susan Wanderi (KALRO-Embu) for their guidance and advice during my research.

My gratitude goes to the Alliance for Green Revolution in Africa (AGRA) the organization for supporting me financially throughout this training.

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LIST OF ABBREVIATIONS

AGRA	Alliance for a Green Revolution in Africa
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
ASL	Above Sea Level
ANOVA	Analysis of variance
CIAT	Centro Internacional de Agricultura Tropical
Cm	Centimeter
DRC	Democratic Republic of Congo
FAO	Food and Agricultural Organization
Fe	Ferus
GCA	General combining ability
GxE	Genotype by Environment
ICPOES	Inductively Coupled Plasma Optical Emission Spectrometry
Kg	Kilogram
LSD	Least Significant Difference
M	Meter
Mg	Milligram
Ppm	Part per million
RCBD	Randomized Complete Block Design
SDG	Sustainable Development Goal
SCA	Specific combining ability
Zn	Zinc

ABSTRACT

Around the world today, legumes are among the most consumed crops and the species especially *Phaseolus vulgaris*. Bean yields vary due to varietal differences and also the environmental conditions. Its nutrient content varies greatly among the genotypes. For example, the concentrations of iron and zinc minerals are not the same in the different varieties grown around the world today. Most of the varieties grown by farmers in the different communities in Kenya have lower or no zinc or iron minerals. Therefore, bio fortification of this crop is necessary to help in fighting mineral deficiency among people who depend on dry beans as a staple food. The broad objective of this research was to improve the mineral (iron and zinc) content of some elite dry bean varieties while maintaining a higher yield. The specific objectives of the study were: (i) To determine the effects of environments on the yield of selected dry bean varieties and lines that have higher concentrations of iron and zinc (ii) To determine the combining ability of selected parents for yield and minerals (iron and zinc) concentration.

For the study of the effects of environment yield, thirty-six genotypes of dry beans were set up in an experiment in two environments, Kenya Agricultural and Livestock Research Organization (KALRO) in Embu and Mwea, Kirinyaga County. Alpha lattice design was used and was replicated three times. The first experiment was planted in May of 2018 and the second one was planted in November of 2018. Meanwhile, for the second objective, eight parents including four high-yielding varieties and four bean lines with higher iron and zinc minerals were selected for crossing. They were mated in half diallel design and the parents were included. After crossing, twenty-eight F1 progenies were produced and planted to generate F2 progenies. The F2 progenies were evaluated together with the parents in two environments of KALRO Mwea and KALRO Embu using alpha lattice design between March and July of 2019. Agronomic data plant health data, and yield data were collected and analyzed. Significant variation of the traits among the genotypes, and between locations in terms of both agronomic and yield traits was reobserved. Genotype KATRAM matured in 74 days while NUA692 matured in 86 days for NUA692, pods number per plant which ranged from 8 pods NUA686 to 10.4 pods in NUA680, and grain yield which ranged from 352 Kg/ha for NUA595 to 697Kg/ha for NUA680, days to flowering varied from 38 days for NUA636 to 48 days for NUA666 in Mwea site. In Embu, days to flowering ranged from 38 days (NUA636) to 47 days (NUA666), days to maturity varied from 67.4 to 77.5 for KATRAM and NUA692 respectively. Pod number per plant ranged between 8.1 and 12 pods. Among the genotypes,

yields varied from 318Kg/ha for NUA666 to 642Kg/ha for EMBEAN14. The correlation between the pod number and seed yield in Kg/ha was positive. Five genotypes (EMBEAN14, NUA680, CHELALANG, WAIRIMU, and TASHA) showed stability in performance across the two sites and the two seasons.

Evaluation of the twenty-eight progenies generated after crossing showed that the environmental effects were significant for percentage emergence; while genotypes had a highly significant effect also on all the agronomic traits. The Genotype by Environment (GxE) interaction showed significant effects also on all agronomic traits plus both root rot and bean fly incidence. There was a significant general combining ability (GCA) ($P<0.05$) effect for yield on genotypes WAIRIMU, CIANKUI, NUA604, and NUA640. There was a significant GCA effect for both Iron and Zinc concentration with genotypes NUA680 and NUA640 ranking as the first and second in GCA for iron concentrations while genotypeNUA604 and NUA 680 ranked first and second in GCA for zinc concentration. For SCA, the genotypes WAIRIMU x KATB9, NUA730 x CIANKUI, NUA730 x NUA640, CIANKUI x NUA640, WAIRIMU x NUA680, WAIRIMU x NUA604, showed higher specific combining ability for seed yield, weight of 100 seed and number of seeds per pod. Across the two sites, among the 28 crosses studied, concentrations of Fe and Zn in the seeds were found to be high with Fe (>70 ppm) and Zn (>30 ppm). The variation among the material's general combining ability (GCA) and specific combining ability (SCA) that showed that there was both additive gene effects and non-additive gene effects. This is because high GCA indicates additive gene action while SCA indicates non additive gene action.

CHAPTER ONE: INTRODUCTION

1.1 Back ground information

Common beans (Phaseolus vulgaris L.) is a very important leguminous crop cultivated and consumed by many people around the world. The production of common beans in Kenya and east Africa is lower than expected with a mean of 895 kg/ha compared to 2.5 to more than 4 tons/ha which is achievable. The shortfall in yield is attributed to many production constraints including poor field operation, inadequate use of inputs, biotic and abiotic factors among others. Plant breeding is one of the approaches used to overcome these challenges (Huertas *et al*,.2022).

Bean breeding programs aim at developing bean varieties that are high-yielding, tolerant to drought, pests and diseases, with good taste and better cooking quality coupled with high micronutrient quality especially iron and zinc content (Huertas *et al*,.2022). Bean provide both proteins and carbohydrates to the human body in good proportions (Santos *et al.*, 2017). Common beans also represent a reliable source of minerals, and other micronutrients for most people in the Americas, Africa, and Asia. For people in East Africa, it provides over 60% of the total protein required by the body and 34% of the total energy needed by the body especially for people with low income (Kasankala *et al.*, 2018).

Kenya is the second leading producer in East Africa (Duku 2020) and common beans come second after maize in level of importance as a staple food crop in Kenya and the region. It is cultivated by over one and half million farmers with yields of about 0.5 t/ha. The main production areas include Western Kenya, Central regions, the Rift Valley, Eastern, and Lake Victoria region. Consumption of common bean in Kenya stands at about 756,000 tons annually but the annual national production stands at about 610,000 tons. Per capita consumption of

beans in Kenya is 14.5 kg yearly, it can reach up to 66 kg per year especially in the western regions of the country. The production of bean in Kenya has been reducing gradually from 714,492 tons in 2013 to 615,992 tons in 2014 (Duku 2020). Over the years, it has been concluded by researchers that, varieties of common beans that have a higher concentrations of iron and zinc can supply an adequate amount of the minerals required by human body for its normal function since dry bean seeds can contain up to about 162 ppm, iron, and about 66 ppm zinc, in the seeds (Zemolin *et al.*,2016).

Hence, breeding common beans for improved zinc and iron content is necessary because common bean is one of the most consumed legumes and a staple diet for most communities in this region and it contains minerals in good proportion as needed by human body. Therefore, consuming dry bean varieties with a sufficient concentration of iron and zinc helps reduce deficiency among minors of five years and below in countries of this region where around 74% of infant mortality is said to be caused by malnutrition and deficiency diseases (Jha *et al*, 2020). Demands of consumers are now becoming a major factor that determines the marketability of a common dry bean variety. The demands may be based on characteristics such as seed color, seed size and shape, cooking time, and taste (Cichy *et al.*, 2012)

1.2 Statement problem

Most of the bean varieties grown in the country have insufficient content of zinc and iron and the yield of common bean in Kenya has been in decline for long now yet the consumption is increasing due to the steady growth of the population The production of bean has reduced from about 714,492 tons annually in the last ten years to about 615,992 tons in recent years (Hawkes *et al.*, 2017). Because of this, malnutrition is prevailing in the country and the region and the effects is seen in one in every three people you meet in the

region today (Hawkes *et al.*, 2017). Malnutrition is one of the leading cause of birth defects and infant ill-health it also reduces human working ability and energy (Harika *et al.*, 2017) Mineral deficiency especially iron and zinc is due to the intake of diets with low concentrations of those important minerals because of inability to afford diets that contain higher minerals. In that case, a cheaper source of iron and zinc such as bio-fortified common bean can be a solution to deficiency (Kasankala *et al.*, 2018).

1.3 Justification of the study

Industrial food fortification rarely impacts rural and low-income households. But since the consumption of common beans in Kenya is among the highest in the region, and since bio fortification of the crop is possible, it can be used as one of the tools in the fight against prevalent malnutrition. A lot of research have been done to help this course, for example the bio fortification breeding of beans at Rwanda Agricultural Board (RAB) that was done through single crosses in 2002, and the populations were developed and advanced up to F7 after pedigree selection method (Jha *et al,*. 2020). This kind of efforts are deemed necessary because hundreds of millions of people namely adult and children are suffering from deficiency related diseases like anemia and stunted growth. The higher percentage of this number is found in Africa and Asia according to the global Nutrition Report of 2017. Those who are affected the most by malnutrition or deficiency are in low-income communities and cannot afford regular diets that supply most of the micro nutrient minerals. Therefore a cheaper diet like bio-fortified dry bean which can be grown by the local farmers themselves and can supply micronutrients like zinc and iron minerals can be used in the alleviation of micronutrient deficiency since it is widely available in Africa and east Africa (Głowacka *et al.,* 2015). The best way to achieve this is through Bio-fortification of common dry bean varieties which are high yielding and attractive to both

farmers and marketers. Such bio fortification can only be done through plant breeding which can be conventional or molecular breeding being the best ways of developing such varieties (Martins *et al.,* 2016).

This study aims at improving some high yielding varieties for higher concentration zinc and iron through plant breeding as a mean bio fortification. This will push forward toward achieving one of the United Nations Sustainable Development Goals SDG agenda of fighting malnutrition for better health since a good balanced diet means better health, therefore less demand on health systems that provide treatment and prevention

1.4 Objectives

The main objective is to improve local elite varieties of common beans for high iron and zinc concentration and high yield. The specific objectives are:

i. To evaluate common dry bean genotypes rich in zinc and iron for agronomic performance and seed yield in different environments.

ii. To define the combining ability for yield, zinc and iron concentration of those selected bean genotypes.

1.5 Research hypothesis

i. The yield of micronutrient-rich beans is not affected by the environment.

ii. The trait for high micronutrient concentration in common beans is not heritable.

CHAPTER TWO

LITERATURE REVIEW

1.6 Origin of common beans

Common beans are known to have originated from South and Central America Andean and Mesoamerican centers of origins. Evidenced by the closest relatives of Phaseolus genus which are spread all over Mesoamerica (Arkwazee *et al.*, 2017). These centers Andean and Mesoamerica form two gene pools namely, the Andean gene pool, and the Mesoamerican gene pool (Alladassi *et al.*, 2018). Common beans are among the five domesticated species from the genus *Phaseolus* and it is a diploid organism with a genome formula (2n = 2x = 22). Common beans, (*Phaseolus vulgaris L)* are in the family Leguminosae. It belongs to the Kingdom Plantae, Phylum Spermatophyta, Class Dicotyledonae, Order Fabales, Genus *Phaseolus*, Species *vulgaris* and scientific name is *Phaseolus vulgaris* grows either as determinate or indeterminate and it is an annual plant. The leaves are trifoliate; and its flower color ranges from white to violet-purple to red (Alladassi *et al.*, 2018) Its pods are about 10 to 26 cm long, straight or slightly curved in shape, and the seeds are, elongated and nearly kidney-shaped though some are round shaped; it has seeds with white, red, purple ,black, gray, or mottled seed coat.

1.7 Importance of common beans

Common bean (*Phaseolus vulgalis L)* is a very important crop in Kenya. It is considered both as food and cash crop in Kenya. Over the last decade, the production was at about 417,000 tons a year, an equivalent of over US$ 199,000,000. The consumption of common bean in Kenya is high and in most regions of the country, it constitutes a part of everyday dietary requirements of proteins, complex carbohydrates, plus some micronutrients and is a reliable source of income in sub-Sahara Africa (Beebe *et al.*, 2014).

They play critical role in combating malnutrition (Mukai Mughi *et al.,* 2017). Its high profitability makes it highly marketable both in the local and international markets. It has lower labor and agricultural input requirement hence minimum investment. Common bean performs well in less fertile soils and its use as green manure for nitrogen fixation it improves soil structure and fertility and growth of soil microorganism. On gender responsiveness, common bean is a woman's crop enabling them to engage in farming for competitive produce markets (Akibode, 2011).

Production of common beans in Kenya is concentrated in the highlands and midlands. About 70% of annual bean farming is done in western Kenya, Nyanza province, Rift valley region, and Eastern Province. The rift valley contributes about 30% of production, in Kenya; followed by Nyanza province which contributes about 23%. Eastern Kenya and the coast have the lowest production percentage due to environmental factors which are not favorable for growing beans in that part of Kenya (Larochelle *et al.,* 2016).

1.8 Production of common bean in Kenya

The main production areas include Western Kenya, Central regions, the Rift Valley, Eastern, and Lake Victoria region. Consumption of common bean in Kenya stands at about 756,000 tons annually but the annual national production stands at about 610,000 tons which leaves a deficit of about 146,000 tons (Duku 2020). Per capita consumption of beans in Kenya is 14.5 kg yearly, it can reach up to 66 kg per year especially in the western regions of the country. Despite the many efforts being put to improve production of beans globally and regionally, the production in Kenya has been reducing gradually by about 98500 tons yearly since the year 2013 (Duku 2020).

There are two types of common beans that are currently being grown in Kenya and the region, these are bush beans, and climbing beans. Bush beans are generally early maturing with yield of about 2.8 t/ha in a season. In other parts of the world like in Latin America the yield range 650 and 850 kg/ha, and in East and Southern Africa, yields are even lower than the above range. Climbing beans however, are slightly late maturing growing season 105-120 days; some varieties can even go up to above 235 days. The yield potential of climbing beans stands at about 4.6 t/ha. In east Africa, common beans is extensively cultivated and Kenya has the most cultivated area in Africa followed by neighboring Uganda while Tanzania is third, Ethiopia is the eighth in Africa followed by Malawi which is ninth (Beebe *et al.*, 2014). However, Uganda produces more beans as compared to Kenya though Kenya uses more land area to grow beans than Uganda uses. Uganda produces more beans in smaller land area compared to Kenya because the environments in Uganda are better for bean production than in Kenya. The world bean production stood above 24 million tones, and out of that, 24.4% came from Latin America while 17.7% was from Africa. The difference in yield around the world is explained by the many stresses that exist. For example, extreme temperatures, moisture stress and also biotic stresses plus many others. This results in reduction in the yield of the crop. Bad soil conditions like too low soil pH may cause poor development of the plant. Biotic stresses like pest and diseases also affect yield of beans especially pests like bean fly or white fly and diseases including root rot, powdery mildew, and angular leaf spot (Manandhar *et al.*, 2016).

1.9 Challenges to common bean production

There are a lot of challenges faced by bean producers in East Africa and in Kenya. The challenges include climate change which has interrupted the weather patterns making it difficult for farmers to plan their bean planting. In some cases, it has resulted in low or

unreliable rainfall, which has led to reduced yield of the crop. Another challenge facing bean production is the farmers' lack of adequate information on the variety that can yield well in their location and ecological zones. Common bean diseases like bean root rot disease, and pests like stem maggots which attack the crop are also major contributors to yield reduction.

Lastly, imported bean from countries with low production costs creates unfair market competition for local bean producers (Katungi *et al.,* 2017).

To mitigate those challenges, researchers have identified some common dry bean genotypes with tolerance to diseases but with high productivity. Such varieties are now being used in bean breeding process to improve yields (Katungi *et al.,* 2017). Varieties of common beans that are tolerant to diseases and mature early can help with problems of unreliable rainfall. However, despite these efforts, the adaptation of the new improved bean varieties remains poor due to farmers' lack of awareness of the existence of such varieties or because the new varieties may be lacking the farmers' preferred trait. therefore, a lot of effort should be placed on the improvement of locally adapted varieties for yield and nutrient content (Zulu *et al.,* 2013).

1.10 Improvement of common bean for yield

Bean improvements in the region began in the early 1960s, and it involved both breeding and better agronomic practices. In the 1980s, with support from CIAT, breeding programs were extended to major bean-producing areas. Between 1996 and 2004, over 10 high-yielding varieties were released in Kenya and more research to develop high-yielding common bean varieties have been progressing well since then. Breeding common beans for better micronutrient content and tolerance to increased temperatures has also been done leading to development of a number of lines (Beebe *et al.,* 2014).

2.5.1 Bean improvement for Iron and Zinc content

One of the ways of improving iron and zinc concentration in common beans is Plant breeding approaches and specialized agronomic practices. Zinc is one of the minerals that are important for plants' growth and development, as it helps in the formation of enzymes, formation of hormones, and development of reproductive cells. Soil zinc deficiency may lead to plant physiological malfunction and, reduced tolerance to environmental stress (Xue *et al.*, 2017).

In humans, zinc plays an important role in the proper building of the immune system, and development, body healing, and metabolism (Liu *et al.*, 2017). Zinc deficiency however may lead to poor immune system response, fertility problems, and stunted growth. The deficiency occurs when food and supplements cannot supply enough of the mineral as demanded by the body. Utilizing foods with low nutritional zinc content is the main contributor to zinc deficiency in sub-Saharan Africa. Low household income and limited access to foods derived from animals and fish that are high in zinc have a negative impact on the availability and affordability of these foods for the majority of populations in sub-Saharan Africa, causing them to consume a majority of cereals, legumes, roots, and tubers that are low in zinc bioavailability. The consumption of fruits and vegetables, which are foods high in vitamin C and have been shown to promote the absorption of zinc in the human stomach, is also low. Phytic acid, tannins, dietary fiber, and calcium are some of the inhibitors that negatively affect zinc bioavailability from plant meals in the human stomach. 5.5% to 56.5% of zinc in plant-based meals is bioavailable. Despite the measures being taken to alleviate zinc deficiency prevalence in sub-Saharan Africa, its effect among the populations showed no significant decrease thus there is a need to apply

supplementation, food chemical fortification and currently bio fortification so that there is a complementation of one another as there is no single existing method that can alleviate micronutrient deficiency in sub-Saharan Africa.

Plant breeding approaches could aid in developing common bean varieties with higher Zn and Fe concentrations by exploiting the existing difference in micronutrient concentrations among bean varieties (Petry *et al.*, 2015). Breeding for higher zinc and iron in common beans is deemed possible because of the high variability that exists among bean varieties and that enables the breeder to have a wider genetic base to start with (Beebe *et al.*, 2014). Iron content of more than 70 ppm in common dry bean seed is taken as high while zinc content of more than 30 ppm is considered high in common dry bean. The human body requires Fe level of >94 ppm and the approximate zinc level is >47 ppm in seeds (Zulu, 2013). The bioavailability of iron is limited by the polyphenol content in common beans. The polyphenol content is indirectly screened for by seed coat color since it is affected by the presence of tannins anthocyanin, and flavonols. Dark-colored seed coat has a very high content of anthocyanin (Zulu *et al.*, 2013). Varieties with black, red, and pink seed coats get those colors because of anthocyanin. While the yellow seed coat colors are based on the presence of condensed tannins (Ganesan *et al.*, 2017).

1.11 Genetics of Iron and Zinc content in seeds of common beans

For an efficient common breeding, the range of genetic variability as far as iron and zinc content is concerned, must be established. Zinc and iron inheritance have a positive correlation suggesting that the two minerals are inherited together. Many literatures have shown that the trait for higher Fe and Zn in beans is quantitative. This means many genes control this trait. (Blair *et al.*, 2009). Monogenic inheritance for Zn content depends largely on the origin of the genotype; for example, the Mesoamerican beans have been reported to have lower

concentrations of Fe than Andean beans, but higher Zn concentration (Blair *et al.*, 2013). Both the gene and environment affect the concentration of iron and zinc in beans hence the selection of elite cultivars adapted to wide regions (Nchimbi-Msolla *et al.*, 2010; Tryphone *et al.*, 2010).

1.12 Mating designs used in the breeding of common bans

Mating arrangements are a way of arranging parent material to produce progenies that plant breeders and geneticists want to exploit for research. There are very many diverse types of mating arrangements or designs currently being used in plant breeding programs. The choice of method usually depends on some factors like the type of pollination the crop undergoes, the method of crossing that is going to be used when hybridization is being done, and the mean of pollen transfer (Luka *et al.*, 2018). Mating designs are one of the tools that help plant breeders assess genetic variance and understand more about the genetics of traits under study, it also is a tool used to generate a base population which is required for the development of new varieties; it gives assessments of genetic gain (Nduwumuremyi *et al.*, 2013).

1.12.1 Bi-parental mating arrangement

This is one of the designs or an arrangement where a number of plants (n) are selected by plant breeders for hybridization and then paired off and mated once in pairs to get half of (n) first-generation families. The progenies of the families are tested for variations which when observed are partitioned by ANOVA within family and between families (Mukamuhirwa *et al.*, 2015). However, data generated by biparental mating design cannot generate enough data for every parameter that may be needed because just two statistics are available namely the progenies which are either full sibs or unrelated no other relationship exists among them (Luka *et al.*, 2018).

1.12.2 North Carolina mating design

Comstock and Robinson created the North Carolina arrangement or designs in 1952; among them is North Carolina designs one, two, and three, which are easier to implement than other designs (Luka et al., 2018).

The North Carolina Design I is a multipurpose design that is used in plant breeding to evaluate additive and dominant variations as well as progeny evaluation (Figure 2.1). When compared to other designs, this design calls for more seeds. and so not suitable for use in breeding programs where the crops involved produce few seeds (Acquaah, 2012).

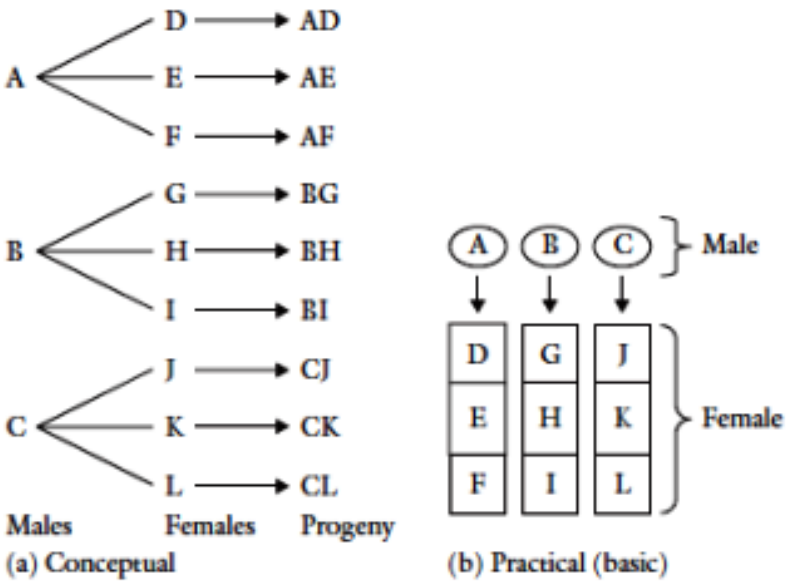

Figure 2. 1: North Carolina Design I (Source: Acquaah, 2012).

The parents are divided into groups of males and females in North Carolina Design 2, a factorial mating system. Then, every female group member gets crossed with every male group member. This design is recommended, especially when assessing the combining capability of inbred lines. It is also appropriate for usage in crops that produce a large number of flowers, allowing for the recurrent use of each plant during hybridization. Plant breeders can now estimate both of the two forms of combining ability because to this design. The design's one significant flaw is that it cannot be utilized to conduct an epistasis test or even analyze genotype by environment

relationships (Kearsey and Pooni, 1996). The systematic crossing operation with the n1n2 progeny groups resulted in the progenies having half-sibling relationships.

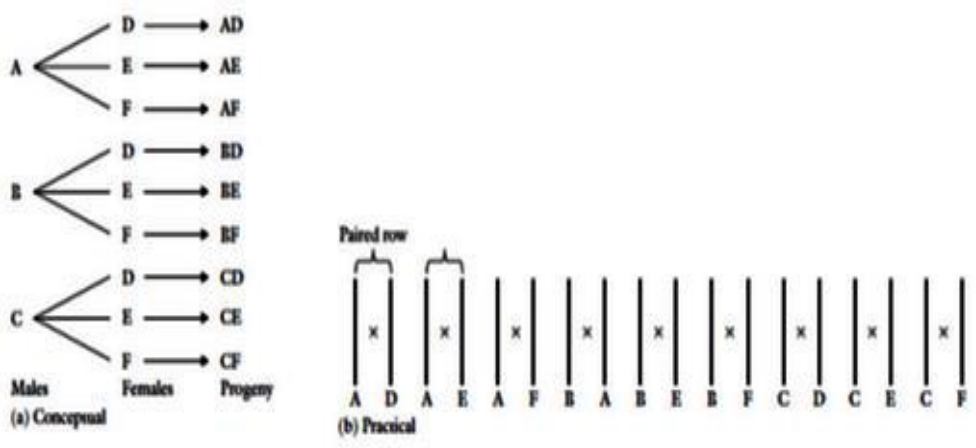

Figure 2. 2: NC II Design (factorial design with paired rows) Source: Acquaah, 2012

North Carolina Design III: In this design, an undetermined number of F2 plants are backcrossed with the inbred lines that produced the F2 materials. The F2 progenies are evaluated against the two inbred lines, which also serve as testers, and because they are the progenitors, they are even more special testers. This design is thought to be more potent than the previous two designs because a third tester was added (Acquaah, 2012). By allowing for the testing of sensitive and ambiguous non-allelic interactions, this inclusion considerably boosts the design's power. (Nduwumuremyi *et al.*, 2013).

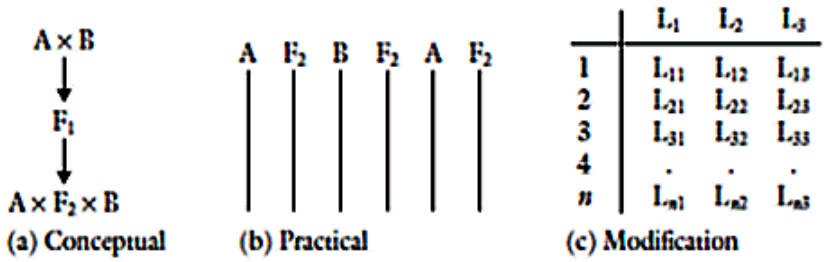

Figure 2. 3: NC III design with conceptual, practical and modified. Source: Acquaah, 2012

Plant breeders use Schmidt's 1919 parallel mating method to produce crosses from different parent types, including inbred lines and broad genetic variants. Full diallel involves both the parents' forward and reciprocal crossing and the reciprocal mating of their offspring to produce F1 progenies. Eisenhart's 1947 model I (fixed) and model II (random) were employed in diallel

mating setups. Only GCA and SCA effects are estimated by Fixed Model I, which assumes that the parents utilized have undergone selection to create a population.

1.13 Methods of breeding common beans

Plant breeders have experimented with various techniques over time to create superior cultivars. The type of pollination that the crops experience self-pollinated or cross-pollinated determines the method that is chosen for use. The following are appropriate techniques that can be utilized for self-pollinated plants, such as common beans.

1.13.1 Pedigree Method

In the process of developing new bean varieties, this is one of the methods used more commonly to select plants that are superior in desirable features after hybridization. When using this approach, the second generation of plants is selected. In the first stage of selection, 250 to 500 plants are usually picked, and their progeny are then tested until they are genetically pure. From the third to the fifth generation, offspring rows are planted with the selected seeds from the previous generation. At this stage, only the best plants are selected and planted, with enough space between them to allow for their natural development. Selection is done using this method both within and between progeny families. Information on biotic stress and seed quality is acquired starting with the fourth generation (F4 generation). Uniformly related families can be grown, picked, and bulked in F6 family rows before the experiment uses a different seed lot. In F7, preliminary yield testing is carried out using the elite variety as the standard check (Welch *et al.*, 2016).

The pedigree method of breeding plants has numerous advantages, including being an excellent approach to improve features like growth patterns, plant height, and leaf size that are readily visible and highly heritable. It is possible to identify each plant's parents and obtain details on features and inheritance as needed because pedigree records are preserved. Early selection of

superior heterogeneous populations increases breeding efficiency and producing a new variety using this strategy takes less time than using other plant breeding strategies. Since progeny tests are performed, genotypic values rather than phenotypic ones are used in the procedure. Although there are many advantages to pedigree plant breeding, there are also some disadvantages. For example, pedigree record keeping is time-consuming, expensive, labor-intensive, and requires professional and experienced staff (Welch *et al.*, 2016).

1.13.2 Back-Cross Method:

Plant breeders use this method when transferring one or two character-related genes from a subpar land race variety to a superior variety. The elite variety is crossed with the donor parent in this manner, and the offspring that carries the desired gene is crossed back to the elite parent. At least four backcrossing rounds are required (Breseghello *et al.*, 2013).

Backcross breeding starts with a variety that needs to be improved by the introduction of the desired gene and a donor parent that carries it. between the two parents crossed. The offspring of this cross will be crossed again with the recurrent parent after being picked for the required trait. Once the donor parent's gene of interest has been retained and all of the traits of the recurrent parent have been recovered, selection and back crossing will proceed (Breseghello *et al.*, 2013).

The back cross method of plant breeding has the following advantages: It can be used repeatedly to get the same outcomes; The elite variety's genotypic basis, performance, and adaptability have barely changed; For interspecific gene transfer, it is the sole available technique; It involves less record keeping, which makes management simpler; It is independent of its surroundings.

1.13.3 Mass selection

In this breeding technique, seeds from suitable individual plants within a population are initially collected, and then the following generation is planted with a stock of mixed seeds. In this procedure, each plant's genetic worth is estimated, and then plants that will serve as the parents of the following generation are chosen based on this estimate (Breseghello et al., 2013). The advantages of the mass selection approach over other plant breeding techniques include, among other things, the wide genetic diversity and stability of the varieties created by mass selection. This method is one of the simplest, and least expensive methods (Graham 2004). The approach's drawbacks include the non-uniformity of the varieties produced by it compared to those produced by the pure line method, its limited utility in improving self-pollinated crops, and its effectiveness for only highly heritable traits (Welch *et al.*, 2016).

1.13.4 Pure-line selection

This method of bean breeding entails choosing numerous attractive, robust, or outstanding plants from a population. The offspring of such selections are subsequently assessed in the field and tracked for several years (Breseghello et al., 2013). Trials are set up to undertake measurements once no further segregation is visible, to conclude whether the selections are high-yielding and performing well in comparison to their parents in terms of other qualities. Any offspring that is determined to be better than the current variety will then be regarded as a new "pure-line" (Breseghello et al., 2013).

The pure line selection approach has many benefits, including being simpler to use and being a low-cost crop improvement technology that fixes materials genetically more quickly, allowing for the immediate conduct of yield studies. Varieties created using this technique

exhibit stability across a range of environmental conditions. In other words, look and performance are consistent.

However, this method has several drawbacks, such as poor adaptability due to a limited genetic base. In contrast to the varieties created through mass selection, this method requires a lot of time and space, and it is more expensive to undertake yield experiments.

1.14 Combining ability and yield, zinc and iron content

Combining ability refers to a parent's capacity to combine during hybridization in such a way that the intended trait is passed on to their offspring. This ability affects whether or not the hybrid produced by crossing two parents carries the desired gene. Heterosis, however, is the ability of the hybrid to do better than the parents (Olfati *et al.*, 2011). The capacity of the mother who is employed as a parent in crossing and the selection of desirable parents have a significant role in a plant breeding program's success. To determine which parent combination will create the desired progeny, it is vital to know which parents can produce early developing offspring as well as the estimated GCA and SCA as well as gene action early in the effort to improve a plant character. Additionally, knowledge of the desirable parental pairings is crucial because it can indicate a high level of heterotic response. (Susanto *et al.*, 2018).

1.15 Breeding beans for high micronutrient concentration

Bio fortification is the process of nutritionally improving food crops using agronomic practices, biotechnology techniques, and conventional plant breeding. Around the world today, agricultural systems have been focusing more on increasing grain yield and crop productivity designed than promoting human health. This has resulted in the quick rise in micronutrient-deficient food grains, which has resulted in micronutrient malnutrition among people. Now, agricultural systems are designed to promote production of nutrient-rich food crops in sufficient quantities to help in the fight against micronutrient

malnutrition especially in low-income countries (Garg *et al.*, 2016). Bio fortification through conventional plant breeding techniques starts with. Identification of materials with sufficient concentration of the target mineral should be stable in a wide range of environments and climates, high yielding. Consumer preferred traits like taste and cooking quality must be tested in other to promote adaptation. (Welch *et al.*, 2013). After collection and evaluation, of parent materials, they are then used in the breeding process to create progenies which will then be put through the conventional breeding process until the selected ones are released (Boye *et al.*, 2010).

1.16 Genotype by environment interaction

This must be conducted to determine how genetic potentials of materials react to two or more distinct settings because it is well-known that environmental differences greatly affect the production of common beans both globally and in east Africa. Knowledge of the interactions between genotype and environment is crucial in Kenya, where beans are cultivated in various environments.

Nearly most of the environment's influence on common bean gene expression is quantitative. Given that plants will experience morphological changes due to the lack of Fe and Zn, such as improved root growth and the formation of transfer cells, as well as the necessity of acidifying the rhizosphere to obtain Fe and Zn from the soil, the Fe and Zn concentration in legumes does not greatly depend on the environment (Ghandilyan *et al.*, 2006). Phenotypic plasticity refers to the degree to which the environment alters a crop's phenotype. This phenomenon happens as a result of the crop's gene expression being highly sensitive to changes in its growing environment. A common bean variety having the gene for, for instance, was developed by taking advantage of specific environmental factors (Santos *et al.*,2017).

The goal of plant breeding is to enhance crop yield, hence plant breeders must be aware of all the genetic and environmental factors that have an impact on crop growth. Depending on the environment's conditions and a gene's capacity to express itself in that context, the impact of these factors may be favorable or negative (Santos *et al.*,2017). Therefore, understanding the interplay between genotype and environment is important for conducting breeding research. By conducting experiments in various contexts, it is possible to understand how genotypes respond to various situations. In this situation, the genotypes that will demonstrate the most stability under various environmental conditions can be chosen and used to genetically enhance the other varieties.

CHAPTER THREE:

EVALUATION OF IRON AND ZINC-RICH COMMON BEAN GENOTYPES ACROSS DIFFERENT ENVIRONMENTS

1.17 Abstract

The majority of the population in various East African nations grows and consumes dry beans as one of the most important sources of protein and minerals. However, the majority of common dry bean varieties grown by local farmers are poor in iron and zinc content, which means a larger proportion of the population that relies on common beans as a source of iron and zinc will inevitably experience mineral deficiencies. The main goal of this study was to increase the yield and iron and zinc content of the locally adapted dry bean varieties which included 24 advanced breeding dry bean lines with high concentrations of iron and zinc, as well as 12 released varieties. The experimental design was a six-by-six alpha lattice with a plot size of 2x2 meters with a spacing of 40 cm and 20 cm between each row, respectively and three replications. Data on agronomic, disease, and yield characteristics were obtained and analyzed. Between locations and seasons, there were significant differences in emergence percentage, time to 50% flowering, and days to maturity (p 0.05). In Embu, genotype variations had a large impact on emergence, vigor, days to flower, and days to maturity. In Mwea, genotype differences for emergence, days to flowering, and days to maturity were significant. The incidence of root rot, bean flies, and aphids was significantly affected by the locations and seasons. However, because the genotypes responded the same way in all of the different environments and seasons, it may be concluded that the genetic variations had little to no influence on the prevalence of pests and diseases. In both seasons and sites, the genotypes EMBEAN14, KATX56, EMBEAN118, NUA604, and CIANKUI produced high yields.

1.18 Introduction

Common bean (*Phaseolus vulgaris* L.) from the genus Phaseolus is among the five currently cultivated species of legume crop, and it is ranked third in importance after soybean and peanut and the first in human consumption. Plant breeding works are always centered on gene or trait transfer from one parent to the other. Once the transfer is successful, the material has to pass another test of stability and how it reacts to different environments. (Mukamuhirwa *et al*,. 2015). Unfortunately, genotype by environment interactions has very significant effects on the breeding for both yield and nutritional traits, especially zinc and iron. Therefore, there is a need to understand and estimate the scale of genotype and environment interactions for yield and high iron and zinc content in beans and to identify genotypes that are very stable, widely adapted, and can endure unpredictable environmental variations. Dry bean cultivars with high concentrations of the elements iron and zinc are important since these two minerals are deficient in many individuals and are a major source of public health problems worldwide. Anemia, overall weakness, abnormal birth weight, weakened immunity, and diarrhea are just a few of the symptoms brought on by these deficiencies. For people who rely on beans as a staple food, producing dry bean cultivars that have good concentrations of iron and zinc elements can considerably help in the reduction of deficiency disorders. Such bean varieties can be developed through biotechnology, the standard plant breeding techniques of crossing and selection, and materials that already exist and have a wide range of iron and zinc concentrations among beans. Therefore, the goal of this study was to increase the zinc and iron content of elite local bean varieties that farmers have adopted, through plant breeding as a bio-fortification method. Fighting malnutrition to improve health is one of the Sustainable Development Goals (SDG) agenda, and by doing so, we can put less burden on our healthcare systems to provide prevention and treatment.

1.19 Materials and methods

1.19.1 Description of the experimental sites

The experiments were conducted at two sites, namely Kenya Agricultural and Livestock Research Organization Food Crop Research Institute KALRO-Embu in Embu County and at KALRO-Mwea Industrial Crop Research Institute in Kirinyaga County in Kenya.

Table 3. 1 The study sites

Site	Latitude	Longitude	Altitude	Average rainfall	Temp.	Soil type
Mwea	0 37' S	37 20' E	1159m (ASL).	850 mm	22ºC.	Nitisols
Embu	0˚ 08' 35''S	37°27′02″ E.	1350m (ASL)	1,000-1,200 mm	21º C	Eutric Astosol

Source: Abuli, 2016

1.19.2 The materials used in the study

The materials used in this experiment included 24 advanced breeding lines with greater concentrations of iron and zinc, as well as 12 released varieties of dry beans with good agronomic qualities, high yielding, and seed quality. Both types with low concentrations of the minerals iron and zinc and some newly released varieties with concentrations of the two minerals above average

Table 3. 2 Parent materials used in the evaluation

S/n	Variety code	Source	Year release	Zn and Fe	Special attributes
1.	KATX 69	KALRO Katumani	1995	Low	High-yielding with high zinc and iron content
2.	KSW 13	KALRO Katumani	2015	Low	Drought tolerant, Early Maturing resistant to bean Rust, and Bean common Mosaic Virus (BCMV)
3.	KAT B9	KALRO Katumani	1998	Low	High yielding
4.	(KAT-RM01) KATRAM	KALRO Katumani	2014	Low	tolerant to moisture stress, yield highly, with the most preferred seed type highly resistant bean Rust
5.	EMBEAN 14 (MWENDE)	KALRO Embu	2014	Low	Tolerant to most fungal diseases, Marketable seed type and high potential of nitrogen fixation
6.	EMBEAN18	KALRO Embu		Low	High yield, Attractive seed colour and Good taste
7.	KATX 56	KALRO Katumani	1995	Low	High yielding
8.	TASHA	Egerton University	2008	High	High yield, Attractive seed colour and Good taste
9.	CIANKUI	Egerton University	2008	High	High yield, Attractive seed colour and Good taste
10.	CHELALANG	Egerton University	2008	High	High yield, Attractive seed colour and Good taste
11.	WAIRIMU(GLP585)	KALRO	2008	High	Tolerant to heat
12.	ROSCOCO (GLP 2)	KALRO Embu	1982	High	High yield, Attractive seed colour and Good taste
13.	NUA695	CIAT line	To be released	High	High yielding
14.	NUA595	CIAT line	To be released	High	High yielding
15.	NUA692	CIAT line	To be released	High	High yielding
16.	NUA654	CIAT line	To be released	High	High yielding
17.	NUA718	CIAT line	To be released	High	High yielding

Table 3. 2 Parent materials used in the evaluation

S/n	Variety code	Source	Year release	Zn and Fe	Special attributes
18.	NUA619	CIAT line	To be released	High	High yielding
19.	NUA596	CIAT line	To be released	High	High yielding
20.	NUA669	CIAT line	To be released	High	High yielding
21.	NUA739	CIAT line	To be released	High	High yielding
22.	NUA686	CIAT line	To be released	High	High yielding
23.	NUA640	CIAT line	To be released	High	High yielding
24.	NUA636	CIAT line	To be released	High	High yielding
25.	NUA680	CIAT line	To be released	High	High yielding
26.	NUA730	CIAT line	To be released	High	High yielding
27.	NUA700	CIAT line	To be released	High	High yielding
28.	NUA728	CIAT line	To be released	High	High yielding
29.	NUA709	CIAT line	To be released	High	High yielding
30.	NUA612	CIAT line	To be released	High	High yielding
31.	NUA662	CIAT line	To be released	High	High yielding
32.	NUA611	CIAT line	To be released	High	High yielding
33.	NUA593	CIAT line	To be released	High	High yielding
34.	NUA666	CIAT line	To be released	High	High yielding
35.	NUA604	CIAT line	To be released	High	High yielding
36.	NUA690	CIAT line	To be released	High	High yielding

1.19.3 Experimental design

Thirty-six genetic materials were tested for two seasons the long rains and the short rains in two evaluation blocks in KALRO-Mwea and KALRO-Embu to measure how well they performed in terms of grain yield and other yield attributes. The plot was 2 by 2 meters in size, with an alpha lattice layout and a 40 cm by 20 cm inter and intra-row spacing, respectively. The treatments were replicated three times, with thirty-six plots in each replicate. The distance between each replication and each plot was one meter. Di-ammonium phosphate (DAP) was administered at a rate of fifty kilos per hectare during planting. (Kiptoo *et al.,* 2016). Pests were controlled by spraying with insecticides and fungicides weekly from after germination until the flowering stage in both seasons.

1.19.4 The assessment of agronomic parameters.

Data collection for the agronomic traits involved the following:

i. Plant count: this was determined by counting the number of plants in each plot. It was collected at least three times throughout the growing season namely a week after emergence, during flowering, and at harvest.

ii. Early vigor was graded on a scale of 1 to 9, where 1 denoted excellent vigor and 9 denoted very poor vigor or stunting (Agrios, 2005).

iii. 50% Flowering: this was recorded as the number of days it took for each plot to reach 50% flowering

iv. Plant height: The measurement was made from the surface of the ground to the tip of the growth point just before bloom initiation and at maturity, the height was measured as from the ground up to its highest node, which had a dry pod-bearing seed.

v. Days to 75% maturity: this depicted the number of days from planting to the day that 75% of the plants in a plot have reached physiological maturity.

vi. Lodging was rated at maturity, with one representing 100% of plants standing upright and five representing 100% of plants lying flat

vii. 100 seeds at a random weight of 100 grams each, with a moisture content of 16%, were used (Blair *et al.*, 2013).

1.19.5 Assessment of disease incidence and severity.

The incidences of root rot, early blight, and leaf rust diseases were assessed using the global method of disease scoring created by CIAT in 1987. The number of infected plants in each plot was counted to determine the prevalence (incidence) of the disease. It was calculated by multiplying the population's total number of infected plants by 100 and then dividing that result by the total number of plants in the population. It provides information on the prevalence of a disease in the region or the population of plants. (Sharma *et al.*, 2017).

The international method of disease severity scoring scale of 1-9 which was developed for screening of crop varieties and lines for disease resistance and host resistance (Manandhar *et al.*, 2016) was used to score for disease severity which was then considered as the total of disease ratings x100, divided by total number of rating x maximum disease grade (Mwebaze *et al.*, 2016).

1.19.6 Assessment of pest incidence and severity.

The percentage of plants containing the pest's pupa was taken into account as the infestation rate when calculating the incidence of pests like the bean fly. On a scale of 1, 3, 5, 7, and 9, the severity was determined. Where 1 is the condition where the infected plant is as vigorous as the healthy plants. 3. This is the stage at which the infected plant begins to somewhat stunt or halt its growth. The infected plant begins to exhibit significant growth slowing or delay around

day five. When the plant is afflicted, it reaches stage 7 and begins to severely hinder or postpone its growth. 9 is the point at which the infected plants are either dead or nearly so (Centro Internacional de Agricultura Tropical) in 1987.

1.19.7 Assessment of yield and yield components

This data includes the number of plants during harvest, the number of pods, the number of seeds per pod, the yield per plot, and the weight in grams of 100 seeds at 16% moisture. Biomass Yield (BY), measured at 16% moisture and rounded to the nearest whole number, is used to determine harvest index as the percentage-based relationship between seed yield (SY) and biomass yield (BY).

The seed yield for each plot was extrapolated in hectares using the formula.

Yield in $Kg/ha = \frac{\text{weight per plot X ha area}}{plot\ area}$

1.20 Data analysis

The data was analyzed using the GENSTAT 15th edition program to generate ANOVA and to find out the differences among traits and then separate genotype means using Fisher's protected least significant difference (LSD) at 5% probability level (Gomez and Gomez, 1984).

1.21 Results

1.21.1 Agronomic performance of the genotypes across seasons in the two locations

There were significant differences among those 36 entries for emergence percentage, time to 50 percent flowering, and days to maturity across locations and seasons. Interactions between the two locations and seasons had an impact only on the days until blossoming. Only the duration to 50% flowering and the number of days to maturity were significantly influenced by genotype variation, as well as location, and season. On emergence percentage, vigor, and days

to flowering, the influence of season by genotype was not significant; however, the effect of the season by genotype interaction was significant only for days to maturity. (Table 3.3).

The two seasons in Mwea had no statistically different effects on vigor, but they did have significant effects at p 0.05 on emergence percentage, the length of time it required to reach flowering and finally maturity in each location. In Embu, genotype differences had a substantial impact on emergence percentage, vigor, days to flowering, and days to maturity. The genotype differences were significant or significant for Mwea's percentage emergence, time to flowering stage, and days to maturity. There was, however, no observable difference in vigor. The genotype by season interaction had a significant impact on the days to flowering and days to physiological maturity in the two sites. (Table 3.4).

Table 3. 3 : Analysis of variance for agronomic parameters combined across two locations and two seasons

Source of variation	D.f.	%Em.	Vigor	50%Flwr	DTM
Location	1	5889**	106.0093**	1825.3**	6502.26**
Season	1	9760**	0.083	1825.3**	7617.12**
Location. Season	1	152.9	4.898	296.7**	5.79^{NS}
Genotype	35	76.6	0.856	72.01**	64.57**
Location. Genotype	35	49.1	1.178	56.12**	63.48**
Season. Genotype	35	50.1	0.378	42.80	78.11**
Loc.Season.Genotype	35	61.7	1.150	108.0**	290.62**
Residual	268	69.8	0.853	32.62	11.79
Total	411	116.3	1.118	55.23	73.91

%Em= ,percentage emergence, per plot, Vigor, = early ,vigor, 50%,Flwr ,= the duration from planting to when ,half of the number of plants in a plot has at least one open flower DTM= ,Days from planting to ,maturity * =shows levels of significance

Table 3. 4: Analysis of variance for agronomic parameters across two seasons in Mwea and Embu

Source of Variation	D.f.	%Em	Vigor	50%Flwr	DTM	%Em	Vigor	50%Flwr	DTM
		Embu				Mwea			
Season	1	6176.04**	3.1296*	1796.89**	4021.41**	3733.35**	1.852^{NS}	325.116**	3601.5**
Replication	2	4122.04**	2.0417*	598.35**	80.977**	131.12*	2.116^{NS}	28.625NS	2.14^{NS}
Block	17	241.19**	0.648^{NS}	127.07**	10.195**	99.83**	2.262*	28.184**	60.54**
Rep.Block	10	34.4^{NS}	0.5813^{NS}	98.7**	6.658NS	85.11*	1.165^{NS}	49.473**	167.24**
Genotype	35	77.4**	0.839*	92.95**	22.841**	62.97*	0.856^{NS}	47.734**	74.5**
Season.Gen	35	39.98^{NS}	0.3247^{NS}	10.97**	7.119^{NS}	67.79^{NS}	0.813^{NS}	148.28**	376.05**
Residual	115	42.19	0.5809	36.94	5.157	43.53	1.163	6.914	13.84
Total	215	129.58	0.6132	65.5	28.291	76.14	1.135	36.725	89.63

%Em= percentage emergence per plot, Vigor = early vigor, 50%Flwr = the duration from planting to when half of the number of plants in a plot have at least one open flower DTM= Days from planting to maturity *= levels of significance

The genotypes NUA666, NUA700, EMBEAN118, and NUA728 had the lowest percentage emergence over two sites and seasons, while CIANKUI, NUA690, NUA680, NUA596, NUA739, EMBEAN118, NUA718, WAIRIMU, ROSCOCO, and NUA669 had greater emergence percentages overall. The genotypes ROSCOCO, WAIRIMU, KATRAM, KATX69, and NUA593 flowered first across the two sites and the seasons, while EMBEAN118, CIANKUI, and KSW13 flowered last. The genotypes ROSCOCO, NUA730, NUA593, NUA636 and KATRAM displayed the earliest maturity, whereas genotypes EMBEAN118, KSW13, and CIANKUI exhibited the most recent maturity. (Table 3.5).

In Mwea, higher percentage emergence was seen for the following genotypes: CIANKUI, NUA690, NUA680, NUA596, NUA739, EMBEAN118, NUA718, WAIRIMU, ROSCOCO, KATB9, NUA669, and TASHA. In Embu, higher percentage emergence was reported for the following genotypes: NUA654, NUA680, NUA666, EMBEAN18, KAT. In Mwea, genotypes KSW13, NUA700, and TASHA flowered later throughout the two seasons than genotypes NUA612, NUA662, NUA666, KATB9, and KATRM. Meanwhile, Genotypes NUA596, KATB9, NUA636, KATRAM, AND NUA680 had early flowering in Embu. Genotypes KATRAM, KATB9, ROSCOCO, NUA636, NUA596, NUA718, and NUA595 matured first in Mwea and Embu, followed by genotypes NUA692, NUA593, TASHA, NUA700, KSW13, NUA669, and NUA604. (Table 3.6).

Table 3. 4: means of agronomic parameters across two locations Embu and Mwea in two seasons

Genotype	%Em	Vigor	50%Flwr	DTM
ROSCOCO	85.42	2.97	33.68	64.85
NUA730	77.00	2.79	38.91	71.92
NUA593	76.76	2.69	37.52	73.11
NUA636	81.83	7.46	39.09	73.63
KATRAM	86.30	2.86	35.28	74.86
NUA709	73.32	6.69	37.69	74.98
CHELALANG	72.95	3.53	40.27	75.17
KATX56	71.06	3.17	38.43	75.61
NUA666	84.09	5.55	38.57	76.31
NUA690	72.50	3.99	44.98	76.53
NUA680	83.79	6.47	42.09	76.59
NUA611	88.36	3.06	41.48	76.87
KATX69	75.59	6.17	36.91	76.97
WAIRIMU	85.21	6.27	34.00	77.18
NUA619	81.97	4.41	37.62	77.19
NUA669	73.77	6.26	40.95	77.54
NUA718	86.24	5.82	42.31	77.66
NUA654	90.24	4.67	39.35	78.22
TASHA	85.85	8.19	42.63	78.34
NUA686	70.47	5.48	41.71	78.35
NUA662	72.41	4.39	42.54	80.78
NUA700	81.20	6.33	44.52	81.58
EMBEAN14	81.65	4.77	45.84	81.63
CIANKUI	78.05	3.38	47.19	81.66
KSW13	70.58	4.13	47.18	81.80
EMBEAN118	87.50	8.76	48.50	85.53
Grand mean	79.50	4.87	41.20	77.78
CV%	20.39	17.48	10.66	4.41
LSD (5%)	14.26	5.35	6.83	5.31

%Em= percentage emergence per plot, Vigor = early vigor, 50%Flwr = the duration from planting to when half of the number of plants in a plot have at least one open flower DTM= Days from planting to maturity

Table 3. 5: Means for agronomic parameters across two seasons in each location

Parameters	%Em	Vigor	50%Flwr	DTM	%Em	Vigor	50%Flwr	DTM
Genotype	Mwea				Embu			
CIANKUI	80.1	3.2	34.8	72.5	63.48	3.3	38.4	73.6
NUA690	80.0	3.0	35.7	70.9	60.45	3.1	40.6	71.8
TASHA	79.9	2.9	34.5	73.3	69.7	3.0	42.9	74.1
NUA718	79.9	2.5	39.8	70.0	69.55	2.6	35.2	70.4
NUA596	79.7	2.0	39.3	69.7	63.48	2.1	30.9	69.6
NUA662	79.7	2.5	31.9	71.8	61.21	2.7	37.2	72.2
ROSCOCO	79.7	2.9	39.5	69.3	64.24	3.0	34.0	70.1
NUA612	79.7	2.2	31.9	72.5	64.39	2.3	40.6	72.6
KATB9	79.6	2	31.7	68.8	70.76	2.1	31.2	68.7
NUA654	79.6	2.5	32.3	70.8	74.55	2.6	36.4	71.2
EMBEAN18	79.2	2.5	47.9	72.2	69.85	2.7	40.5	72.6
KATX56	79.4	2.5	35.1	71.8	58.94	2.6	38.3	72.2
KATRAM	79.2	1.9	31.0	67.4	68.79	2	32.0	67.2
NUA680	79.4	2.5	44.5	71.5	71.36	2.6	32.4	71.9
NUA669	79.1	2.5	31.4	73.7	61.67	2.6	37.9	74.1
WAIRIMU	79.1	2.5	47.1	71.8	68.79	2.6	37.5	72.1
NUA739	79.1	2.9	43.1	69.6	66.82	3.0	35.9	70.4
NUA695	78.4	3.2	38.5	72.2	61.36	3.3	39.9	73.2
NUA686	78.9	2.5	33.0	70.4	63.18	2.7	37.2	70.9
KSW13	78.9	2.5	45.2	72.6	58.94	2.7	37.8	73.0
KATX69	78.7	2.9	41.2	73.0	60	3.0	34.6	73.8
EMBEAN14	77.8	2.9	36.5	70.9	67.58	3	32.6	71.7
NUA728	77.8	3	34.8	73.6	63.79	3.1	44	74.4
NUA636	77.8	1.9	32.9	69.6	69.7	2	31.2	69.3
CHELALANG	77.7	2.9	35.5	71.6	63.18	3	39.1	72.4
NUA611	77.1	2.5	32.7	73.1	69.85	2.7	38.4	73.5
NUA666	76.6	1.9	30.8	71.1	70.76	2	32.4	70.9
G. Mean	78.8	2.6	36.5	69.3	65.9	2.7	37.5	72.1
LSD	3.9	2.0	4.3	4.5	5.5	2.0	4.3	4.5
CV	17.5	32.6	7.5	7.4	16.2	32.6	7.5	7.4

% ،Em= percentage emergence per plot, ،Vigor is the plant appearance 50%Flwr = the duration from planting to when half of the number of plants in a plot have at least one open flower ،DTM= Days from planting to ،maturity

1.21.2 Incidence of root rot bean fly and aphid among the genotypes across seasons in the two locations, and in each location

The locations and seasons had a big effect on the occurrence of root rot, bean fly, and aphids. Root rot, bean flies, and aphid incidence were all significantly influenced by the interactions of location by season and genotypes by location. However, across the two locations and seasons,

the genotype differences had no significant impact on either diseases or pests, demonstrating that genetic variation among the genotypes had no significant impact on disease prevalence implying that the genotypes were not resistant. (Table 3.7).

The incidences of bean flies and root rot in Embu were significantly affected by the two seasons, during the long rainy season, there was less incidence if the disease and pests compared to the short rain season. The seasons had a big impact on how often bean flies and root rot occurred in Mwea. Genetic variations had no appreciable impact on the frequency of diseases and pests throughout the course of the two seasons. Aphid incidence in Mwea was unaffected by genotype changes, whereas root rot and bean fly occurrence in Embu were significantly impacted. (Table 3.8).

1.21.3 Yields of the genotypes across two seasons in the two environments.

The genotype by season and location by genotype interactions had a substantial impact on the number of seeds in each pod, the weight of 100 seeds, and the seed yield (Table 3.9). While genotype by location interactions only significantly affected the weight of one hundred seeds, location by season interactions significantly affected the number of pods on each plant. Each plant's number of pods, number of seeds inside each pod, weight of one hundred seeds, and grain yield all differed significantly across the two locations and seasons (p 0.05). The weight of 100 seeds and seed yields were significantly impacted by genotype variations (Table 3.9). The number of pods on each plant, the number of seeds per pod, the weight of one hundred seeds, as well as the general genotype, season, and location interactions, all had a significant influence on the amount of seeds produced (Table 3.9).

The seasons had a big impact on Embu's seed yield, number of pods on each plant, number of seeds per pod, weight of 100 seeds, and number of pods per plant. In Mwea, however, the two seasons had a substantial impact solely on the weight of one hundred seeds. While genotype by season had a significant impact on seed yield and the weight of 100 seeds in Embu, it had a significant impact on the number of pods on each plant, the number of pods per plant, and the weight of 100 seeds in Mwea (Table 3.10).

EMBEAN14, KATX56, EMBEAN118, NUA604, and CIANKUI demonstrated greater yield performance in the two locations in both seasons, yielding above the checks. The yield in Mwea was not statistically significant. However, there were notable genotypic variances in Embu. The genotypes NUA666, NUA612, NUA669, and KATB9 produced much less than the mean throughout the course of the two seasons in Mwea. On each plant, the pod counts were lowest for the genotypes KATB9, NUA612, and NUA666 and highest for the genotypes NUA680, CHELALANG, NUA604, EMBEAN118, EMBEAN14, and WAIRIMU. KATX69 and KSW13 genotypes had the lowest 100-seed weight, while EMBEAN14, NUA595, and NUA695 genotypes had the greatest (Table 3.11).

Table 3. 6: Analysis of variance for pest and disease across two locations and two seasons

Sources of variation	D.f.	Root rot	Bean fly	Aphid
Location	1	8094.68**	2556.95**	34.4537**
Season	1	28162.37**	15134.84**	36.75**
Replication	2	41.03^{NS}	13.24^{NS}	0.8819^{NS}
Loc.season	1	22044.9**	30552.52**	34.4537**
Genotype	35	23.99^{NS}	45.33^{NS}	0.6113
Location. Genotype	35	51.08**	70.38**	1.977**
Season. Genotype	35	24.39^{NS}	22.39^{NS}	0.2396^{NS}
Location.Season.Genotype	35	26.91^{NS}	26.95^{NS}	0.9401^{NS}
Residual	268	20.02	38.2	0.6474
Total	413	159.12	151.87	0.9727

NS=non-significant ($p>0.05$) at 5% **= highly significant ($p<0.005$) at 5% *= significant ($p>0.005$) at 5%

Table 3. 7: Analysis of variance for pests and disease parameters across two seasons in each location

		Root rot	Bean fly	Aphid	Root rot	Bean fly	Aphids
Sources of variations	D.f.		Embu			Mwea	
Season	1	50020.3**	44347.4**	0NS	187.1**	1340.1**	146.7**
Replication	2	1.67^{NS}	194.25*	0.167^{NS}	74.06**	98.6*	1.685^{NS}
Block	17	35.2^{NS}	83.25*	0.092^{NS}	40.13**	45.21*	3.545^{NS}
Rep.Block	10	16.77^{NS}	56.73^{NS}	0.094^{NS}	12.86^{NS}	31.64^{NS}	0.867^{NS}
Genotype	35	35.42^{NS}	54.95^{NS}	0.148^{NS}	29.55**	42.79**	2.393^{NS}
G x S	35	27.72^{NS}	26.9^{NS}	0.001^{NS}	12.91^{NS}	21.8^{NS}	1.716^{NS}
Residual	115	23.26	42.93	0.084	13.92	24.28	1.956
Total	215	259.01	253.64	0.083	22.32	38.92	2.825

NS=non-significant (p>0.05) at 5% **= highly significant (p<0.005) at 5% *= significant (p>0.005) at 5% G = genotype S =season Rep = replication

Table 3. 8: ANOVA for yield parameters combined across two locations and two seasons

Source of variation	D.f.	NOP	NSP	SW	SY
Location	1	2488.08**	3.0839**	1337.04**	21453131**
Season	1	1869.8**	11.8339**	15.56^{NS}	70340676**
Location. Season	1	2959.71**	7.1302**	592.68**	40941115**
Genotype	35	15.74^{NS}	0.2447^{NS}	254.48**	960847**
Location. Genotype	35	7^{NS}	0.2767^{NS}	48.61*	154414^{NS}
Season. Genotype	35	7.17^{NS}	0.4296*	88.5**	574602*
Location.Season.Genotype	35	22.88^{NS}	1.1226**	149.92**	391462*
Residual	268	44.75	0.298	33.13	356961
Total	411	60.48	0.3808	83.67	792151

NOP=, number of pods, per plant, NSP= Number, of seeds per pod, SW= ,Weight of 100 seeds, SY= seed yield per plot

Table 3. 9 mean squares for yield parameters across two seasons in Mwea and Embu

sources of variation	D.f.	NOP	NSP	SW	SY	NOP	NSP	SW	SY

		Embu				Mwea			
Season	1	62.296**	18.6678**	208.07**	27602575**	6.168^{NS}	0.2963^{NS}	400.17**	198787^{NS}
Rep.	2	64.875**	4.126**	118.02*	498487**	33**	2.3519**	157.53**	2399308**
Block	17	10.259**	0.8964**	98.79**	83049*	5.273^{NS}	0.2522^{NS}	100.76**	309988**
Rep.Block	10	5.801^{NS}	0.2948^{NS}	111.46**	163319**	7.967*	0.3417^{NS}	29.71^{NS}	218905**
Genotype	35	6.742^{NS}	0.246^{NS}	228.63**	255181**	6.63**	0.2815^{NS}	133.61**	186032**
G X S	35	5.284^{NS}	0.2165^{NS}	53.07**	150297**	6.714*	1.3075**	193**	92410^{NS}
Residual	115	4.125	0.1988	28.35	53467	3.603	0.226	28.66	80401
Total	215	6.151	0.3917	77.02	242729	5.455	0.357	84.5	162459

NOP= ،number of pods per plant, NSP= Number of seeds per pod, SW= ،Weight of 100 seeds, SY= seed yield ،per plot

Table 3. 11: Means for yield parameters across two seasons in each location

Genotype	NOP	NSP	SW	SY	NOP	NSP	SW	SY
	Mwea				Embu			
EMBEAN14	9.2	3.7	41.0	695.0	10.0	4.0	43.0	641.6
KATX56	8.0	4.0	42.7	692.2	8.8	3.3	42.7	586.5
EMBEAN18	12.8	3.3	49.6	661.3	9.8	3.7	58.3	585.8
KATRAM	8.1	4.0	46.0	649.7	9.6	4.0	55.1	585.2
TASHA	8.4	4.0	42.8	648.1	11.1	3.0	49.1	579.5
CHELALANG	9.0	4.0	45.2	645.8	8.7	4.0	50.0	568.9
WAIRIMU	11.4	3.5	48.4	641.8	8.8	3.9	34.8	552.4
CIANKUI	8.4	4.0	43.6	613.2	10.3	3.3	45.2	502.0
NUA619	8.3	3.6	44.3	476.8	8.2	3.7	51.0	501.3
NUA640	8.0	3.5	46.6	525.8	10.3	4.0	41.4	494.2
NUA709	9.0	3.7	44.4	541.1	8.4	3.3	45.9	488.0
NUA604	7.6	4.0	43.9	559.9	10.9	3.7	38.9	472.8
NUA593	8.7	3.9	40.7	351.6	9.0	3.3	41.4	472.1

Table 3. 11: Means for yield parameters across two seasons in each location

Genotype	NOP	NSP	SW	SY	NOP	NSP	SW	SY
	Mwea				Embu			
KSW13	10.6	3.7	30.7	603.0	9.6	4.0	32.2	463.3
NUA728	8.4	3.5	48.4	541.0	10.3	4.0	47.5	460.6
NUA739	11.4	3.3	46.5	555.0	9.6	4.9	42.7	441.5
NUA636	8.1	3.7	47.3	556.6	11.2	3.3	45.9	439.1
NUA718	9.5	4.0	46.9	558.5	10.5	3.3	44.0	434.9
NUA654	8.0	3.5	48.4	496.6	9.6	3.3	45.6	434.3
NUA662	10.1	4.0	48.3	573.3	9.7	4.0	47.8	423.4
NUA700	9.2	3.7	48.6	560.6	9.2	3.7	40.1	363.0
NUA611	8.4	3.3	42.0	523.8	9.7	3.4	39.4	350.5
NUA669	8.8	3.5	46.2	459.8	8.3	3.7	36.6	346.5
KATB9	7.6	3.5	43.0	511.1	8.8	3.3	45.9	334.0
NUA666	8.1	4.0	42.0	487.5	9.9	3.3	41.7	317.3
NUA612	9.0	3.7	47.5	523.9	9.3	3.7	38.6	292.6
G. Mean	9.1	3.7	43.9	562.9	9.6	3.7	43.8	448.5
LSD	2.1	1.2	8.0	259.0	2.9	1.2	2.7	115.1
CV (%)	18.1	13.4	15.0	52.7	16.4	17.8	5.2	24.2

NOP= number of pods per plant, NSP= Number of seeds per pod, SW= Weight of 100 seeds, SY= seed yield per plot

1.21.4 Correlation among all the traits observed in this study

Correlation analysis was used to identify the correlations between the attributes. Between the two locations, a strong positive correlation between bean flies and root rot incidence was seen showing that the condition that favoured both pests existed. However, there was a negative correlation between the prevalence of root rot and bean fly and the number of pods and seeds per plant, as well as the weight of 100 seeds and seed production. Time to 50% flowering and days to maturity revealed a significant positive correlation. The amount of pods on each plant and seed output are significantly correlated. (Table 3.12).

A significant positive link between the prevalence of bean flies and root rot was observed in Mwea. The number of pods on each plant and the amount of seeds they produced were negatively correlated. Days to maturity and the amount of time till 50% flowering were strongly correlated. The number of pods on each plant was a reliable indicator of seed yield (Table 3.12). In Embu, root rot incidence and the prevalence of bean flies were significantly positively correlated. While the number of pods on each plant, the number of seeds per pod, the weight of a hundred seeds, and the number of seed produced all showed a negative connection with root rot and bean fly incidence. Time until 50% flowering and days to maturity revealed a significant positive correlation. The number of pods on each plant and the number of seeds per pod are significantly correlated. (Table 3.13)

Table 3. 12: correlation analysis among agronomic, disease, and yield parameters across the two locations and seasons

Traits	%Em	Vigor	RtRt	Bean fly	50%Flwr	DTM	NOP	NSP	SW	SY
%Em	-									
Vigor	0.20	-								
RtRt	0.21	0.01	-							
Bean fly	0.18	0.10	0.86**	-						
50%Flwr	0.15	-0.05	0.30	0.10	-					
DTM	0.05	-0.14	0.47	0.32	0.67**	-				
NOP	-0.09	0.06	-0.14	-0.10	0.02	0.10	-			
NSP	-0.35	-0.07	-0.23	-0.19	-0.02	0.15	0.36	-		
SW	0.02	0.04	-0.17	-0.12	0.07	0.09	0.31	0.32	-	
SY	-0.27	-0.22	-0.53*	-0.50*	-0.10	-0.07	0.55*	0.33	0.23	-

% Em= emergence percentage, Vigor is the plant appearance 50%Flwr = Days to 50% flowering DTM= Days to maturity, NOP= number of pods per plant, NSP= Number of seeds per pod, SW= Weight of 100 seeds, SY= seed yield per plot, * =significant, **highly significant

Table 3. 12: correlation analysis among agronomic, disease, and yield parameters across seasons in Mwea

Traits	%Em	Vigor	RtRt	Bean fly	50%Flwr	DTM	NOP	NSP	SW	SY
%Em	-									
Vigor	0.2	-								
RtRt	0.21	0.01	-							
Bean fly	0.18	0.1	0.86**	-						
50%Flwr	0.15	-0.05	0.3	0.1	-					
DTM	0.05	-0.14	0.47	0.32	0.67*	-				
NOP	-0.09	0.06	-0.14	-0.1	0.02	0.1	-			
NSP	-0.35	-0.07	-0.23	-0.19	-0.02	0.15	0.36	-		
SW	0.02	0.04	-0.17	-0.12	0.07	0.09	0.31	0.32	-	
SY	-0.27	-0.22	-0.53*	-0.51*	-0.1	-0.07	0.50*	0.32	0.23	-

% Em= emergence percentage, Vigor is the plant appearance 50%Flwr = Days to 50% flowering DTM= Days to maturity, NOP= number of pods per plant, NSP= Number of seeds per pod, SW= Weight of 100 seeds, SY= seed yield per plot

Table 3.13: correlation analysis among agronomic, disease, and yield parameters across seasons in Embu

Traits	%Em	Vigor	RtRt	Bean fly	50%Flwr	DTM	NOP	NSP	SW	SY
%Em	-									
Vigor	0.23	-								
RtRt	0.46	0.18	-							
Bean fly	0.41	0.13	0.88**	-						
50%Flwr	0.41	0.17	0.29	0.09**	-					
DTM	0.49	0.18	0.17	0.11	0.50*	-				
NOP	-0.24	0.00	-0.21	-0.16	-0.12	-0.17	-			
NSP	-0.49	-0.14	-0.46	-0.36	-0.33	-0.39	0.49	-		
SW	-0.07	-0.09	-0.14	-0.07	-0.07	-0.14	0.31	0.43	-	
SY	-0.32	-0.19	-0.68**	-0.60**	-0.29	-0.59*	0.24	0.50*	0.24	-

% Em= emergence ,percentage, Vigor is the plant appearance 50%Flwr = Days to 50% flowering DTM= Days to maturity, NOP= ,number of pods per plant, NSP= Number of seeds per pod, SW= Weight of 100 seeds, SY= seed yield per plot * =level of significance

1.22 Discussion

Variations in the environment, especially the weather at several experimental sites, were the primary causes of the significant changes in percentage emergence between the genotypes. (Table on weather information on Appendix 1 & 2). Warm soil temperatures are necessary for the consistent emergence of bean seeds. At maturity, the temperature should be between 16 and 30 degrees Celsius, with the ideal temperature being around 26 degrees Celsius during the flowering period. According to Masangwa *et al.* (2017), the ability of the seeds of that genotype to absorb water may also be the reason why bean germination and percentage emergence rose with increase in soil temperature till the ideal degree of roughly 26 degrees Celsius. Variability in the percentage of beans that emerge is also a result of unfavorable climatic conditions such dry soil, waterlogging, and uneven moisture availability. (Masangwa *et al.*, 2017) According to Abubakar et al., environmental factors including water stress and diseases that have a detrimental impact on the time it takes for plants to reach flowering stage were also a factor in the variation in the number of days to reach the flowering stage. Due to the threat posed to the plant by its environment, these conditions have an impact on how physiologically the plant functions by sending stress signals that force the plant to start the reproductive process earlier

than usual. When the environment provides abundant, plants tend to stay in the vegetative stage longer before transitioning to the reproductive stage because there is no hint of stress and thus no threat to its life from the environment (Masangwa *et al.,* 2017). Environmental temperature has a significant impact on the time it takes a plant to reach physiological maturity, which can vary from 60 to 145 days on average. If the temperature is warmer, or between 25 and 35 degrees Celsius, the rate of metabolic reaction in the plant increases, which shortens the time it takes to reach physiological maturity, and vice versa. thus, different varieties exhibit different growth habits, (Tadesse *et al.*, 2016).

In this study pests and disease were prevalent and the genotypes responded differently across season and sites. The disease incidence was lower in the short rain season which was also characterized by low relative humidity in both locations. The level of diseases and pests' incidences are influenced by the type of soil moisture, temperature, and humidity. This matched the information in the report by Belete *et al.,* (2017). Warm and highly humid environment predisposes beans to high diseases and pests' incidence while less humid and colder environment lowers the pathogen's ability to infect the host plants (Olango *et al.,* 2017).

The materials used in this study displayed significant variability in yield and other yield-related characteristics suggesting genetic diversity. Sufficient yield is a function of the ability of the leaf to induce adequate light interception, proper nutrition, plant density and or spacing (Kelly *et al.*, 1998). With proper spacing, there is reduced intra and inter-plant competition for nutrients and light hence boosting photosynthetic activity (Amanuel *et al.*, 2018). Yield is a product of the number of pods on each plant with variances attributed to genetic heterogeneity across different genotypes and their capacity to absorb nutrients from the soil (Tadesse *et al.*, 2014; Darkwa, 2016). Addition of phosphorous fertilizer has been reported to support pod

formation hence increase yield (Alemu *et al.,* 2018). The traits days to flowering, pod formation, and grain filling varied greatly across sites and this has been associated with the different soil moisture (Asfaw et al., 2012). The quantity of seeds per pod did not seem to have any effect on yield across locations or seasons. Similar findings have been reported by Yoseph et al. (2014) and Zelalem et al. (2014). Disparity in weights of a hundred seeds reported in this study could be associated with reduced photosynthate assimilation hence low carbohydrate distribution to developing pods. Under soil moisture stress, drought-tolerant genotypes have shown effective assimilation of photosynthates and mobilization of carbohydrates hence seeds with higher weights ensue (Darkwa *et al.,* 2017). Previous studies have reported similar variations in performance due to genotype effects (Zelalem *et al.,* 2014; Safapour *et al.,* 2011; Yoseph *et al.,* 2014; Narayan *et al.,* 2013).

A negative correlation between the quantity of pods on each plant and yield, as well as root rot and bean fly incidence was observed in the study. The bean fly and root rot in common beans reduces the number of pods on each plant, the quantity of seeds inside the pods, and all other yield-related variables (Zongo et al., 2017). Heavy yield declines have been reported in previous studies on bean and sunflower (Lemessa et al. 2011). With root rot, root dysfunction hinders the absorption of nutrients hence lowering the yields (Zongo et al., (2017).

1.23 Conclusion

The significant differences between the various agronomic traits, as well as between yield and yield component traits, were largely caused by variations in the genotypes' genetic make-up and variations in the environmental conditions (both atmospheric and soil) in the two locations where the experiment was conducted. Some of the superior bean genotypes included KATRAM, NUA666, NUA636, and KATB9 which exhibited early flowering. The study also found genotypes EMBEAN14, KATX56, EMBEAN18, KATRAM, TASHA, CHELALANG, and WAIRIMU to be high-yielding across the locations in both seasons.

CHAPTER FOUR

EVALUATING THE COMBINING ABILITY FOR YIELD AND ZINC AND IRON CONCENTRATION AMONG SELECTED BEAN GENOTYPES

1.24 Abstract

Prior to any bean variety release and recommendation, the nutrition composition must be established to meet the needs of the target demographic. In this study, F1 bean crosses resulting from eight parents including locally adapted varieties and micronutrient-rich bean lines were evaluated at Kenya Agricultural and Livestock Research Organization (KALRO) -Embu and Mwea in a 6 x 6 alpha lattice design. Data was collected on some traits namely agronomic, disease and pest intensity, and yield and its related components and subjected to analysis of Variance (ANOVA). The highest yield performance was observed on the cross NUA604 x Ciankui followed by Wairimu x NUA680, NUA730 x Ciankui, NUA604 x NUA640, and Wairimu x NUA640. The agronomic traits showed high heritability whereas that of the disease and pest incidences was very low. The yield and yield related traits exhibited high heritability values. With regard to the combining, the WAIRIMU, CIANKUI, NUA604, NUA640, and NUA730 had the highest general combining ability (GCA) effect for yield whereas the parents NUA680 and NUA640 had the highest GCA for both iron and zinc concentrations. The parents KATX56, KATB9, and WAIRIMU had the lowest GCA effects for both iron and zinc concentrations. The genotypes WAIRIMUxNUA604 had the highest SCA effects yields followed by genotypes WAIRIMUxNUA730, NUA730xCIANKUI, KATB9xNUA640 among others. The genotypes CIANKUIxNUA680, WAIRIMUxNUA604 and NUA730xKATB9 had highest SCA for iron concentration whereas the genotypes WAIRIMUxNUA640, CIANKUIxNUA680, NUA730xCIANKUI, and NUA730xKATB9 had the highest SCA for zinc concentration. High heritability values were reported for the yield and micronutrient concentrations implying that these traits were under genetic control. Thus,

it is possible to select for parents with superior zinc and iron content coupled with excellent yield performance for incorporation into a bean breeding program.

1.25 Introduction

Today, most of the food crop varieties including common bean varieties grown by farmers in Kenya are deficient in micronutrients this is true because farmers most of the time consider only the yield factor when choosing varieties to grow. This has led to widespread health issues related to malnutrition due to lack of micronutrients like birth defects and infant ill-health it also led to reduces human working ability and energy. This issue is prevalent among members of low income societies in Asia, Sub-Saharan Africa, east Africa and Kenya in particular where majority of the rural farming communities cannot access or afford rich and fortified diet (Hawkes *et al.,* 2017), (Harika *et al.,* 2017). Many studies have been conducted in effort to combat this problem and many varieties of food crops with improved nutritional values were developed. For example, the NUA bean lines developed by CIAT which have higher iron and zinc concentration. Other crops targeted for improvement for various nutritional values by breeding programs in this region and Kenya include crops like sorghum, wheat, maize rice, sweet potato, and tomato (Garg *et al.,* 2018).

In this study, some popular and elite bean varieties which are liked by farmers and the market (Wairimu, Ciankui, KATB9, KATX56) were targeted for improvement of micronutrients (iron and zinc) content through plant breeding technique. This will help push towards achieving one of the Sustainable Development Goals (SDG) agenda's goals, which is to combat malnutrition in order to improve health. Since better nutrition leads to better health, there will be less demand placed on our already overburdened healthcare systems to provide prevention and treatment.

1.26 Materials and methods

1.26.1 Common Bean Germplasm used in the study

An assortment of eight parent including locally adapted varieties and advanced micronutrient beans were grown in the screen house at the Field Station, University of Nairobi. F1 crosses were produced using partial diallel mating design including the parents and later advanced in F2 lines. These bean parents were assembled from Kenya Agricultural and Livestock Research Organization, Embu and Katumani, Egerton University, and CIAT (Table 4.1).

Table 4. 1: Descriptions of parental genotypes used to generate F1 and F2.

S/N	Genotype	Source	Descriptions
1	WAIRIMU (GLP585)	KALRO Embu	Low Fe and Zn content
2	NUA680	CIAT Breeding line	High Fe and Zn content
3	NUA 730	CIAT Breeding line	High Fe and Zn content
4	KAT B9	Katumani	Low Fe and Zn content
5	NUA 604	CIAT Breeding line	High Fe and Zn content
6	CIANKUI	Egerton University	High Fe and Zn content
7	KATX 56	KALRO Katumani	Low Fe and Zn content
8	NUA 640	CIAT Breeding line	High Fe and Zn content

1.26.2 Generation of Crosses and their Evaluation

During planting, the parents were placed in a screen house using polyethene bags filled with a mixture of soil, manure, clean sand, and 20g of DAP fertilizer per pot. The seed rate was two seeds per polyethene bag, which were afterwards pruned to one plant per hill. To synchronize flowering, the seeds were planted in groups of 80 pots every two weeks. After germination till maturity, pesticide was administered every week. The crossing took place in the morning from 6 am to 10am and again in the late afternoon from 4:00 pm to 6:00 pm. The male pollen was then brushed on the stigma after the female parents had been emasculated. On the female parent, flower buds that would open in one or two days were chosen. From the male parents, flowers that had just begun to open that morning were chosen. This was done a day before the female flower opened, and after pollination, flowers were marked and allowed to develop. The 6x6 alpha lattice structure was used to

analyze the F1 seeds from the crosses in the field after they had been harvested, proceeded to F2, and evaluated. (Miller *et al.*, 2013).To evaluate the F1s and F2s, the crosses were grown at KALRO-Embu and Mwea during the second season in 2018 in plots measuring 2 by 2 meters in size in a six by six alpha lattice pattern in three replications. The distance between each replication and each plot was one meter. On crop husbandry, 50 kg/ha of di-ammonium phosphate (DAP) fertilizer was applied during planting. Bean fly was managed by weekly spray with pesticide from germination until flowering.

In order to compare the performance of 28 offspring and their 8 parents in terms of yield and yield components, as well as micronutrient content, two assessment blocks were set up in Mwea and Embu. The plots measured 2 by 2 meters and were arranged in a 6 by 6 alpha lattice with a 40 cm and 20 cm space between each row. The treatments were replicated three times, with 36 plots or treatments in each replicate. The distance between each replication and each plot was one meter. 50 kg/ha of di-ammonium phosphate (DAP) fertilizer was applied during planting. (Kiptoo *et al.*, 2016). Bean fly was managed by weekly spray with pesticide from germination until flowering.

1.26.3 Assessment of agronomic parameters.

i. The Emergence percentage: the number of plants found in each plot two weeks following planting was counted

ii. Early Vigor was assessed within three weeks after germination and scored on a scale of 1 to 9, with 1 denoting excellently vigorous growth, 2 denoting very good growth, 3 denoting decent growth, 5 denoting intermediate growth, 7 denoting poor growth, and 9 denoting extremely stunted growth (Agrios, 2005).

iii. Days to 50% flowering: From the date of planting until half the number of plants in the plot had opened flowers

iv. Plant height of the plant was measured in centimeters whereby during the initiation of the flower, the measurement was taken from the ground level to the tip of the growing point, and later, during maturity, it was taken from the ground level to the uppermost node with a dry pod containing seed. On a scale of 1 to 5, where 1 represents a hundred% of the plants in the plot standing upright and 5, represents a hundred% of the plants lying flat on the ground, lodging was graded at harvest.

v. One hundred Seeds Weight was measured as the weight in grams of one hundred randomly selected undamaged seeds with 16% moisture content. (Blair *et al.,* 2013).

1.26.4 Assessment of pests and diseases incidence.

According to the Centro Internacional de Agricultura Tropical's standard operating procedures, the number of plants infected by the pathogen in each plot was counted to determine the disease incidence rate (1987). It is determined as the population's total number of infected plants multiplied by a hundred and then divided by the total number of plants. It provides information on the prevalence of a disease in the area or the population of plants (Sharma *et al.,* 2017). The global method of disease severity scoring scale of 1-9, developed for on-station screening of crop genotypes and breeding lines for disease resistance and for in-depth study of host resistance (Manandhar *et al.,* 2016), was utilized to score for disease severity, which will then be calculated as the sum of disease ratings x100, divided by the total number of ratings x maximum disease grade (Sharma *et al.,* 2017).

When determining the prevalence of a pest, such as the bean fly, the number of plants with and without the pest's pupa was counted, and the percentage of plants that had the pupa was taken into account as the infestation rate. The severity is graded on a scale of 1, 3, 5, 7, and 9, and is only assessed if there is incidence. When a plant is infected, it is at 1 when it is equally active

as healthy plants. The infected plant begins to exhibit modest growth stunting or a delay at stage 3. The infected plant begins to show noticeable growth stunting or delay. 7 is when an infected plant begins to seriously reduce or stop its growth. 9 is when the infected plants are dead or nearly dead (Manandhar *et al.*, 2016).

1.26.5 Assessment of yield and yield components

i. Number of plants at harvest

ii. Mean number of pods and seeds per pod

iii. Weight in grams of a hundred seeds at 16% moisture,

iv. Yield per plot

vi. Harvest index is calculated as the ratio of SY/BY stated in percentage using the biomass yield, or BY, which is the total plant dry weight recorded at 16% moisture and rounded up to the next whole number using the formula shown.

Yield in $Kg/ha = \frac{\text{weight per plot X ha area}}{plot\ area}$

1.26.6 Estimation of heritability of agronomic traits and yield traits

Broad sense heritability was calculated as H = VG/VP, and narrow sense heritability, h2 = VA/VP where H^2 and h^2 are broad sense heritability and narrow sense heritability respectively. VA is additive variance; VG is genotypic variance and VP is phenotypic variance.

1.26.7 Estimation of GCA and SCA among the parents for yield parameters across environment

The fixed effect model I was used to analyze general and specific combining abilities in order to estimate SCA and GCA effects for the hybrids and parents, respectively. Yij = + GCAi + SCAij + error, where = mean, GCA i=the effect of male I and SCA ij= the interaction effect of female I when crossed to j (Olfati et al., 2011).

1.26.8 Assessment of micronutrient concentration

After the crop was harvested, the seed was cleaned and allowed to sun dry for a week in order to lower the moisture content to roughly 13%. A 400g seed sample was drawn at random from each plot, which represented all the entries. The samples were subsequently transferred to the College of Agriculture and Veterinary Science (CAVS) University of Nairobi's food science laboratory. To remove all of the dust from the seed samples' surfaces and minimize any contamination with aluminum or iron, the samples were cleaned in the lab using moistened cotton towel sprayed with distilled water. (Petry *et al.*, 2015). After the cleaning was done, the samples were put in oven at 65ºC for a period of about 10 hours. The samples were then crushed and ground in a Sunbeam Conical Burr Mill Grinder first at 20 setting and then after that, the samples were ground finer at below 5 setting. The grinder was cleaned with brush and vacuum between each of the samples. (Stangoulis, 2010). Each of the samples was then packed in a moisture proof paper bags separately ready for XRF analysis. Each of the prepared samples was loaded into sample cup of the X-ray fluorescence (XRF) machine for analysis and the samples were scanned for two minutes each and the content of Fe and Zn was determined by spectrophotometry (Oxford Instruments, 2009). 213.9nm and measure the absorbencies. Plot a calibration curve from the readings of the standard series and determine the concentration of the unknown.

1.27 Results

1.27.1 Performance of the genotypes material in different locations as per agronomic parameters, and pest and disease incidence

Site differences did not alter vigor, time to flower or even maturity time; only emergence, root rot, and bean fly incidence were significantly impacted. However, genotypes vary between sites in terms of emergence percentage, vigor, flowering duration, and maturity time. Incidence of root rot and bean flies were significantly influenced by genotype, while percentage of emergence, time to flowering, and days to maturity were not significantly impacted. Environment and genotype interactions also have an impact on the two traits. (Table 4.2).

1.27.2 Performance of the crosses and their parents in terms of yield and yield components across environments

The yield and number of seeds per pod were not significantly impacted by location, however the number of pods on each plant and the weight of 100 seeds were. Genotype had a substantial impact on the total yield component characteristic, the number of pods on each plant, the number of seeds in each pod, the weight of a hundred seeds, and the overall yield of seeds per plot across the two sites. Additionally, the genotype and environment interactions had no discernible effects on any of the yield parameters between the two sites. (Table 4.2).

1.27.3 Estimate of heritability for agronomic, yield and micronutrient traits

The majority of agronomic variables were highly heritable, with the exception of percentage emergence, which showed a relatively low heritability. While it was found that vigor was only somewhat heritable, the time it needed to attain 50% flowering and maturity was highly heritable. The traits for root rot and bean fly both exhibited extremely low heredity, however yield and yield component variables had very high heritability, with seed yield per plot demonstrating the largest heritability value of one. Furthermore, it was found that the concentrations of iron and zinc are both highly heritable traits, with heritability values of more than 0.9 for each variable. (Table 4.2).

Table 4. 2 Analysis of variance for agronomic traits, pest and disease incidence, and yield traits across the two locations

Source of variation	D.f.	%Em	EV	Flwr	DTM	RtRt	Bean Fly	NOP	NSP	SW	SY
Loc	1	249.18**	1.04	8.17	2730.67	298.685**	3.6296*	247.042**	0	242.782**	24342
Rep	2	5.38	2.04**	6.34	3.63**	0.01	1.34	12.042*	0.3	15.042**	4001
Bloc	8	16.714**	0.43	59.2**	52.32	7.56	2.8864**	33.324**	0.752**	83.371**	90280**
Rep.Bloc	16	8.3	0.48	18.18**	12.8**	4.86	0.04	6.65*	0.12	27.97**	27558**
Genotype	35	16.292**	0.73**	41.2**	35.1**	18.216**	1.35**	14.479**	0.9205**	92.603**	72166**
Loc.Gen	35	13.098**	0.32	6.41**	7.78**	12.799**	1.925**	0.61	0.15	0.95	224
Residual	118	5.35	0.35	1.77	1.23	5.39	0.62	3.08	0.12	1.97	5471
Total	214	10.17	0.44	12.37	23.28	10.04	1.01	7.14	0.28	22.76	20348
δ^2_G		0.53	0.07	5.8	4.55	0.9	-0.1	2.31	0.13	15.28	11953.67
δ^2_{GE}		2.58	-0.01	1.54	2.18	2.47	0.43	-0.82	0.01	-0.34	-1749
δ^2_ε		5.35	0.35	1.77	1.23	5.39	0.62	3.08	0.12	1.97	5471
H^2		0.2	0.56	0.84	0.78	0.3	-0.47	0.96	0.84	0.99	1

% Em= percentage emergence per plot, Vigor is the plant appearance 50%Flwr = the duration from planting to when half of the number of plants in a plot have at least one open flower DTM=Days to maturity, NOP= number of pods per plant, NSP= Number of seeds per pod, SW= Weight of 100 seeds, SY= seed yield per plot, δ^2G=genotypic variance δ^2GE=genotype by environment interaction variance, $\delta^2\varepsilon$ = Residual error variance, H^2=broad sense heritability

1.27.4 Performance of the crosses and their parents in different locations as per iron and zinc concentrations in their seeds.

Environmental factors had no real effect on the Fe and Zn concentrations in the crosses and their parents, but genotype differences had a real affect at (P 0.001) on the Fe and Zn concentrations in the crossings' and their parents' seeds. Fe concentration in the seeds of the crosses was significantly impacted by genotype by environment interactions since the results of their performance was not the same across the two environments. Whereas Zn concentration was unaffected so much by the environment since their performance in both environments was similar, it was discovered that Fe and Zn concentrations are strongly heritable traits. (Table 4.3).

Table 4. 3 Analysis of variance for iron and zinc concentration across the two locations

Sources of variation	D.f	Fe	Zn
Location	1	1.716	0.222
Rep	2	0.152	2.419
Block	8	243.027**	58.443**
Genotype	35	2387.564**	695.623**
Genotype.env	35	12.069**	3.204
Residual	125	1.138	4.592
Total	213	410.525	121.077
δ^2_G		197.826	217.111
δ^2_{GE}		3.64367	-0.4627
δ^2_ε		12.069	3.204
H^2		0.98993	0.99755

D.f = Degree of freedom, Fe = Iron, Zn = Zinc, δ^2G=genotypic variance, δ^2GE=genotype by environment interaction variance, $\delta^2\varepsilon$ = Residual error variance, H^2=broad sense heritability

1.27.5 The average performance of the crosses and the parents for agronomic traits, pests, disease and yield across the two locations

In terms of emergence % across the two sites, the genotypes Wairimu x KATB9, KATX56 x KATB9, Ciankui x KATB9, NUA680 x KATX56, KATX56 x NUA640, and NUA604 x Wairimu did particularly well. genotype KATB9, Wairimu x NUA640, NUA730 x NUA640,

NUA730 x Ciankui, NUA604 for days to 50% in the two places while Ciankui and Ciankui x NUA640 had greater performance, and they flowered first.

The genotypes WB9, X56B9, X56640, CIANKUIX56, and CIANKUIB9 exhibited lower incidence of the disease than the other genotypes for root rot across the two locations. However, genotypes NUA680, NUA730, NUA604 x CIANKUI, NUA640 x CIANKUI, Wairimu x NUA680, and Wairimu x NUA604 had lower incidence of bean flies in both settings than the other genotypes. The genotypes 730640, W640, KATX56, B9, CIANKUI x NUA604, and NUA730 had shorter days to maturity than the other genotypes in both locations, as measured by days to 75% maturity. The genotypes B9 x NUA604, CIANKUI x NUA604, WAIRIMU x NUA680, NUA680 x NUA604, KATB9, and CIANKUI x NUA680 performed better in each of the two locations; they also produced the most pods per plant. The genotypes that performed best for number of seeds per pod were KATB9 x NUA730, CIANKUI x NUA604, NUA730 x NUA640, NUA680 x NUA604, NUA680, and NUA604. Genotypes WAIRIMU x NUA604, CIANKUI x NUA730, CIANKUI x NUA604, NUA680 x NUA604, WAIRIMU x NUA680, and NUA604 x NUA640 showed higher weight in grams per a hundred seed weight between the two locations than the other genotypes. genotypes NUA604 x CIANKUI, NUA680 x NUA604, WAIRIMU x NUA604, W680, CIANKUI x NUA730, and NUA604 x NUA640 have the highest seed output in Kg/ha between the two environments. (Table 4.4).

Table 4. 4 The mean performance of the parents and the best crosses for agronomic traits, pests, diseases and yield traits across two locations

Genotypes	%Em	Flwr	DTM	RtRt	BeanFl	NOP	NSP	SW	SY
Wairimu	79.5	34.6	74.9	2.1	2.6	16.1	4.9	49.5	1771
NUA730	68.5	33.4	73.3	6.5	0.9	11.6	4.7	47	1763
Ciankui	68.5	37.4	77.6	4.9	1.7	11.6	5.3	49.3	1752
NUA640	61.1	37.2	77.7	7.5	1.7	13.6	5.3	48.8	1746
KATB9	66.5	31	73.2	7.3	1.6	16.2	4.8	44.4	1731
NUA604	66.4	34.8	74.5	5.4	1.7	11.9	6	44.6	1655
NUA680	86.4	37.3	77.4	2	0.4	14	6	43.1	1559
KATX56	83.6	33	73.1	3.4	2.1	12.8	5.2	41.4	1499
Parental mean	72.57	34.8	75.2	4.9	1.6	13.5	5.3	46.0	1684.5
Ciankui x UA604	89.1	32.1	73.3	2.5	1.3	17.2	4.8	54.2	1993
NUA680 x UA604	88.8	35.7	75.7	1.8	2.5	17.5	5.4	53.6	1973
WAIRIMU xNUA604	92.5	39.3	79.3	3.5	1.4	11.6	5.7	57.7	1959
WAIRIMU xNUA680	72.4	35	75.9	4.6	1.3	17	5.3	52.4	1917
CIANKUI x NUA730	76.4	32	73.4	3.5	3	13.2	5.8	54.6	1910
NUA604 x NUA640	69.9	34.7	74.7	6.3	1.9	10.9	4.9	51.3	1838
Progeny mean	82.63	36.2	76.42	3.28	2.13	13.9	5.06	46.9	1712.3
G.mean	80.2	35.9	76.1	3.7	2	13.8	5.1	46.5	1700.3
LSD 5%	0.6	3.7	3	0.6	0.2	0.5	0.1	3.9	20.3
%CV	14.4	3.7	1.5	63	39.6	12.8	6.9	3	4.4

% Em= percentage emergence per plot, EV = Plant vigor, 50%Flwr = Days to flowering DTM=Days to maturity, NOP= number of pods per plant, NSP= Number of seeds per pod, SW= Weight of 100 seeds, SY= seed yield

1.27.6 The mean performance of the crosses and their parents for Zinc and Iron concentrations

The outcomes of seed study showed that the genotypes performed differently with regard to the levels of iron and zinc in their seeds. For iron concentration throughout the two locations, parents NUA680, NUA604, NUA640, and NUA730 fared well, each providing a mean above 79 ppm. Progeny CIANKUI680, 730604, 730640, 680730, 604W, 680604, W680, and 730B9 similarly displayed greater iron concentration in both locations, with each of them providing a mean of more than 79ppm. While genotype NUA604,730604, CIANKUI680, 680604, 680B9, and NUA680 performed well in both locations for zinc content, with means of over 36.5 ppm. (Table 4.5)

Table 4. 5 Mean performance for iron and zinc across two locations

Genotype	Fe (ppm)	Zn (ppm)
CIANKUI	38.17	10.91
KATB9	35.98	10.64
KATX56	37.22	11.55
NUA604	86.52	38.78
NUA640	80.32	35.7
NUA680	90.61	36.51
NUA730	81.59	32.32
WAIRIMU	34.98	11.17
Parental mean	60.67	23.45
NUA604 x NUA640	78.89	35.12
KATB9 x NUA604	76.47	35.39
CIANKUI x NUA604	78.74	36.01
WAIRIMU x NUA604	83.39	33.95
NUA604 x KATX56	79.59	36.44
KATB9 x NUA640	78.23	33.86
NUA680 x NUA604	82.13	36.89
NUA680 x NUA640	80.29	36.41
NUA680 x NUA730	83.82	35.55
KATB9 x NUA680	78.18	36.58
KATX56 x NUA680	77.75	36.15
NUA730 x NUA604	85.59	37.45
NUA730 x NUA640	85.19	35.25

Table 4. 5 Mean performance for iron and zinc across two locations

Genotype	Fe (ppm)	Zn (ppm)
KATB9 x NUA730	80.9	34.46
NUA730 x CIANKUI	78.05	34.62
NUA730 x KATX56	73.26	31.53
CIANKUI x NUA640	77.4	34.31
CIANKUI x NUA680	85.71	36.96
CIANKUI x KATB9	36.42	11.27
WAIRIMU x CIANKUI	36.82	11.72
KATX56 x CIANKUI	37.17	11.77
WAIRIMU x NUA640	76.92	36.13
WAIRIMU x NUA680	80.65	34.29
WWAIRIMU x NUA730	78.68	30.55
WAIRIMU x KATB9	34.1	11.42
WAIRIMU x KATX56	37.85	11.19
KATX56 x NUA640	78.71	32.14
KATX56 x KATB9	36.89	12.76
Progeny mean	70.64	30.01
Grand Mean	68.42	28.55
LSD	3.04	1.84
CV	1.58	7.11

Fe = iron content, Zn = zinc content, ppm = part per million, LSD = least significant difference, CV = coefficient of variation.

1.27.7 Estimate of GCA among the parents for yield and yield components across the two environments

On genotypes WAIRIMU, CIANKUI, NUA604 and NUA640, there was a significant general combining ability (GCA) effect on seed number per pod, pod number on each plant, and seed yields characteristics. Only for pod number on each plant did the genotype NUA730 have significant general combining ability effects. While KATB9 and NUA604 had the lowest GCA effect for pod number on each plant, genotype WAIRIMU and NUA730 had the greatest significant GCA effect for pod number on each plant.

Table 4. 6 The estimate of GCA among the parents for yield and yield components

Parent	NOP	NSP	SW	SY
WAIRIMU	0.8**	0.10*	2.0**	44.11**
NUA 730	0.7**	-0.04	0.3	2.75
KATX 56	-0.2	-0.07	2.9**	-83.75
KAT B9	-0.1	0.08	-2.1	46.14**

CIANKUI	0.3	0.08	0.9**	27.16**
NUA 640	0.5*	0.14**	0.5*	9.42
NUA680	0.4*	0.13**	0.4*	-10.54
NUA 604	-0.1	0.06	1.6**	56.98**

NOP= number of pods per plant, NSP= Number of seeds per pod, SW= Weight of 100 seeds, SY= seed yield

1.27.8 Estimate of GCA among the parents for pests, disease, and agronomic parameters across environment

The general combining ability effect on days to 75% maturity for the genotypes NUA730, KATX56, and NUA680 was significantly different between the two environments. The duration of flowering to 50% was significantly impacted by the general combining ability of genotypes KATB9 and NUA680. But across the two environments, there was no significant overall combining impact for either root rot incidence or bean fly for any of the genotypes.

Table 4. 7 The estimate of GCA among the parents for pests and disease

Parent	Bean fly	Rtrt	Flwr	DTM
WAIRIMU	-0.1458	0	0.1375	0.1375
NUA 730	-0.0792	0.13333	-0.5958	-0.7958*
KATX 56	0.17083	0.06667	-0.6625	-0.7292*
KAT B9	0.1375	0.1	0.80417*	0.5375
CIANKUI	0.2375	0	-1.3458	-0.4625
NUA 640	-0.1792	-0.45	-0.0458	0.1375
NUA680	-0.0958	-0.3167	2.1375**	1.67083**
NUA 604	-0.0458	0.46667	-0.4292	-0.4958

DTM= days to 75% maturity, Flwr=days to 50% flowering, Rtrt=root rot

1.27.9 Estimate of GCA among the parents for iron and zinc concentrations across environment

Both the Fe and Zn concentrations exhibited a significant GCA effect, with genotype NUA680 ranking first and genotype NUA640 second in terms of iron concentrations. When it comes to zinc content, genotype NUA604 comes in first and genotype NUA680 comes in second. The least GCA effect was seen for the genotypes KATX56, KATB9, and WAIRIMU on the concentration of iron and zinc in the seeds. (Table 4.7)

Table 4. 7 The estimate of GCA among the parents for Fe and Zn concentration

Parent	GCA Fe	GCA Zn

WAIRIMU	-11.8	-6.6
NUA 730	11.4**	4.7*
KATX 56	-12.1	-6.2
KAT B9	-12.3	-6.0
CIANKUI	-11.0	-5.9
NUA 640	10.1*	5.8**
NUA680	13.5**	6.9**
NUA 604	12.3**	7.2**

GCAFe=general combining ability for iron, GCAZn=general combining ability for zinc

The genotypes WAIRIMU x NUA604, WAIRIMU X NUA730, NUA730 x CIANKUI, KATB9 x NUA640, WAIRIMU x NUA680, NUA730 X NUA680, WAIRIMU x NUA604, KATX56 x NUA604, and NUA 640 x NUA604 exhibited stronger specific combining ability impacts on yield and yield components. The strongest specific combining ability impacts on seed yield and a hundred seed weight were found in the genotypes CIANKUI x NUA640, NUA680 x NUA604, NUA730 x CIANKUI, WAIRIMU x NUA680, and WAIRIMU x NUA604. (Table 4.8).

Table 4. 8 Specific combining ability (SCA) for yield and yield components

Male	Female	SY	Rank	SW	Rank	NSP	Rank	NOP	Rank
NUA730	WAIRIMU	-64.87*	25	-1.02	22	0.41*	5	-3.78	36
WAIRIMU	KATX56	-46.45	24	-1.97	26	-0.22	29	1.69*	6
NUA730	KATX56	45.61	12	1.19**	12	-0.62**	35	-0.64	25
WAIRIMU	KATB9	-118.75**	30	-3.27**	31	-0.2	27	0.12	17
NUA730	KATB9	76.43*	7	4.89**	5	0.06	13	1.62	7
KATX56	KATB9	-46.12	23	-0.72	19	0.26	9	-0.24	10
WAIRIMU	CIANKUI	-70.86*	27	-2.79**	28	-0.04	18	1.16	20
NUA730	CIANKUI	181.24**	3	6.38**	3	-0.1	21	2.32**	3
KATX56	CIANKUI	-69.40*	26	-2.91**	29	-0.40**	33	-1.71*	31
KATB9	CIANKUI	-72.65*	28	-0.87	21	0.45**	4	-1.61*	29
NUA640	WAIRIMU	18.05	18	0.68	15	-0.49**	34	0.67	13
NUA730	NUA640	-202.39**	36	-5.99**	35	0.45**	4	1.51*	8
NUA640	KATX56	-101.66**	29	-2.27**	27	-0.02	17	-0.36	21
NUA640	KATB9	74.61*	8	0.93	13	0.33*	8	3.74**	1
NUA640	CIANKUI	236.38**	1	7.24**	1	0	16	-1.23	27
NUA680	WAIRIMU	161.63**	4	4.58**	7	0.25	10	2.42**	2
NUA730	NUA680	51.92	11	2.41**	10	-0.15	22	-2.24**	33
KATX56	NUA680	107.52**	6	4.79**	6	-0.29	32	-0.94	26
KATB9	NUA680	42.48	13	-1.84*	25	-0.27	31	-0.51	23
CIANKUI	NUA680	-139.14**	32	-4.52**	32	0.40*	7	-1.64*	30
NUA640	NUA680	-153.68**	34	-5.06**	33	-0.72**	36	0.87	12
WAIRIMU	NUA604	160.77**	5	7.18**	2	0.65**	1	-2.39**	34
NUA730	NUA604	-153.44**	33	-6.49**	36	-0.09	20	2.11*	5
KATX56	NUA604	35.83	15	-0.61	17	-0.22	29	2.24**	4
KATB9	NUA604	55.87*	10	2.59**	9	-0.2	26	-1.83*	32
CIANKUI	NUA604	-9.1	20	-0.09	16	-0.2	26	0.21	16

Table 4. 8 Specific combining ability (SCA) for yield and yield components

Male	Female	SY	Rank	SW	Rank	NSP	Rank	NOP	Rank
NUA640	NUA604	73.24*	9	2.71**	8	0.01	15	-2.44**	35
NUA80	NUA604	183.47**	2	5.94**	4	0.41*	6	1.14	11

NOP= number of pods per plant, NSP= Number of seeds per pod, SW= Weight of 100 seeds, SY= seed yield

Table 4. 9 Specific combining ability (SCA) for zinc concentration

MALE	FEMALE	SCAZn	RANK
WAIRIMU	NUA640	8.17722	1
KATB9	NUA680	7.91556	2
NUA730	CIANKUI	7.29389	3
KATX56	NUA640	7.16056	4
CIANKUI	NUA680	7.04222	5
KATX56	NUA604	6.96889	6
WAIRIMU	NUA680	6.82722	7
CIANKUI	NUA604	6.73389	8
KATB9	NUA604	6.50722	9
KATX56	NUA680	6.27722	10
CIANKUI	NUA640	5.77556	11
NUA730	KATB9	5.35056	12
WAIRIMU	NUA604	4.85222	13
KATB9	NUA640	4.79889	14
NUA730	KATX56	4.64556	15
WAIRIMU	NUA730	4.19556	16
NUA730	NUA604	-2.5678	17
NUA730	NUA640	-3.6761	18
KATX56	KATB9	-4.1461	19
WAIRIMU	KATB9	-4.3294	20
NUA604	NUA604	-4.4111	21
WAIRIMU	WAIRIMU	-4.8511	22
NUA730	NUA680	-4.8594	23
NUA640	NUA640	-4.8778	24
WAIRIMU	CIANKUI	-4.9528	25
WAIRIMU	KATX56	-5.0678	26
KATB9	CIANKUI	-5.1478	27
NUA730	NUA730	-5.1911	28
KATX56	KATX56	-5.2178	29
NUA640	NUA680	-5.3611	30
KATX56	CIANKUI	-5.4028	31
KATB9	KATB9	-5.4744	32
NUA680	NUA680	-5.6444	33
CIANKUI	CIANKUI	-5.6711	34
NUA680	NUA604	-6.5528	35
NUA640	NUA604	-7.1194	36

SCA Zn = Specific combining ability for zinc

Table 4. 10 Specific combining ability (SCA) for iron concentration

MALE	FEMALE	SCAFe	RANK
WAIRIMU	NUA680	15.7122	1
WAIRIMU	NUA604	14.2939	2
CIANKUI	NUA680	13.6806	3
KATX56	NUA640	12.6256	4
KATB9	NUA640	12.3306	5
KATX56	NUA604	11.2022	6
WAIRIMU	NUA730	10.0256	7
CIANKUI	NUA640	9.85222	8
CIANKUI	NUA730	9.76056	9
WAIRIMU	NUA640	9.68389	10
KATB9	NUA680	9.20889	11
CIANKUI	NUA604	9.02889	12
NUA730	KATB9	8.93889	13
KATB9	NUA604	8.00722	14
NUA730	KATX56	6.60056	15
KATX56	NUA680	5.00389	16
NUA730	NUA640	-3.9094	17
NUA680	NUA680	-5.2278	18
NUA730	NUA604	-5.5328	19
KATX56	KATB9	-6.2928	20
NUA604	NUA604	-6.3311	21
KATB9	KATB9	-6.7544	22
KATX56	KATX56	-6.7644	23
WAIRIMU	KATX56	-7.3061	24
NUA640	NUA640	-7.8344	25
KATB9	CIANKUI	-7.8828	26
NUA730	NUA730	-8.2344	27
KATX56	CIANKUI	-8.3044	28
CIANKUI	CIANKUI	-8.4111	29
WAIRIMU	CIANKUI	-9.3128	30
NUA730	NUA680	-9.4144	31
WAIRIMU	KATB9	-10.801	32
WAIRIMU	WAIRIMU	-11.148	33
NUA680	NUA604	-11.579	34
NUA640	NUA680	-12.156	35
NUA640	NUA604	-12.758	36

SCA Fe = specific combining ability for iron

1.28 DISCUSSIONS

For the three main qualities that made up the study's objective yield, iron concentration, and zinc concentration there were significant GCA and SCA effects, indicating that both additive and non-additive gene effects were active in regulating the observed. This outcome is comparable to what Mukamuhirwa et al. (2015) found regarding the inheritance of the two micronutrients. The genotypes NUA604, KATB9, and WAIRIMU showed the highest positive GCA significant impacts for seed yield (56.98 P0.001, 46.14 P0.001, and 44.11 P0.001, respectively). The majority of the parental genotypes are thought to be good combiners with those parents that have highly substantial GCA effects, making them good genotypes for use in breeding programs (Jacinto *et al.*, 2003).

To breed for high iron concentration, genotypes NUA680, NUA604, and NUA730 were ranked first, second, and third in SCA; consequently, they make good parents. To improve zinc concentration, genotypes NUA604, NUA680, and NUA640 were identified as first, second, and third; this was done because they demonstrated significance in SCA effects on concentration of Fe and Zn. This suggests that a more than one gene may play a role in determining the amounts of iron and zinc present in the seeds of the F2 progenies (Da Rosa *et al.*, 2010).

In the seeds of the F2 progenies, Fe and Zn concentrations were found to be strongly positively correlated (R = 0.96), suggesting that the genes that cause higher Fe concentrations co-segregate with those that cause higher Zn concentrations. This was in line with earlier research that was described in (Blair et al., 2010). When a breeder wants, he may simultaneously select for those minerals.

The finding or results of this research were similar to some prior studies done by other scientists as discussed here. Days to flowering and the incidence of bean flies varied significantly between the parents and the offspring in the two different locations. These variations suggest that the features are influenced by both genetic and environmental influences. This conclusion

supports a previous study by Nkhata *et al.* (2019), which found that bean fly occurrence varies by location and depends on the environment and the availability of host plants. It has been reported that bean flies cause more crop damage in arid climates or during the dry season. Higher temperatures, lesser humidity, and unpredictable rainfall are typically conducive to the pest's ability to reproduce. (Nkhata *et al.*, 2019).

The number of seeds in each pod, weight of a hundred seeds, and the seed yield all had high heritability values. Heritability had high values of 0.84 and 0.78, respectively, for time to reach flowering stage and the time needed to reach physiological maturity. These suggest that those traits are controlled by fewer genes compared to the other traits and early generation selection can be effective to these two traits. The heritability was much higher for iron and zinc concentrations for the two characteristics (0.94 and about 0.99, for iron and zinc respectively) (Mukamuhirwa *et al.*, 2015). As previously mentioned by Blair *et al.* (2010) and Da Rosa *et al.* (2010), the higher the heritability value for a trait, the higher the probability that the trait is controlled by genetic factor and very few genes are involved in controlling the traits. Since traits are controlled by genes more so than by environment, selection for increase in micronutrient concentration is highly achievable in the early generations.

1.29 Conclusion

This study found that the traits for higher iron and zinc can be highly heritable because some of the parent used in the study showed very good combining for the trait. Selection for high iron and zinc during breeding can start at very early generation since the trait is highly heritable

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUTIONS, AND RECOMMENDATION

The age of the seeds, their viability, changes in the moisture content of the soil, and the temperature of the soil were all blamed for the variation in percentage emergence among genotypes. Previous authors, such as De Ron in De Ron et al., (2016); Pattung et al., (2016), claim that dry bean seeds need warm soil temperatures for uniform emergence, with the bottom temperature being around 15.6 °C and the ideal temperature being around 26 °C. Variability in the percentage of beans that emerge is also a result of unfavorable climatic conditions such dry soil, waterlogging, and uneven moisture availability. (Masangwa *et al.*, 2017; Azimi *et al.*, 2014).

In both locations, there was a pest and disease incidence among the bean genotypes, indicating that the environment was favorable for the development of pest and disease on the crops. The incidence was lower during the relatively short rainy season, when the humidity was lower, indicating that disease development was influenced by environmental factors, particularly humidity, soil temperatures, and moisture content of the soil, in a way that higher humidity and warmer temperature This was in line with the findings of the report by Belete et al (2017). Warm and highly humid environment favors disease incidences but drier and colder environment lowers the pathogen ability to infect the plants (Olango *et al.*, 2017).

The duration of flowering, which varied greatly between genotypes across the two sites and in individual locations, also varied widely. This was attributed to genetic variances between genotypes as well as differences in the temperature, amount of rainfall, and diseases between the two locations. Bean genotypes with diverse genetic origins mature at different times, as previously discussed by Masangwa et al. (2017), because some genotypes mature earlier than

others even when they are grown under the same environmental conditions. However, as discussed in Masangwa *et al.*, 2017; Azimi *et al.*, 2014; and Abubakar *et al.*, 2008, the time it takes for plants to flower is also influenced by environmental factors. For example, crops start flowering more quickly when it is warmer and less humid outside than when it is cooler and more humid. Minimum and ideal temperatures for flowering are 16°C and 30°C, respectively. (Masangwa *et al.*, 2017).

The genetic makeup of the materials used and the environmental conditions had a significant impact on the variation in the time taken by different bean genotypes to reach physiological maturity, which ranged on average from 60 to around 100 days. When the temperature was warmer, the crop took a shorter time to reach physiological maturity, and when the temperature was cooler, the crop took a longer time to reach physiological maturity. Because some of the kinds utilized in the experiment have genes for early maturity and others may have genes for late maturity, this variation can also be attributable to genetic variation among the types (Tadesse *et al.*, 2016).

Because the genotypes performed so differently for different yield components, it was clear that the genotypes were genetically distinct. This is due to genotypes' varying growing habits, which affects their capacity to produce sufficient leaf area for light interception, which in turn affects how well they can perform photosynthesis (Kelly et al., 1998). However, under this notion, space, plant density, and yield are also significant factors that cannot be neglected. First off, a vigorous plant with plenty of healthy leaves for optimal light absorption during the growth and reproductive stages can produce the highest possible output. Second, the suggested and uniform distance between plants has a good effect on yield by promoting air circulation, increasing leaf exposure to light, and preventing nutrient competition. Therefore, the maximum yield might not be achieved unless the plant distribution was at the required rate (Amanuel et

al., 2018). Given that plants depend on nutrients being available at the appropriate times for normal operation, soil fertility and fertilizer application in the correct amount and timing must also have played a role in the yield variation and grain quality. However, other environmental aspects including location, climate, and soil type all play a role. In comparison to other factors like the number of seeds in each pod and the weight of a hundred seeds, the number of pods on each plant is the factor of yield that has the greatest impact on yield (Tadesse et al., 2014). According to Darkwa (2016), variations in the number of pods on each plant can mostly be linked to the genetics of the common bean plants and the degree of nitrogen uptake from the soil. The quantity of pods on each plant is typically closely related to the ideal environmental conditions. Crop fertilization with phosphorus aids in the crop's growth and reproduction (Alemu *et al.,* 2018). The genotypes showed difference in their response to the environmental factors such as soil moisture content especially during Flowering, pod formation, and grain filling (Asfaw *et al.,* 2012). For all genotypes, the number of seeds in each pod is shown to be insignificant across all locations and seasons. This indicates that the environment has no impact on it. Yoseph et al. previously reported on the non-significance differences in the genotype-specific seed number in each pod (2014). Similar to other studies by Yoseph et al. (2014) and Zelalem et al. (2014), this study revealed a substantial difference in 100 seed weight. The weight of 100 seeds varied depending on the size of the seeds. The variance in seed weight is related to the amount of soil moisture present, which, when insufficient, slows down the rate of photosynthate assimilation and results in poor carbohydrate partitioning to the developing grain in drought. However, genotypes that are tolerant to drought have genes that enable them to build greater defenses against drought stress. As a result, they continue to maintain high seed weight despite moisture stress (Darkwa et al., 2017). In earlier research on common beans, genetic influences have been shown to result in variation in performance (Zelalem *et al.,* 2014; Safapour *et al.,* 2011; Yoseph *et al.,* 2014).

1.30 CONCLUSION

This work has unequivocally shown that it is possible to develop a common dry bean genotypes with high concentrations of the minerals iron and zinc without sacrificing yields. Early generations and simultaneous selection can lead to greater iron and zinc concentrations in cultivars of common beans since the genes for both micronutrients have been found to co-segregate together in contrast to other features. In addition, compared to yields, the concentration of micronutrients did not differ considerably across the environments.

1.31 RECOMMENDATIONS

It is recommended that the genotypes NUA604 x CIANKUI, NUA604 x NUA680, and NUA604 x WAIRIMU be utilized to increase yield in Kenyan market-class dry beans due to their high yield and high levels of iron and zinc concentrations. Given the high heritability values for yield, iron, and zinc content, it follows that breeding for these traits can be accomplished using these parents in a conventional breeding. It is advised to assess and research each new piece of breeding program material for the reasons listed below.

1. The stability of these promising genotypes selected for yield and high iron and zinc concentration should be tested by evaluating it across different environments so that the most stable ones can be selected for further use in breeding programs while the least stable ones are discarded.

2. More studies on combining ability and mode of inheritance of the genes for high yield and high iron and zinc concentration should be done.

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Appendix 1 WEATHER DATA DURING THE EXPERIMENT AT KARLO EMBU				
May 2018 - Daily weather Observations Embu				
Time	**Temperature**	**Humidity**	**Wind Speed**	**Precipitation**
May	° C | ° F	%	Kph | Mph	Total (mm/in)
01/05/2018	20 | 68.0	86	6 | 3.73	18.4 | 0.72
02/05/2018	20 | 68.0	86	6 | 3.73	14.6 | 0.57
03/05/2018	21 | 69.8	82	7 | 4.35	16.8 | 0.66
04/05/2018	21 | 69.8	86	6 | 3.73	20.2 | 0.8
05/05/2018	20 | 68.0	91	4 | 2.49	18.3 | 0.72
06/05/2018	21 | 69.8	80	6 | 3.73	1.7 | 0.07
07/05/2018	20 | 68.0	87	5 | 3.11	9.3 | 0.37
08/05/2018	21 | 69.8	84	6 | 3.73	5.7 | 0.22
09/05/2018	20 | 68.0	86	6 | 3.73	4.7 | 0.19
10/05/2018	20 | 68.0	85	5 | 3.11	6.7 | 0.26
11/05/2018	21 | 69.8	83	6 | 3.73	6.6 | 0.26
12/05/2018	21 | 69.8	82	6 | 3.73	7.5 | 0.3
13/05/2018	21 | 69.8	83	6 | 3.73	21.4 | 0.84
14/05/2018	21 | 69.8	86	6 | 3.73	20.0 | 0.79
15/05/2018	20 | 68.0	88	5 | 3.11	8.5 | 0.33
16/05/2018	20 | 68.0	87	6 | 3.73	14.5 | 0.57
17/05/2018	21 | 69.8	83	6 | 3.73	10.4 | 0.41
18/05/2018	21 | 69.8	80	6 | 3.73	10.8 | 0.43
19/05/2018	20 | 68.0	84	5 | 3.11	5.5 | 0.22
20/05/2018	20 | 68.0	86	5 | 3.11	2.0 | 0.08
21/05/2018	19 | 66.2	91	4 | 2.49	13.8 | 0.54
22/05/2018	19 | 66.2	92	3 | 1.86	17.5 | 0.69
23/05/2018	20 | 68.0	85	5 | 3.11	2.6 | 0.1
24/05/2018	20 | 68.0	85	5 | 3.11	7.0 | 0.28
25/05/2018	20 | 68.0	82	5 | 3.11	1.6 | 0.06
26/05/2018	20 | 68.0	84	6 | 3.73	6.0 | 0.24
27/05/2018	20 | 68.0	81	7 | 4.35	0.5 | 0.02
28/05/2018	21 | 69.8	78	7 | 4.35	0.5 | 0.02
29/05/2018	21 | 69.8	70	6 | 3.73	0.0 | 0.0
30/05/2018	21 | 69.8	67	7 | 4.35	0.0 | 0.0
31/05/2018	21 | 69.8	77	6 | 3.73	5.5 | 0.22
October 2018 - Daily weather Observations Embu				
Time	**Temperature**	**Dew Point**	**Humidity**	**Precipitation**
October	° C | ° F	° C | ° F	%	Total (mm/in)
01/10/2018	22 | 71.6	9 | 48.2	52	0.0 | 0.0
02/10/2018	21 | 69.8	12 | 53.6	61	1.2 | 0.05
03/10/2018	22 | 71.6	14 | 57.2	69	9.3 | 0.37
04/10/2018	22 | 71.6	14 | 57.2	66	1.2 | 0.05
05/10/2018	23 | 73.4	14 | 57.2	62	1.3 | 0.05

Appendix 1 WEATHER DATA DURING THE EXPERIMENT AT KARLO EMBU				
06/10/2018	23 | 73.4	14 | 57.2	66	4.1 | 0.16
07/10/2018	23 | 73.4	15 | 59.0	66	4.2 | 0.17
08/10/2018	21 | 69.8	15 | 59.0	74	35.1 | 1.38
09/10/2018	21 | 69.8	15 | 59.0	73	12.2 | 0.48
10/10/2018	21 | 69.8	13 | 55.4	65	2.0 | 0.08
11/10/2018	22 | 71.6	13 | 55.4	65	2.0 | 0.08
12/10/2018	23 | 73.4	13 | 55.4	61	1.2 | 0.05
13/10/2018	23 | 73.4	15 | 59.0	70	16.1 | 0.63
14/10/2018	23 | 73.4	14 | 57.2	63	1.3 | 0.05
15/10/2018	24 | 75.2	15 | 59.0	64	3.0 | 0.12
16/10/2018	24 | 75.2	15 | 59.0	66	2.5 | 0.1
17/10/2018	22 | 71.6	15 | 59.0	73	10.1 | 0.4
18/10/2018	22 | 71.6	15 | 59.0	72	9.3 | 0.37
19/10/2018	22 | 71.6	15 | 59.0	75	2.2 | 0.09
20/10/2018	23 | 73.4	12 | 53.6	61	0.1 | 0.0
21/10/2018	23 | 73.4	11 | 51.8	55	0.0 | 0.0
22/10/2018	22 | 71.6	12 | 53.6	62	1.6 | 0.06
23/10/2018	19 | 66.2	15 | 59.0	79	17.6 | 0.69
24/10/2018	21 | 69.8	13 | 55.4	68	0.7 | 0.03
25/10/2018	21 | 69.8	13 | 55.4	68	12.1 | 0.48
26/10/2018	21 | 69.8	16 | 60.8	81	16.5 | 0.65
27/10/2018	20 | 68.0	16 | 60.8	81	4.2 | 0.17
28/10/2018	21 | 69.8	16 | 60.8	81	4.2 | 0.17
29/10/2018	21 | 69.8	15 | 59.0	79	10.0 | 0.39
30/10/2018	21 | 69.8	16 | 60.8	78	7.9 | 0.31
31/10/2018	21 | 69.8	15 | 59.0	74	7.5 | 0.3
Source Embu, KE Climate Zone, Monthly Weather Averages and Historical Data (tcktcktck.org)				

Appendix 2 WEATHER DATA DURING THE EXPERIMENT AT KARLO MWEA				
May 2018 - Daily weather Observations Kirinyaga				
Time	**Temperature**	**Humidity**	**Precipitation**	
May	° C | ° F	%	Total (mm/in)	
01/05/2018	20 | 68.0	86	18.4 | 0.72	
02/05/2018	20 | 68.0	86	14.6 | 0.57	
03/05/2018	21 | 69.8	82	16.8 | 0.66	
04/05/2018	21 | 69.8	86	20.2 | 0.8	
05/05/2018	20 | 68.0	91	18.3 | 0.72	
06/05/2018	21 | 69.8	80	1.7 | 0.07	
07/05/2018	20 | 68.0	87	9.3 | 0.37	
08/05/2018	21 | 69.8	84	5.7 | 0.22	

Appendix 2 WEATHER DATA DURING THE EXPERIMENT AT KARLO MWEA				
09/05/2018	20 | 68.0	86	4.7 | 0.19	
10/05/2018	20 | 68.0	85	6.7 | 0.26	
11/05/2018	21 | 69.8	83	6.6 | 0.26	
12/05/2018	21 | 69.8	82	7.5 | 0.3	
13/05/2018	21 | 69.8	83	21.4 | 0.84	
14/05/2018	21 | 69.8	86	20.0 | 0.79	
15/05/2018	20 | 68.0	88	8.5 | 0.33	
16/05/2018	20 | 68.0	87	14.5 | 0.57	
17/05/2018	21 | 69.8	83	10.4 | 0.41	
18/05/2018	21 | 69.8	80	10.8 | 0.43	
19/05/2018	20 | 68.0	84	5.5 | 0.22	
20/05/2018	20 | 68.0	86	2.0 | 0.08	
21/05/2018	19 | 66.2	91	13.8 | 0.54	
22/05/2018	19 | 66.2	92	17.5 | 0.69	
23/05/2018	20 | 68.0	85	2.6 | 0.1	
24/05/2018	20 | 68.0	85	7.0 | 0.28	
25/05/2018	20 | 68.0	82	1.6 | 0.06	
26/05/2018	20 | 68.0	84	6.0 | 0.24	
27/05/2018	20 | 68.0	81	0.5 | 0.02	
28/05/2018	21 | 69.8	78	0.5 | 0.02	
29/05/2018	21 | 69.8	70	0.0 | 0.0	
30/05/2018	21 | 69.8	67		
October 2018 - Daily weather Observations Kirinyaga				
Time	**Temperature**	**Humidity**	**Wind Speed**	**Precipitation**
October	° C | ° F	%	Kph | Mph	Total (mm/in)
01/10/2018	22 | 71.6	52	7 | 4.35	0.0 | 0.0
02/10/2018	21 | 69.8	61	7 | 4.35	1.2 | 0.05
03/10/2018	22 | 71.6	69	6 | 3.73	9.3 | 0.37
04/10/2018	22 | 71.6	66	5 | 3.11	1.2 | 0.05
05/10/2018	23 | 73.4	62	5 | 3.11	1.3 | 0.05
06/10/2018	23 | 73.4	66	6 | 3.73	4.1 | 0.16
07/10/2018	23 | 73.4	66	6 | 3.73	4.2 | 0.17
08/10/2018	21 | 69.8	74	6 | 3.73	35.1 | 1.38
09/10/2018	21 | 69.8	73	5 | 3.11	12.2 | 0.48
10/10/2018	21 | 69.8	65	6 | 3.73	2.0 | 0.08
11/10/2018	22 | 71.6	65	5 | 3.11	2.0 | 0.08
12/10/2018	23 | 73.4	61	6 | 3.73	1.2 | 0.05
13/10/2018	23 | 73.4	70	5 | 3.11	16.1 | 0.63
14/10/2018	23 | 73.4	63	6 | 3.73	1.3 | 0.05
15/10/2018	24 | 75.2	64	6 | 3.73	3.0 | 0.12
16/10/2018	24 | 75.2	66	5 | 3.11	2.5 | 0.1
17/10/2018	22 | 71.6	73	6 | 3.73	10.1 | 0.4
18/10/2018	22 | 71.6	72	7 | 4.35	9.3 | 0.37

Appendix 2 WEATHER DATA DURING THE EXPERIMENT AT KARLO MWEA				
19/10/2018	22 | 71.6	75	7 | 4.35	2.2 | 0.09
20/10/2018	23 | 73.4	61	9 | 5.59	0.1 | 0.0
21/10/2018	23 | 73.4	55	9 | 5.59	0.0 | 0.0
22/10/2018	22 | 71.6	62	7 | 4.35	1.6 | 0.06
23/10/2018	19 | 66.2	79	6 | 3.73	17.6 | 0.69
24/10/2018	21 | 69.8	68	7 | 4.35	0.7 | 0.03
25/10/2018	21 | 69.8	68	7 | 4.35	12.1 | 0.48
26/10/2018	21 | 69.8	81	5 | 3.11	16.5 | 0.65
27/10/2018	20 | 68.0	81	5 | 3.11	4.2 | 0.17
28/10/2018	21 | 69.8	81	5 | 3.11	4.2 | 0.17
29/10/2018	21 | 69.8	79	6 | 3.73	10.0 | 0.39
30/10/2018	21 | 69.8	78	6 | 3.73	7.9 | 0.31
31/10/2018	21 | 69.8	74	8 | 4.97	7.5 | 0.3
Source Kirinyaga, KE Weather In May, 2018 (Weather History May, 2018) (tcktcktck.org)				