# GENOTYPE x ENVIRONMENT INTERACTION OF COMMON BEAN (Phaseolus vulgaris L.) GENOTYPES ON REACTION TO FOUR BEAN DISEASES

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A DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS

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#### **ABSTRACT**

This study was carried out to assess the performance of advanced common bean lines (BC<sub>2</sub>F<sub>6</sub>) to diseases (Angular leaf spot, Common bean blight and Bean common mosaic virus/Bean common mosaic necrosis virus) and yield and yield components in three different locations; Arusha, Mbeya and Morogoro. The experiment was arranged in split plot, main plots were locations (Arusha, Morogoro and Mbeya) and sub-plots were 32 genotypes (28 lines and 4 checks). The results from the experiment revealed high significant differences (p < 0.05) among genotypes and Genotypes x Environment interaction in days to 85 % maturity, pods per plant, seeds per pod, weight of seeds per plant, 100 seed weight and grain yield. There were no significant differences in diseases (Angular leaf spot and Common bean blight) scores and few genotypes had the Bean common mosaic virus/Bean common mosaic necrosis virus symptoms basing on the score of presence and absence. Among the lines that performed well basing on both diseases and yield were KT 13-1b, KT 4g-3c, KT 6-2d, KT 6-2c, KT 10-2d, KT 11-1a, KT 15a, KT 9-2b, KT 11-2d and KT 2b. Grain yield had a positive significant correlation with days to 50 % flowering, days to 85 % maturity, pods per plant, seeds per pod, weight of seeds per plant, 100 seed weight. This suggests that the traits have to be considered when breeding for higher grain yield. This study showed that the introgression of genes for resistance to the three diseases to preferred bean variety Kablanketi was successful since most of the lines were resistant to the diseases. However, this study needs to be repeated to verify the performance of these promising lines and planting should be done at the beginning of the season to ensure that there are favourable environments for diseases progress.

#### **DECLARATION**

I, Baraka Mvile, do hereby declare to the Senate of Soko	oine University of Agriculture that
this dissertation is my own original work and has never	r been submitted nor concurrently
being submitted for a higher degree award in any other i	nstitution.
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The above declaration is confirmed by;	
The above declaration is committed by,	
Prof. S. Nchimbi-Msolla	Date

(Supervisor)

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#### **DEDICATION**

To my family

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#### LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

ALS Angular leaf spot

ANOVA Analysis of variance

ARI Agricultural Research Institute

BCMNV Bean common mosaic necrosis virus

BCMV Bean common mosaic virus

CBB Common bean bacterial blight

CEC Cation exchange capacity

CIAT International Centre of Tropical Agriculture

DAP Diammonium phosphate

DNA Deoxyribonucleic acid

G x E Genotype and Environment interaction

Hrs Hours

Kg Kilogram

masl Meters above sea level

MDR Multiple disease resistance

PCR Polymerase chain reaction

pH Logarithmic hydrogen ion concentration

QTL Quantitative trait loci

SUA Sokoine University of Agriculture

t/ha Tonne per hectare

XAP Xanthomonas axonopodis pv. phaseoli

YDC Yeast extract-dextrose-calcium carbonate

#### **CHAPTER ONE**

#### 1.0 INTRODUCTION, JUSTIFICATION AND OBJECTIVES

#### 1.1 Introduction

Common bean (*Phaseolus vulgaris* L.) belongs to the *Fabaceae* family and originated from South America. It is a staple food for more than 100 million people in Africa with per capita consumption of 60 kg/person/year in the Great Lakes Regions (CTA, 2010). It is a major source of protein for many people in Tanzania and other East African countries. It provides protein of approximately 20 - 25 % in form of phaseolin, vitamins, minerals (Ca, Cu, Fe, Mg, Mn and Zn) and caloric requirement of 12 - 16 %. It is produced mainly for its green shelled and dry seeds and young tender leaves are often used as fresh vegetables (Fivawo and Nchimbi-Msolla, 2011).

The fresh form of grain is the most preferred due its fresh flavour, good taste, and it requires little time to cook (approximately 40 minutes). However, fresh beans are difficult to keep, and as such they are consumed for a short time only in season before they dry (Katungi *et al.*, 2009).

Latin America is the largest producer, with 5.5 million metric tons, with Brazil and Mexico being by far the major producers. Africa is the second most important region, producing about 2.5 million metric tons, with Uganda, Kenya, Rwanda, Burundi, Tanzania, and Congo playing major roles. Main producers in Tanzania are the Northern Zone (particularly Arusha, Kilimanjaro and Manyara regions), the Great Lakes region in the West (Kigoma) and the Southern Highlands (Mbeya and Ruvuma). Tanzania's bean production is constrained by several biotic and abiotic factors; most important ones are

diseases, insect pests, low soil fertility and periodic water stress in the lowlands (Mwang'ombe *et al.*, 2007).

#### 1.2 Justification

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume in human diets. It provides protein, carbohydrates and valuable nutrients for more than 300 million people in the tropics. It is estimated that over 75 % of rural households in Tanzania depend on it for daily dietary requirements (CIAT, 2008). Despite its importance, bean yield in developing countries like Tanzania is among the lowest in the world with average of 0.717 t/ha (FAO, 2010) while under optimal management the yield of common bean ranges from 2 - 3 t/ha (Kanyeka *et al.*, 2007). Among the constraints to bean productivity are diseases like Angular leaf spot (ALS), Common bacterial blight (CBB), Bean common mosaic virus (BCMV)/Bean common mosaic necrosis virus (BCMNV) and others causing quality and yield losses of 20 - 100 % of the common bean worldwide to a susceptible variety.

Smallholder common bean farmers mainly rely on pesticides application to reduce production and post-harvest losses associated with diseases and insect pests (Wasonga *et al.*, 2010) and some do not apply at all. Chemicals increase the cost of production and negative effect on environment and human health (Burkett-Cartena *et al.*, 2008). The use of cultivars possessing multiple disease resistance with desirable agronomic characters is the most adoptable, requires few inputs, environment-friendly and is sustainable method for smallholder farmers. Selecting common bean genotypes for multiple disease resistance (MDR) provide better protection than a single disease resistance (Fininsa and Tefera, 2006). Remarkable efforts have been made so far to improve bean production by developing and releasing improved varieties like SUA 90, Rojo, Selian 97 which are still

susceptible to the pathogens which keep on changing to new races. Development of cultivars which have multiple resistance to diseases is preferably a better approach rather than resistance to a single disease. Therefore this study aims to evaluate the performance of the advanced common bean lines on both disease resistance and yield in different environments (G x E).

#### 1.3 Objectives

#### 1.3.1 Overall objective

To determine Genotype x Environment (G x E) effects on disease severity, yield and yield components on advanced common bean lines containing resistance to BCMV, BCMNV, ALS and CBB.

#### 1.3.2 Specific objectives

- To identify common bean lines that is resistant to BCMV, BCMNV, ALS and CBB by using molecular markers.
- ii. To determine G x E interaction on diseases, yield and yield components on 28 advanced common bean genotypes.
- iii. To determine the severity of diseases (BCMV, BCMNV, ALS and CBB) on 28 advanced common bean genotypes under field conditions.
- iv. To determine yield and yield components levels on 28 advanced bean lines.

#### **CHAPTER TWO**

#### 2.0 LITERATURE REVIEW

#### 2.1 Economic Importance of Diseases

Angular leaf spot (*Phaeoisareopsis griseola*) causes a yield losses of up to 80 % to susceptible varieties and under severe conditions of infection (Stenglein *et al.*, 2003). Losses due to ALS result from premature defoliation that mainly occurs at the beginning of the flowering and pod-filling stage (Mwang'ombe *et al.*, 1994). The disease significantly reduces the number of seeds per pod as well as seed weight. Yield losses caused by CBB incited by *Xanthomonas axonopodis pv. phaseoli (XAP)* vary from place to place. In Colombia yield losses has been estimated to be 22 % by natural infection and 45 % by artificial infections (Yoshii *et al.*, 1976). Wallen and Jackson (1975) reported losses of 38 % in Ontario Canada. For BCMV, field infections have been reported as high as 100 percent in susceptible bean varieties. Yield losses differ, depending on varieties, environment and time of infection, and whether the disease originates as a seed-borne infection or is carried in later by aphids (Mukeshimana *et al.*, 2003) but BCMNV cause the hypersensitive reaction to the plants with *I* gene resulting to death of the whole plant causing a loss of up to 100 percent.

#### 2.2 Symptomatology

#### 2.2.1 Angular leaf spot

Angular leaf spot is characterized by all aerial plant parts, including leaves, petioles, stems and pods being infected but symptoms are most recognizable on leaves (Mndolwa, 2008). Lesions on leaves appear as brown spots with a silvery appearance which are initially confined to tissues between major veins, which gives it an angular appearance. Lesions on stems and petioles appear dark brown and elongated but on pods appear

circular, black and sunken. Under severe infection, lesions coalesce and premature defoliation occurs. On pods, symptoms appear as large circular to ovoid reddish-brown lesions with a slightly darker perimeter. Infection may spread to the underlying seeds, which then become discoloured and malformed.

#### 2.2.2 Common bean bacterial blight

Common bean bacterial blight symptoms exhibit a burned appearance on leaf tissue and contain water-soaked spots. These small, irregularly shaped lesions often enlarge and form dark brown lesions along the edge of the leaflet. A narrow lemon-yellow margin often surrounds these lesions and large portions of the foliage can be infected (Schwartz, 2011). Infected pods exhibit circular, water-soaked areas that often produce yellow masses of bacterial ooze. Pod infection often causes discoloration, shriveling and bacterial contamination of seeds; however some seeds may appear healthy.

#### 2.2.3 Bean common mosaic virus and Bean common mosaic necrosis virus

Symptoms produced by both viral species are very similar. The representative symptoms seen on susceptible plants include mottling, curling and disfigured leaves, accompanied with stunting of the plants. Some BCMV strains cause hypersensitive reaction to cultivars possessing the dominant resistant gene (*I* gene), resulting to black root disease (Mukeshimana *et al.*, 2003). Black root begins as small, red-brown spots that expand into a dead tissue leading to death of plant. The death of plants as a result of the hypersensitive reaction prevents the plants from serving as a source of inoculum or infected seed for the next generation.

#### 2.3 Diseases Control

#### 2.3.1 Common bean bacterial blight

The best control method of bacterial bean diseases is prevention. Only certified, disease-free seeds should be planted. The chance of obtaining seed-borne bacteria can be significantly reduced by using certified bean seed produced in arid climates. Since moisture is required by these pathogens for reproduction and spread, seeds are more likely to become contaminated with bacteria when seed production fields have been exposed to summer thunderstorms, rains, and overhead irrigation (Dillard and Legard, 1991).

Chemical control is an important option in the management of bean diseases because of wide spread occurrence of these foliar diseases and the susceptibility of the available cultivars (Jesus *et al.*, 2004). Seed treatment with the antibiotic streptomycin can help to reduce contamination of the surface of the seed coat. Streptomycin seed treatment do not provide 100 % control, but it has been very effective against surface contamination. Copper-based bactericides reduce epiphytic populations of bacterial pathogens on bean foliage, and also reduce disease severity when applied as a preventative measure. However, these compounds cannot eradicate the pathogens once the plants are infected. If wet weather is persistent, bacterial populations can increase rapidly and are difficult to control unless several applications of copper-based bactericides are made (Dillard and Legard, 1991).

#### 2.3.2 Angular leaf spot

Application of fungicides to the bean plants may be beneficial if applied early. Fungicides can be applied during early bloom (10 - 30 % flowering) when environmental conditions are conducive for disease spread (Mndolwa, 2008). Second fungicide application seven

days later at late bloom (50 - 70 % flowering) if environmental conditions favouring infection and disease development occur between early bloom and late bloom, or are predicted after late bloom. Always it is recommended to read and follow the fungicide label before applying (Celetti, *et al.*, 2006).

Genetic resistance is considered most appropriate, safe and cost effective for small scale bean growers. Choice of bean genotypes resistant to ALS is important for improvement of common bean production in terms of both quality and quantity. Thus breeding for high resistance levels to ALS is important (Tryphone *et al.*, 2012). The introgression of resistant genes in locally adapted cultivars optimizes resistance levels of the variety since the mode of gene action and inheritance is subject to the background used. The resistance sources identified in the inter-specific crosses between *P. vulgaris* and *P. coccineus/P. polyanthus* have potential for durable ALS resistance and these genes must be sufficiently characterized (Mahuku *et al.*, 2003).

#### 2.3.3 Bean common mosaic virus and bean common mosaic necrosis virus

These are seed borne virus which also can be spread with aphids. Chemical control of the aphid vector is ineffective but the most effective control is to plant resistant varieties. Resistant varieties with the dominant *I* gene can be used against BCMV. Some varieties possess recessive genes like *bc-1*, *bc-2*, *bc-3* which impart resistance to both BCMV and BCMNV. Combination of these recessive genes with the dorminant *I* gene in new bean varieties is the appropriate way to achieve a long-lasting resistance against a large number of strains of both BCMV and BCMNV (Mukeshimana *et al.*, 2003). The use of clean, virus-free seed is critical if there is no choice of resistant varieties.

#### 2.4 Breeding for Multiple Disease Resistance

Genetic resistance is considered as the most appropriate, safe and cost effective for small scale bean growers to control diseases in common beans. Selection of common bean genotypes which possess MDR is important for raising bean production in terms of quality and quantity (Mahuku et al., 2009). Host plant resistance is simple to be used with the small holder farmers due to their application which does not require any other expertise in handling of the seeds. There are genes found to impart resistance against the diseases (BCMV/BCMNV, ALS and CBB). The dominant I and recessive bc-2, bc-3 genes confer resistance to both BCMV and BCMNV. These genes can be obtained from UBR (95)25, Mshindi and MCM5001. The dominant phg-2 gene confer angular leaf spot resistance. The genes can be obtained from Mexico 54. Common bean bacterial blight resistance genes or QTL obtained from interspecific crosses between P. vulgaris with P. coccineus and P. vulgaris with P. acutifolius respectively. Breeders transfer a target allele from a donor variety to a popular cultivar by a repetitive backcrossing (Joshi and Nayak, 2010). Multiple disease resistance breeding has been done in Kenya to produce genotypes that are resistant to angular leaf spot (Phaeoisareopsis griseola), anthracnose (Collectotrichum lindemuthianum) and rust (Uromyces appendiculatus). This study was carried out in two different locations in Kenya. Among the advanced lines, two bush lines (KSB 10 W and KSB 10 BR), and one climbing line (HAV 130) had consistent multiple resistance to angular leaf spot, anthracnose and rust at both locations. The multiple disease resistant lines were not the highest yielders but had the highest number of pods per plant (Wahome et al., 2011). Finisa and Tefera (2006) evaluated 201 genotypes from International Center for Tropical Agriculture (CIAT) to see their response against anthracnose, angular leaf spot and common bacterial blight. Out of 201 genotypes planted in 1996, 171 genotypes were found resistant to anthracnose, 117 to ALS, and 161 to CBB but 26 genotypes (13 %) were commonly resistant to all the three diseases. The

aforementioned 26 genotypes were re-evaluated in the next year where only 10 genotypes were resistant to all the three diseases.

#### 2.5 Molecular Markers Associated with Disease Resistance

Identification of plants carrying two or more resistance alleles of different genes using standard inoculation test is sometimes impractical because several races would be needed to screen for specific alleles (Yu *et al.*, 2000). In conventional breeding, breeders rely on visual screening of genotypes to select traits of their interest. However, successful application of this method depends on its reproducibility and heritability of the trait. Marker assisted selection has improved the accuracy of identifying the introgression lines or individuals that carry genes of interest has allowed breeders to produce germplasm with combined traits within a short time.

#### 2.5.1 Angular leaf spot

Resistance genes against *Phaeoisariopsis griseola* the causal agent of ALS are controlled by major genes, that are either dominant or recessive, acting singly or duplicated and which may interact in an additive manner with or without epistasis (Mahuku *et al.*, 2003). Resistance genes are *phg*-1 and *phg*-2. The breeders have identified molecular markers linked to angular leaf spot resistance genes in beans. SCAR markers for selecting genes for resistance to ALS include SH13 linked to *phg*-1 gene (Queiroz *et al.*, 2004) and SNO2 was found to be linked to *phg*-2 gene in Mexico 54 (Sartorato *et al.*, 2000).

#### 2.5.2 Common bean bacterial blight

Common bacterial blight (CBB) disease is a seed-borne in nature. The disease resistance is controlled by many minor genes (QTL) with varying degree of action. Resistance of

CBB has been obtained from *Phaseolus acutifolius* and *Phaseolus coccineus* and common bean lines Vax 1, Vax 2, Vax 3 and XAN159 developed by CIAT. There are SCAR markers used for breeding of CBB resistant varieties, these include SU91, BC420 (Yu *et al.*, 2000), SAP6 (Miklas *et al.*, 2000), BAC6 (Jung *et al.*, 1999) and R7313 (Beattie *et al.*, 1998).

#### 2.5.3 Bean common mosaic virus and Bean common mosaic necrosis virus

Resistance to BCMNV is conditioned by independent multi-allelic loci in common bean which is affected by four different loci: *bc*-1, *bc*-2, *bc*-3 and *bc*-u. Resistance controlled by alleles at these loci is inherited as recessive traits. Other common bean plants have dominant *I* gene that confers resistance to BCMV of which when infected with BCMNV cause hypersensitive reaction or black root. *I* gene is linked to SW13 (Melotto *et al.*, 1996), *bc*-3 is linked to ROC11 (Johnson *et al.*, 1997). Bean breeders are looking for combination of the dominant *I* gene with recessive *bc* gene that offer the broad spectrum resistance to BCMV and BCMNV, since the two types of genes have different mechanisms of resistance (Kelly, 1997).

#### 2.6 Importance of Genotype x Environment Interaction

When different genotypes are grown at different locations, their performance might not be the same. One genotype may perform well in some environments and surpass others. Relative performance of genotypes exposed to different environments is called Genotype x Environment (G x E) interaction. The main goal when growing crops everywhere is to maximize net profit by increasing seed yields (Alghamdi, 2004). Currently, the study of G x E interaction has assumed great importance in the variety testing programs. The seed yield performance of a genotype is a result of its interaction with environmental factors such as rainfall, temperature, fertility status and soil characteristics that play an important

role in varietal performance. The variation in environmental factors across the locations influence the yielding ability of the varieties therefore it is difficult to establish the superiority of a cultivar across environments (Aslam *et al.*, 1989).

In a predictable environment (i.e. controllable variables such as fertilization, sowing dates and harvesting methods, spacing), a high level of G x E interaction is desirable to ensure a maximum yield and financial return (Tahir *et al.*, 2013). In case of unpredictable environment (distribution of rainfall, relative humidity and prevailing temperature), low level of G x E interaction is required for uniform performance over a number of locations and years (Khan, 1981). Breeders prefer high yielding genotypes which have low G x E interaction and which are stable to many environments.

#### 2.7 Stability Analysis

The ability of some crop varieties to perform well over a wide range of environmental conditions has long been appreciated by the agronomist and plant breeder. In the cereal belts of southern Australia general adaptability has proved to be of particular importance, because edaphic variation between localities and the seasonal variation in any one locality are very great (Finlay and Wilkinson, 1963). There are cultivars adapted to a wide range of environment while others are more limited in their potential distribution. The cultivars that perform equally in number of environments regardless of the productivity of environment (stable cultivars), others whose performance is influenced by environment (unstable). The regression procedure is used to describe the adaptation response of individual grown over a range of environments to assess adaptation and yield performance. The mean yield of varieties at each site and for each season (hereafter referred to as "site means") provides a numerical grading of sites and seasons and, it is suggested, a useful evaluation of environment (Finlay and Wilkinson, 1963).

Varieties characterized by regression coefficient, approximating to 1.0 have average stability over all environments. For example, the variety shows average stability, with a linear regression coefficient (b) of 0.90 (it can produce above-average yields in all seasons at all sites, then it has general adaptability). On the other hand a regression coefficient approximating 1.0 (b = 1.05) produce below-average yields (It is poorly adapted to all environments). Variety which is sensitive to changes in the environment (below average stability); where small changes in the environment produce large changes in yield. This variety yields very little grain in a low-yielding environment, but as the environment improves, thus favouring higher yields. Under the most favourable conditions it becomes one of the highest-yielding varieties. This is described as being specifically adapted to high-yielding environments, and is characterized by a regression coefficient significantly greater than 1.0 (b = 2.13). Eberhat and Rusell (1966) used the regression model to study the stability of variety in an experiment on an environmental index. According to this model a stable genotype should have a high mean yield, b = 1.0and  $S_{di}^2 = 0$ . It is however specifically the deviation from the regression ( $S_{di}^2$ ) which is used as a measure of a genotype's stability across environments. The model used was:  $Y_{ij} = \mu_i + \beta_i I_j + \delta_{ij} (S^2_{di})$  (1) where  $Y_{ij}$  is the variety mean of the  $i^{th}$  variety at the  $j^{th}$  environment,  $\mu$  is the mean of the  $i^{th}$  variety over all environments,  $\beta$  is the regression coefficient that measures the response of the  $i^{th}$  variety to varying environments,  $\delta_{ij}$  is the deviation from regression of the  $i^{th}$ variety at the jth environment, and Ii is the environmental index obtained as the mean of

all varieties at the j<sup>th</sup> environment.

#### **CHAPTER THREE**

#### 3.0 MATERIALS AND METHODS

#### 3.1 Materials

#### 3.1.1 Source of resistance

Genotypes used for introgression of resistant genes were Vax-3 for CBB, Mshindi for BCMV/BCMNV, Mexico 54 for ALS and Kablanketi a preferred variety (PV)

#### 3.1.2 Generation of the materials

Crosses were made among Mexico 54 and VAX-3 and Mshindi to produce F<sub>1</sub>. F<sub>1</sub> were crossed with PV Kablanketi followed by two backcrosses. Selfing has continued to BC<sub>2</sub>F<sub>6</sub> as per scheme below:

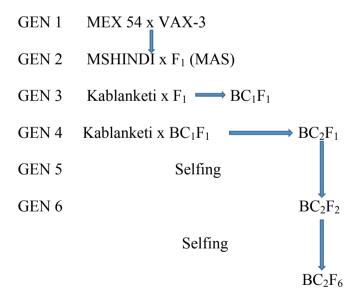


Figure 1: Crossing scheme

Plants (104) from single plant selection were used in the laboratory to select those with three to four genes by using molecular markers.

#### 3.1.3 Genotypes selected for trials

There were thirty two genotypes, twenty eighty (28) test lines and four (4) checks. These were KT 10-1a, KT 4b, KT 9-1a, KT 10-1b, KT 6-2a, KT 6-2b, KT 7d, KT 13-2d, KT 13-2a, KT 10-2c, KT 15a, KT 10-2a, KT 5a, KT 7a, KT10-2d, KT 2b, KT 9-2a, KT 6-2c, KT 11-2d, KT 13-1b, KT 1-2b, KT 13-2b, KT 9-2d, KT 1-2c, KT 6-1c, KT 9-2c, KT 6-2d, KT 11-1d, Vax 3 (CBB resistant check), Mexico 54 (ALS resistant check), Mshindi (BCMV resistant check) and Kablanketi (susceptible check).

#### 3.2 Methods

### 3.2.1 To identify common bean lines that are resistant to diseases (ALS, CBB,

#### BCMV and BCMNV) by using molecular markers

Bean seeds were planted in the plastic containers with sterilized soil. Leaf samples for DNA extraction were collected from the plants which were 20 days old. These young leaves were used for DNA preparation. The leaves were ground using hand held mortar that fits into the 1.5 ml Eppendorf tube with 300 µl of TES for 4 minutes. DNA extraction was done following Mahuku *et al.* (2004) protocol.

PCR amplification was done by using the SNO2, SAP6, ROC11 and SW13 primers for ALS, CBB, BCMNV and BCMV respectively. The PCR water (18μl), 1μl of forward primer, 1 μl of reverse primer and 20 ng of DNA was put in the PCR premix tubes which consisted of 1U of Taq polymerase, 250 μM of dNTPs, 10 mMTris-HCl (pH 9.0), 30 mMKCl and 1.5 mM MgCl<sub>2</sub> together with tracking dye. One PCR reaction had a total 20 μl and PCR conditions were as per Miklas (2010).

For electrophoresis, 1.2 % of gel was prepared by mixing 4.5 g of Agarose and 350 mls of 1xTBE. This mixture was autoclaved for 8 minutes and left to cool at room temperature.

Electrophoretic conditions were 120 V for 2 hours. Gel was stained in the ethidium bromide for 30 minutes and UV transluminator was used to visualize the separation of DNA. Pictures were captured by using a digital camera.

## 3.2.2 To determine the severity of diseases, yield and yield component levels of advanced lines

The study was conducted in three locations, Arusha (ARI Selian), Morogoro (SUA) and Mbeya (ARI-Uyole). The criteria used to classify environments of bean areas were those of Wortman *et al.* (1998), based on three altitudes namely: low altitude <1000 m. a. s. l (Morogoro), medium altitude 1001-1500 m.a.s.l (Arusha) and high altitude > 1500 m.a.s.l (Mbeya). Soil samples were collected from each site before land preparation for analysis. Soil data collected included soil N, P, K, pH, CEC and physical characteristic (Table 1). Experimental area was disc ploughed and harrowed by a tractor. The experiment was laid as split plot in RCBD with three replications. Locations (3) were the main-plot factor and the common bean genotypes (32) were sub plot factor. Thirty two genotypes were sown at a spacing of 50 cm x 20 cm and length of each row was 2 m. The spreader (Canadian Wonder) was sown after every 4 rows of test lines and one seed was placed in each of the ten hills per row. Phosphatic fertilizer (DAP) was applied during planting at the rate of 40 kg P/ha. Other agronomic practices like weeding was done two times by hand hoe. Climatic data like annual rainfall, temperature and relative humidity for the respective cropping season were collected (Table 2).

Table 1: Soil analysis results

			Experimental	sites		
Parameters analyzed before planting	Mbeya	Rating	Morogoro	Ratings	Arusha	Ratings
pH in water (1:2.5)	6.12	Slightly acidic	6.36	Slightly acidic	6.83	Neutral
Total N(%)	0.15	Medium	0.17	Medium	0.15	Medium
Bray-1-P(mg/kg)	4.73	Very low	3.68	Very low	17.12	Medium
CEC (cmol/kg	19.80	Medium	18.40	Medium	32.60	High
Exchangeable K(cmol/kg Particle size analysis	8.95	Very high	1.71	High	3.50	Very high
Clay (%)	32		53		28	
Silt (%)	25		4		17	
Sand (%)	43		49		55	
Soli textural class	Clay		Clay		Sand	
					clay	

Table 2: Means of monthly temperature, total rainfall and RH in three locations for respective cropping seasons

					Locat	ions				
	Mbeya				Morog	oro		Arusha		
Months	ths Mean				Mea	n		Mean		
	DII	Temp	Rainfall	DII	Temp	Rainfall	RH	Temp	Rainfall	
	RH	(°C)	(mm)	RH	(°C)	(mm)	КП	(°C)	(mm)	
March	70	19	136	58	27	111	79	21	160.3	
April	74	18	305	76	25	182	85	21	294.5	
May	59	15	92	65	25	59	90	20	43.6	
June	50	16	0	58	24	5	86	19	10.2	
July	40	17	0	53	23	0	83	18	0	
August	42	17	0	54	23	4	72	19	0	

#### 3.2.3 Data collection

Diseases (CBB and ALS) were scored based on a 1-9 severity scale according to Van Schoonhoven and Pastor-Corrales (1987). Sample of five plants were used for data

collection. The plants were randomly selected and marked with plastic tape. Diseases severity was assessed by scoring three trifoliate leaves sampled at bottom, middle and top of each plant. Disease scores were recorded every two weeks until maturity starting from 25 days of sowing. Mean disease scores were calculated for each plant and used to determine the level of reaction of genotype to the pathogens. Genotypes showing a disease severity rating of 1 - 3 were regarded as resistant, 4 - 6 as intermediate and 7 - 9 as susceptible. Rating of BCMV/BCMNV based on presence and absence of disease symptoms and the score of 1 and 0 were for the presence and absence of the disease respectively (Chilagane *et al.*, 2013). Yield and yield components data included days to 50 % flowering and days to 85 % maturity which were assessed basing on the whole plot, while the number of pods per plant, number of seeds per pod were collected from five plants. Weight of seeds per plant and 100 seed weight were collected from ten plants per genotype.

#### 3.2.4 Data analysis

Analysis of Variance (ANOVA) was performed for all data including days to 50 % flowering, days to 85 % maturity, number of pods per plant, number of seeds per pod, weight of seeds per plant, weight of 100 seeds were analyzed by using the GenStat statistical package  $15^{th}$  edition at p  $\leq$  0.05. Two way ANOVA models were used for single site and combined analysis respectively (Appendix 1). Treatments means were separated by using Duncan's multiple range test.

#### 3.2.5 Stability analysis

Finlay and Wilkinson (1963) model was used to determine the regression coefficient by regressing variety mean on the environmental mean, and plotting the obtained genotype regression coefficients against the genotype mean yields. Varieties characterized by

regression coefficient (b), approximating to 1.0 have average stability over all environments and those with b > 1 are suitable for favourable environment. The regression model used is in Appendix 2.

#### **CHAPTER FOUR**

#### 4.0 RESULTS

4.1 Molecular Marker Analysis for Angular Leaf Spot, Common Bean Blight,

Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus

The results shows the lines analysed for the presence of marker were variable. Some lines had only one marker, some had a combination of two (2) or three (3) genes for resistances. Most of the lines had genes for CBB and ALS but lines with BCMV and BCMNV were few. There were nine lines with combination of three resistance genes, these are important lines for advancing and phenotyping. The lines with combinations of two resistance genes are important for future breeding program and can be recombined to have lines with all resistance genes.

Table 3: Results of Molecular marker analysis

Gel Lane	Genotype	SAP6 - CBB	ROC11 – BCMNV	SN02 – ALS	SW13 – BCMV	Total genes
6	KT 1 – 2b	1	0	1	0	2
7	KT 1 – 2c	1	1	1	0	3
10	KT 2b	1	0	1	0	2
17	KT 4b	1	0	1	0	2
20	KT 5a	1	0	1	1	3
26	KT 6 -1c	1	0	1	1	3
28	KT 6-2a	1	0	1	0	2
29	KT 6-2b	1	0	1	0	2
30	KT 6-2c	1	0	1	0	2
31	KT 6-2d	1	0	1	0	2
32	KT 7a	1	0	1	0	2
35	KT 7d	1	0	1	1	3
40	KT 9-1a	1	0	1	1	3
44	KT 9-2a	1	0	1	1	3
45	KT 9-2b	1	0	0	0	1
46	KT 9-2c	1	0	1	0	2
47	KT 9-2d	1	0	1	0	2
48	KT 10-1a	1	0	0	0	1
49	KT 10-1b	0	0	1	1	2
52	KT 10-2a	1	0	1	1	3
54	KT 10-2c	1	0	1	1	3
55	KT 10-2d	1	0	1	0	2
59	KT 11-1d	1	0	1	0	2
63	KT 11-2d	0	0	1	0	1
69	KT 13-1b	0	0	1	1	2
75	KT 13-2d	0	1	1	0	2
80	KT 15a	1	0	1	0	2
94	KT 4g-3c	1	0	0	0	1_

Key: 1= present, 0= absent

#### 4.2 Analysis of Variance (ANOVA) for Field Experiments

There were significant differences among genotypes and among locations for all variables except for the CBB that did show any the significant difference basing on genotypes. Genotype x Environment was significant for days to 85 % maturity, number of pods per plant, weight of seeds per plant, weight of 100 seeds and yield only as shown in the Table 4.

Table 4: Combined ANOVA table showing the mean square for all variables

Source of variance	df	ALS	СВВ	50% flowering	85% maturity	Pods/plant	Seed/pod	weight of seed/plant	100seed weight	Yield(t/ha)
Replication	2	0.2118	0.4479	0.8576	0.332	0.94	0.2739	2.11	14.216	0.037
Genotype	31	3.1753***	0.6083 ns	28.5479***	34.443***	131.49***	1.3271***	186.39***	113.962***	1.26782***
Environments	2	31.6076***	40.5938***	394.2951***	5549.917***	5069.4***	81.0631***	15681.61***	749.037***	139.51157***
GxE	62	0.6506 ns	0.6009 ns	0.8507 ns	5.471***	23.16***	0.3874ns	52.93***	16.54***	0.34044***
Error	190	0.7908	0.4935	0.9278	2.658	11.65	0.3020	11.40	6.480	0.08358

<sup>\*\*\* =</sup>Significant at 0.001 and ns= not-significant

### 4.3 Diseases Severity

#### 4.3.1 Angular leaf spot

There were significant differences at  $p \le 0.05$  for ALS scores among genotypes and locations except for G x E interaction in all locations (Table 4). The results from Morogoro showed the disease scores ranged from 1.0 (Mexico 54) to 4.0 (KT 9-1a and KT 9-2d). Among the lines KT 4g-3c and KT 9-2a had the lowest scores (1.3 and 1.7 respectively). In Arusha, the lowest score was recorded in Mexico 54 (1.0) and the highest in KT 9-2c (4.7) (Table 5). Among the lines KT 10-1b and KT 9-2a scored the lowest (3.3). In Mbeya the score ranged from 1.0 (Mexico 54) to 5.7 (Kablanketi). Among the lines KT 6-1cand KT 1-2b had the lowest score (2.7) while the rest had scores between 3.0 to 4.3. Location wise, in Arusha the genotypes had the highest score (3.8) followed by Mbeya (3.5) and lastly in Morogoro (2.7). Results for combined means for all locations showed Mexico 54 had the lowest score (1) and Kablanketi the susceptible check had the highest score (4.1). Among the lines KT 9-2a had the lowest score followed closely by KT 1-2b (2.7 and 2.8 respectively) as shown in Table 5. Plate 1 shows the typical symptoms of ALS in the field.

### 4.3.2 Common bean blight

There were significant differences at  $p \le 0.05$  for CBB scores among genotypes and G x E interaction but the significant differences were observed among locations (Table 4). The results from Morogoro shows the disease scores ranged from 1.3 (KT 6-2d, KT 15a and Vax 3) to 3.7 (KT 4b, KT 7d and Mshindi). Plate 2 shows the symptoms of CBB in the field. Among the genotypes, twenty five lines (78 %) had the score that ranged from 1.7 to 3.0. In Arusha, the lowest score (1.0) was recorded in 24 (86 %) genotypes and the highest was 2.3 (KT 9-2b) as shown in Table 5. Among the genotypes, twenty three lines (82 %) scored the lowest (1.0). In Mbeya the genotypes had the scores which ranged from

1.1 to 1.7. Twenty five (89 %) lines had the lowest score (1.0) while the rest had scores that ranged from 1.3 to 1.7. Location wise, in Morogoro the genotypes had the highest score (2.2) followed by Mbeya and Arusha (1.1). Results for combined means for all locations shows KT 6 - 2d and KT 15a had the lowest score (1.1) and KT 9 - 1a had the highest score (2.0). Among the lines, twenty seven (27) lines had scores that ranged from 1.2 to 1.9 as shown in Table 5. In general the disease scores for this disease were low in all locations.

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Plate 1: Symptoms of angular leaf spot



Plate 2: Symptoms of common bean blight

Table 5: Angular leaf spot and common bean blight diseases scores of 32 genotypes grown in Morogoro, Mbeya and Arusha in 2014

		A	LS			(	CBB	
Genotypes	Morogoro	Arusha	Mbeya	Combined mean	Morogoro	Arusha	Mbeya	Combined mean
KT 10-1a	3.3	4.0	3.7	3.7	2.3	1.0	1.0	1.4
KT 4b	3.0	4.0	4.0	3.7	3.7	1.0	1.0	1.9
KT 9-1a	4.0	4.3	3.3	3.9	3.3	1.3	1.3	2.0
KT 10-1b	2.7	3.3	4.0	3.3	1.7	1.0	1.0	1.2
KT 6-2a	3.7	4.3	4.0	4.0	2.7	1.0	1.0	1.6
KT 6-2b	3.0	4.0	4.3	3.8	2.3	1.0	1.0	1.4
KT 7d	3.3	3.7	3.7	3.6	3.7	1.0	1.0	1.9
Mshindi	1.7	3.3	3.7	2.9	3.7	1.0	1.0	1.9
KT 13-2d	2.7	3.7	3.3	3.2	1.7	1.0	1.0	1.2
KT 4g-3c	1.3	3.0	3.0	2.4	1.7	1.0	1.0	1.2
KT 10-2c	3.7	4.0	3.3	3.7	1.7	1.0	1.0	1.2
KT 15a	2.7	4.0	3.7	3.4	1.3	1.0	1.0	1.1
KT 10-2a	2.3	4.0	3.3	3.2	1.7	1.3	1.0	1.3
KT 5a	3.0	4.0	4.0	3.7	1.7	1.0	1.3	1.3
KT 7a	3.0	4.3	4.0	3.8	1.7	1.0	1.3	1.3
KT 10-2d	2.0	4.3	3.0	3.1	2.0	1.0	1.0	1.3
KT 2b	2.3	4.0	4.3	3.6	3.0	1.0	1.0	1.7
KT 9-2a	1.7	3.3	3.0	2.7	2.7	1.0	1.0	1.6
KT 6-2c	2.7	4.0	3.0	3.2	2.0	1.0	1.0	1.3
KT 11-2d	2.7	4.0	3.3	3.3	2.3	1.0	1.0	1.4
Kablanketi	2.7	4.0	5.7	4.1	2.0	1.7	1.7	1.8
KT 13-1b	2.3	4.0	4.0	3.4	2.0	1.0	1.3	1.4
KT 1-2b	2.0	3.7	2.7	2.8	2.0	1.7	1.3	1.7
KT 9-2b	2.0	3.7	3.3	3.0	2.3	2.3	1.0	1.9
KT 9-2d	4.0	4.0	4.0	4.0	1.7	1.0	1.3	1.3
KT 1-2c	3.0	3.7	3.7	3.4	3.0	1.0	1.0	1.7
KT 6-1c	2.3	4.0	2.7	3.0	2.0	1.0	1.0	1.3
Vax 3	2.3	3.7	3.0	3.0	1.3	1.3	1.0	1.2
Mexico 54	1.0	1.0	1.0	1.0	3.0	1.7	1.0	1.9
KT 9-2c	3.7	4.7	3.7	4.0	2.3	1.3	1.0	1.6
KT 6-2d	3.3	3.7	3.0	3.3	1.3	1.0	1.0	1.1
KT 11-1d	3.0	4.0	4.0	3.7	2.0	1.0	1.0	1.3
Grand mean	2.7a	3.8c	3.5b	3.3	2.2b	1.1a	1.1a	1.5
L.S.D	1.824	0.9662	1.374	1.4322	1.073	0.7492	0.5242	1.1314
S.E	1.118	0.592	0.8418	0.8892	1.751	0.459	0.3212	0.7025
CV (%)	41.04	15.6	23.9	26.6	47.9	40.1	29.6	47.2

# 4.3.3 Bean common mosaic virus/bean common mosaic necrosis virus severity

Reaction of genotypes to BCMV was generally low in all locations. For Morogoro, there were 8 genotypes (25 %) infected with BCMV. Among the 28 lines, seven lines (25 %) of advanced lines had BCMV symptoms. In both Arusha and Mbeya only 7 % (two lines) were found to have typical symptoms of BCMV (Plate 3).



Plate 3: Symptoms of bean common mosaic virus in Morogoro

Table 6: Bean common mosaic virus/bean common mosaic necrosis virus severity of 32 genotypes grown in Morogoro, Mbeya and Arusha in 2014

Comotymos		Locations	
Genotypes	Morogoro	Arusha	Mbeya
KT 10-1a	0	0	0
KT 4b	1	0	0
KT 9-1a	0	0	0
KT 10-1b	1	0	0
KT 6-2a	0	0	0
KT 6-2b	1	1	0
KT 7d	0	0	0
Mshindi	0	0	0
KT 13-2d	1	0	0
KT 4g-3c	0	0	0
KT 10-2c	0	0	0
KT 15a	0	0	0
KT 10-2a	0	0	0
KT 5a	0	0	0
KT 7a	0	0	0
KT 10-2d	0	0	0
KT 2b	0	0	0
KT 9-2a	0	0	0
KT 6-2c	1	0	1
KT 11-2d	0	0	1
Kablanketi	0	0	0
KT 13-1b	0	0	0
KT 1-2b	0	0	0
KT 9-2b	0	0	0
KT 9-2d	0	0	0
KT 1-2c	1	0	0
KT 6-1c	1	1	0
Vax 3	0	0	0
Mexico 54	1	0	0
KT 9-2c	0	0	0
KT 6-2d	0	0	0
KT 11-1d	0	0	0

Key: 1 = present, 0 = absent

### 4.4 Days to 50 % Flowering

There were significant differences ( $p \le 0.05$ ) on days to 50 % flowering among genotypes and locations except for G x E interaction (Table 4). For Morogoro, genotype Mshindi had the lowest number of days (32) followed by KT 10-1a (34) and others ranged between 35 and 42 days (Table 7). Mexico 54 took the longest time (42) to reach days to 50 % flowering. In Arusha, the genotype Mshindi was the earliest to achieve 50 %

flowering while others took between 37 to 46 days and Mexico 54 being the latest (Table 7). In all the three locations genotype Mshindi and Mexico 54 had the shortest and longest time to reach days to 50 % flowering respectively. In Morogoro the genotypes flowered earlier than Mbeya and Arusha.

### 4.5 Number of Days to 85 % Maturity

There were significant differences (p  $\leq$  0.05) on number of days to 85 % maturity among genotypes, locations and G x E interaction (Table 4). For Morogoro, Mshindi (69) was the earliest maturing genotype and Mexico 54 (84) was the latest maturing genotype. In Mbeya, Mshindi took 89 days but some other genotypes required between 90 and 98. Mexico 54 was the latest with 102 days. In Arusha again Mshindi was the earliest (83) followed by Vax-3 (90) and Mexico 54 (98). Among the locations, in Morogoro the genotypes reached 85 % maturity earlier (77) compared to Arusha (88) and Mbeya (92). Similar to flowering results, the genotypes Mshindi (82) and Mexico 54 (95) were the earliest and latest maturing genotypes respectively in overall. Among the lines KT 7d was the earliest in 85 % days to maturity (Table 7).

Table 7: Phenological characteristics of 32 genotypes grown in Morogoro, Mbeya and Arusha in 2014

and Arusha in 2014								
Comotomos	Γ	Days to 50%	flowering		Days to 8:	5% physic	ological m	aturity
Genotypes	Morogoro	Mbeya	Arusha	Mean	Morogoro	Mbeya	Arusha	Mean
KT 10-1a	34	40	38	37	77	91	87	85
KT 4b	35	39	38	37	77	93	88	86
KT 9-1a	35	39	38	37	77	91	89	86
KT 10-1b	36	40	38	38	77	92	87	86
KT 6-2a	36	38	37	37	78	90	87	85
KT 6-2b	35	39	37	37	77	92	88	85
KT 7d	35	39	37	37	77	93	87	84
Mshindi	32	37	35	35	69	89	83	82
KT 13-2d	36	40	38	38	77	92	88	86
KT 4g-3c	35	38	38	37	77	93	89	86
KT 10-2c	35	38	37	37	77	93	89	86
KT 15a	35	39	37	37	77	92	85	85
KT 10-2a	35	39	37	37	77	93	89	86
KT 5a	35	39	37	37	77	91	87	85
KT 7a	35	39	38	37	77	91	87	85
KT 10-2d	35	39	38	37	77	92	89	86
KT 2b	35	40	38	37	77	92	89	86
KT 9-2a	35	40	38	38	78	92	89	86
KT 6-2c	35	39	38	37	78	91	86	85
KT 11-2d	36	39	38	37	78	91	89	86
Kablanketi	35	40	38	38	78	92	88	86
KT 13-1b	35	38	37	37	77	90	88	85
KT 1-2b	35	40	38	38	77	90	89	86
KT 9-2b	36	40	38	38	77	93	88	86
KT 9-2d	35	39	39	38	77	92	87	85
KT 1-2c	35	39	38	38	77	93	87	86
KT 6-1c	36	39	38	38	77	92	87	86
Vax 3	39	45	44	42	79	98	93	90
Mexico 54	42	48	46	45	84	102	98	95
KT 9-2c	36	39	38	38	78	90	87	85
KT 6-2d	35	39	38	37	78	91	87	85
KT 11-1d	36	39	38	37	78	91	87	85
Grand mean	35.5a	39.5c	38.1b	37.7	77.4a	92.1c	88.1b	85.9
L.S.D	1.3953	2.109	1.0124	1.5513	1.0764	3.223	3.109	2.626
S.E	0.8549	1.292	0.6203	0.9632	0.6588	1.975	1.905	1.6303
CV (%)	2.4	3.3	1.6	2.6	0.9	2.1	2.2	1.9

### 4.6 Number of Pods per Plant

There were significant differences (p  $\leq$  0.05) in number of pods per plant among genotypes, locations and G x E interaction as displayed in Table 4. In Mororgoro, the highest number of pods was observed in Mexico 54 (19) followed by Vax 3 (17), KT 6-2d (14), KT 6-2b and KT 6-1c (12). The lowest number of pods was observed in KT 10-2d (4). The remaining genotypes had 5 to 11 pods. For Mbeya, Mexico had the highest number of pods (44) followed by Vax 3 (31) and KT 9-2c (29). The remaining genotypes had between 16 and 27 pods. In Arusha, genotype Mexico 54 had the highest number of pods (37) followed by Vax 3 (25) while KT 10-2d (9) had the lowest number of pods. Among the lines (KT 10-1a, KT 10-1b, KT13-2d, KT 4g-3c, KT 15a and KT 6-2c) had the highest number of pods per plant (15). The remaining genotypes produced between 10 and 14 pods (Table 8).

Combined analysis of all the three locations revealed that, Mbeya had the genotypes with highest number of pods (24) followed by Arusha (14) and lastly Morogoro (9). The genotype Mexico 54 had the highest (33) number of pods in combined results for all the three locations followed by Vax3 (24) and KT 6-2d (18) the remaining ranged from 14 to 17 pods. The genotype that did not perform well was KT 10-2c and KT 11-1d each produced only 13 pods (Table 8).

Table 8: Number of pods per plant of 32 genotypes grown in Morogoro, Mbeya and Arusha in 2014

<i>C</i> 1		Locations		Combined
Genotypes	Morogoro	Mbeya	Arusha	Mear
KT 10-1a	9	27	15	17
KT 4b	6	21	14	14
KT 9-1a	9	22	14	15
KT 10-1b	9	18	15	14
KT 6-2a	6	21	14	14
KT 6-2b	12	25	12	16
KT 7d	8	23	13	1:
Mshindi	8	24	16	10
KT 13-2d	11	21	15	16
KT 4g-3c	11	17	15	14
KT 10-2c	5	21	13	13
KT 15a	10	21	15	10
KT 10-2a	9	23	14	10
KT 5a	7	25	13	1:
KT 7a	7	24	11	14
KT 10-2d	4	26	9	1:
KT 2b	10	21	10	1.
KT 9-2a	9	16	12	1:
KT 6-2c	10	26	15	1
KT 11-2d	10	19	14	1:
Kablanketi	10	25	9	1
KT 13-1b	11	22	11	1.
KT 1-2b	8	23	13	1-
KT 9-2b	7	23	13	1-
KT 9-2d	9	20	13	14
KT 1-2c	9	25	13	10
KT 6-1c	12	26	12	10
Vax 3	17	31	25	24
Mexico 54	19	44	37	3.
KT 9-2c	7	29	11	1
KT 6-2d	14	27	12	1
KT 11-1d	8	19	11	1:
Grand mean	9.4a	23.7c	14.0b	10
L.S.D	2.758	8.124	4.3	5.:
S.E	1.687	4.976	2.6	3.4
CV (%)	17.9	21	18.8	21.

# 4.7 Number of Seed per Pod

There were significant differences (p  $\leq$  0.05) in number of seeds per pods among genotypes, and locations except for G x E interaction (Table 4). In Morogoro, genotype Vax 3 was the best performer with four (4) seeds per pod followed by Mexico 54, KT 2b and KT 10-2c (Table 9). The rest had between 2 and 3 seeds per pod (Table 10). In Mbeya, the genotype Mexico 54 was the best with six (6) seeds followed by Vax 3 (5), KT 11-1d (5) and Mshindi (5). The remaining genotypes had four (4) seeds per pod. For Arusha, the genotype Vax 3 had the highest number (6) seeds per pod followed by Mexico 54 (5), KT 10-2a (5) and the rest had four (4) seeds per pod.

Location wise, Morogoro was statistically different from Arusha and Mbeya but Mbeya and Arusha were not significantly different. In both Mbeya and Arusha plants had four (4) seeds per pod followed by Morogoro which had three (3) seeds per pod (Table 9). Combined means showed that, the genotype Vax 3 had the highest number of seeds per pod (5) followed by Mexico 54 and the lowest number was three seeds (3) per pod which was found in three genotypes (KT 10-1b, KT 11-2d and KT 6-1c). Most of the genotypes had four (4) seeds per pod.

Table 9: Number of seeds per pod of 32 genotypes grown in Morogoro, Mbeya and Arusha in 2014

Comotomoo		Locations		Combined
Genotypes	Morogoro	Mbeya	Arusha	Mean
KT 10-1a	3	4	5	4
KT 4b	3	4	4	4
KT 9-1a	3	4	4	4
KT 10-1b	2	4	4	3
KT 6-2a	3	4	5	4
KT 6-2b	2	4	4	4
KT 7d	3	4	4	4
Mshindi	3	5	4	4
KT 13-2d	3	4	5	4
KT 4g-3c	3	5	4	4
KT 10-2c	4	4	4	4
KT 15a	3	4	5	4
KT 10-2a	3	4	5	4
KT 5a	3	4	4	4
KT 7a	3	4	4	4
KT 10-2d	3	5	4	4
KT 2b	4	5	4	4
KT 9-2a	3	4	4	4
KT 6-2c	3	4	4	4
KT 11-2d	2	4	4	3
Kablanketi	3	5	5	4
KT 13-1b	3	5	4	4
KT 1-2b	2	5	5	4
KT 9-2b	2	5	4	4
KT 9-2d	3	4	5	4
KT 1-2c	3	4	4	4
KT 6-1c	2	4	4	3
Vax 3	4	5	6	5
Mexico 54	4	6	5	5
KT 9-2c	2	4	5	4
KT 6-2d	2	4	5	4
KT 11-1d	3	5	4	4
Grand mean	2.8a	4.4b	4.b	4
L.S.D	0.9277	0.9	0.8	0.9
S.E	0.5676	0.6	0.5	0.5
CV (%)	20.1	13.2	10.4	14.2

### 4.8 Weight of Seed per Plant

There were significant differences (p  $\leq$  0.05) among genotypes, locations and G x E interaction at p  $\leq$  0.05 as shown in Table 4. The genotype Mexico 54 had the highest weight of seed per plant (16.7 g) followed by Vax 3 (12.4 g) and the lowest weight was from KT 5a (3.0 g) for Morogoro. Among the lines KT 4g-3c and KT 2b were the best in Morogoro. In Mbeya, genotype Mexico 54 performed better than the rest (51.9 g) followed by Kablanketi (44.6 g) and the lowest was from KT 6-1c (22.0 g). The genotype Mexico 54 was the best yielder (49.9 g) in Arusha followed by KT 13-2d (19.7 g) and the lowest was from KT 6-1c (Table 10).

Combined analysis showed that Mbeya had the highest seed weight per plant (31.5 g) followed with Arusha (15.1 g) and Morogoro (6.3 g). Also there were significant differences among genotypes based on combined means, the control Mexico 54 recorded the highest seed weight per plant (39.5 g), Kablanketi (23.6 g), KT 13-1b (20.3 g) and the lowest yield was recorded in KT 6-1c (12.6 g) as displayed in Table 10.

Table 10: Weight of seed per plant of 32 genotypes grown in Morogoro, Mbeya and

Arusha in 2014

Canatanas		Locations		Combined
Genotypes	Morogoro	Mbeya	Arusha	Mean
KT 10-1a	5.5	31.6	16.7	17.9
KT 4b	4.7	34.6	17.8	19.0
KT 9-1a	5.1	32.7	13.6	17.1
KT 10-1b	3.8	29.3	13.9	15.6
KT 6-2a	3.4	24.3	10.4	12.7
KT 6-2b	4.2	27.8	10.4	14.1
KT 7d	5.0	28.6	13.3	15.6
Mshindi	7.9	24.4	14.3	15.5
KT 13-2d	4.9	26.6	19.7	17.0
KT 4g-3c	10.7	31.8	13.1	18.5
KT 10-2c	4.8	31.2	13.3	16.4
KT 15a	6.5	27.8	17.9	17.4
KT 10-2a	4.1	31.4	16.0	17.2
KT 5a	3.0	32.2	14.6	16.6
KT 7a	5.5	32.7	12.4	16.9
KT 10-2d	3.0	42.9	10.7	18.9
KT 2b	10.7	26.6	15.3	17.5
KT 9-2a	5.2	27.3	14.1	15.5
KT 6-2c	5.7	30.7	16.1	17.5
KT 11-2d	5.2	24.2	15.0	14.8
Kablanketi	10.3	44.6	15.9	23.6
KT 13-1b	8.0	40.2	12.6	20.3
KT 1-2b	4.9	33.8	12.8	17.2
KT 9-2b	3.6	27.8	13.2	14.9
KT 9-2d	7.5	31.3	13.9	17.6
KT 1-2c	6.5	34.2	14.0	18.2
KT 6-1c	5.9	22.0	9.9	12.6
Vax 3	12.4	27.3	16.7	18.7
Mexico 54	16.7	51.9	49.9	39.5
KT 9-2c	3.2	36.6	12.8	17.5
KT 6-2d	7.7	35.5	12.4	18.5
KT 11-1d	7.3	23.9	10.1	13.7
Grand mean	6.3a	31.5c	15.1b	17.6
L.S.D	2.003	8.659	3.274	5.439
S.E	1.225	5.305	2.006	3.377
CV (%)	19.4	16.8	13.3	19.2

### 4.9 100 Seed Weight

There were significant differences among genotypes, locations and G x E interaction at p  $\leq 0.05$  (Table 4). In Morogoro genotype Kablanketi had the highest 100 seed weight (40.3 g), followed by KT 1-2b (36.7 g), KT 2b (36.2 g) while the lowest weight was recorded in Vax 3 (20.5 g). The genotype Kablanketi led (42.8 g) for 100 seed weight in Mbeya followed by KT 1-2b (42.5 g) and KT 9-2d (41.0 g) while Vax 3 recorded the lowest weight (17.6 g). Also in Arusha Kablanketi had the highest 100 seed weight (40.9 g) and the lowest 100 seed weight was recorded from Vax 3 (20.8 g) (Table 11).

Results from combined analysis showed that, Mbeya had the highest 100 seed weight (36.9 g) followed by Arusha (34.6 g) and Morogoro (31.4 g). Genotypes combined means were also significantly different ( $p \le 0.05$ ), the control Kablanketi was the one with highest weight (41.3 g) followed by KT 1-2b (38.4 g) and the lowest was recorded from Vax 3 (19.5 g).

Table 11: 100 seed weight of 32 genotypes grown in Morogoro, Mbeya and Arusha in 2014

C 1		Locations		Combined
Genotypes	Morogoro	Mbeya	Arusha	Mean
KT 10-1a	31.6	35.9	36.1	34.5
KT 4b	30.2	39.1	37.2	35.5
KT 9-1a	30.0	39.8	36.8	35.5
KT 10-1b	28.3	37.7	38.1	34.7
KT 6-2a	29.4	33.6	29.1	30.7
KT 6-2b	35.5	35.9	31.1	34.2
KT 7d	34.5	37.8	35.6	36.0
Mshindi	26.0	28.5	30.7	28.4
KT 13-2d	29.5	38.1	34.0	33.9
KT 4g-3c	33.1	38.0	34.3	35.2
KT 10-2c	30.0	38.0	36.5	34.8
KT 15a	32.9	37.4	34.4	34.9
KT 10-2a	28.8	36.2	39.0	34.7
KT 5a	29.7	37.5	35.3	34.2
KT 7a	34.3	40.9	34.1	36.4
KT 10-2d	33.2	39.0	33.2	35.2
KT 2b	36.2	39.7	37.7	37.9
KT 9-2a	33.1	36.7	37.9	35.9
KT 6-2c	29.6	33.9	31.5	31.7
KT 11-2d	31.5	39.0	33.3	34.6
Kablanketi	40.3	42.8	40.9	41.3
KT 13-1b	35.6	38.0	34.8	36.1
KT 1-2b	37.0	42.5	35.6	38.4
KT 9-2b	33.6	40.8	37.7	37.4
KT 9-2d	29.6	41.0	37.6	36.0
KT 1-2c	31.0	39.3	32.4	34.2
KT 6-1c	32.2	40.8	31.2	34.7
Vax 3	20.5	17.6	20.8	19.5
Mexico 54	30.2	31.6	36.6	32.8
KT 9-2c	30.1	35.7	37.5	34.5
KT 6-2d	29.8	33.1	33.3	32.1
KT 11-1d	28.0	36.8	33.8	32.9
Grand mean	31.4a	37.0c	34.6b	34.3
L.S.D	3.341	4.05	4.619	4.1
S.E	2.044	2.482	2.83	2.546
CV (%)	6.5	6.7	8.2	7.4

# 4.10 Yield (t ha<sup>-1</sup>)

There were significant differences at  $p \le 0.05$  on grain yield among the genotypes, locations and interaction between genotypes and locations (Table 4). For Morogoro, the genotype Mexico 54 had the highest yield (1.5 t/ha) followed by Vax 3 (1.1 t/ha), KT 4g-3c (1.0 t/ha) and the lowest was recorded in KT 5a (0.3 t/ha). For Mbeya, Mexico 54 had the highest yield (4.7 t/ha), Kablanketi (4.0 t/ha), KT 10-2d (3.9 t/ha) and KT 13-1b (3.6 t/ha) while the lowest was 2.0 t/ha (Table 12). For Arusha, the genotype Mexico 54 had highest yield compared to others followed by KT 10-1a, Vax 3 and the lowest yield was recorded in KT 6-1. Location wise, Mbeya had the highest yield, followed by Arusha lastly Morogoro (Table 12). Combined means of all the three locations showed the highest yield was from controls Mexico 54 (3.2 t/ha) followed by and Kablanketi 2.0 t/ha, KT 13-1b (1.8 t/ha) and Vax 3 (1.6 t/ha) and the lowest was obtained from KT 6-1c (1.0 t/ha). Yield for the remaining genotypes ranged from 1.0 to 1.6 t/ha. The combined mean yield was lowered by Morogoro where yield of the genotypes were very low compared to the other two locations due to floods (Plate 4).



Plate 4: Flooded Morogoro trial

Table 12: Yield (t/ha) of 32 genotypes grown in Morogoro, Mbeya and Arusha in 2014

Constance		Locations		Combined
Genotypes	Morogoro	Mbeya	Arusha	Mean
KT 10-1a	0.5	2.8	1.5	1.6
KT 4b	0.4	3.1	1.3	1.6
KT 9-1a	0.5	2.9	1.1	1.5
KT 10-1b	0.3	2.6	0.8	1.3
KT 6-2a	0.3	2.2	0.6	1.0
KT 6-2b	0.4	2.5	0.6	1.2
KT 7d	0.5	2.6	0.8	1.3
Mshindi	0.7	2.2	0.9	1.3
KT 13-2d	0.5	2.4	1.0	1.3
KT 4g-3c	1.0	2.9	0.8	1.5
KT 10-2c	0.4	2.8	0.7	1.3
KT 15a	0.7	2.5	0.9	1.4
KT 10-2a	0.4	2.8	0.9	1.4
KT 5a	0.3	2.9	0.8	1.3
KT 7a	0.5	2.9	1.0	1.5
KT 10-2d	0.3	3.9	0.7	1.6
KT 2b	1.0	2.4	1.0	1.5
KT 9-2a	0.5	2.5	1.0	1.3
KT 6-2c	0.5	2.8	1.0	1.4
KT 11-2d	0.5	2.2	0.9	1.2
Kablanketi	0.9	4.0	1.0	2.0
KT 13-1b	0.7	3.6	1.1	1.8
KT 1-2b	0.4	3.0	0.7	1.4
KT 9-2b	0.3	2.5	1.0	1.3
KT 9-2d	0.7	2.8	0.9	1.5
KT 1-2c	0.6	3.1	0.9	1.5
KT 6-1c	0.5	2.0	0.4	1.0
Vax 3	1.1	2.5	1.3	1.6
Mexico 54	1.5	4.7	3.3	3.2
KT 9-2c	0.3	3.3	0.8	1.5
KT 6-2d	0.7	3.2	0.9	1.6
KT 11-1d	0.7	2.1	0.8	1.2
Grand mean	0.6a	2.8c	1.0b	1.5
L.S.D	0.1984	0.779	0.116	0.466
S.E	0.1214	0.477	0.071	0.289
CV (%)	21.2	16.8	7.2	19.8

# 4.11 Correlation Analysis among Variables

For Mbeya, there were significant correlations at  $p \le 0.05$  in grain yield with days to 85 % maturity (r = 0.6891\*\*\*), number of pods per plant (r = 5178\*\*\*), number of seed per pod (r = 0.3267\*\*\*) and weight of seed per plant (r = 3516\*\*\*) as shown in Table 13. Results of correlation analysis for Morogoro shows there were significant correlation at  $p \le 0.05$  as shown in Table 13. Yield positively correlated with days to 50 % flowering (r = 0.45\*\*\*), days to 85 % maturity (r = 0.2885\*\*), number of pods per plant (r = 0.6826\*\*\*), number of seed per pod (r = 0.5602\*\*\*) and weight of seed per plant (r = 0.9675\*\*\*).

For Arusha, results shows that grain yield was positively correlated (p  $\leq$  0.05) with days to 50 % flowering (r = 7579\*\*\*), days to 85 % maturity (r = 0.5975\*\*\*), number of pods per plant (r = 7861\*\*\*), number of seed per pod (r = 3716\*\*\*) and weight of seed per plant (r = 0.9285\*\*\*). There was a negative correlation between yield and diseases (ALS and BCMV) and grain yield as shown in Table 13. Results of combined simple correlation analysis showed that there was positive correlation between days to 50% flowering(r = 0.6617\*\*\*), days to 85 % maturity(r = 0.726\*\*\*), number of pods per plant(r = 0.8447\*\*\*), number of seed per pod(r = 0.5141\*\*\*), weight of seed per plant (r = 0.9776\*\*\*), weight of 100 seeds (r = 0.4105\*\*\*) and grain yield as shown in Table 13.

Table 13: Summarized correlation coefficients of different character combinations across locations for 32 genotypes grown in 2014

		Locations		Combined
Character combinations	Mbeya	Morogoro	Arusha	locations
ALS vs grain yield	-0.168ns	-0.3253**	-0.5921***	0.0384ns
CBB score vs grain yield	0.1765ns	0.0532ns	0.1971ns	-0.3184***
BCMV score vs grain yield	-0.0478ns	0.1188ns	-0.2239*	-0.3027***
50 % flowering vs grain yield	0.3516***	0.45***	0.7579***	0.6617***
85 % maturity vs grain yield	0.1722ns	0.2885**	0.5975***	0.726***
Pods/plant vs grain yield	0.4916***	0.6826***	0.7861***	0.8447***
Seeds/pod vs grain yield	0.282**	0.5602***	0.3716***	0.5141***
Weight of seed/plant vs grain yield	1***	0.9675***	0.9285***	0.9776***
100 seed weight vs grain yield	0.1485ns	0.0466ns	0.1117ns	0.4105***

<sup>\* =</sup> significant at 0.05, \*\* = significant at 0.01, \*\*\* = Significant at 0.001 and ns = non-significant

### 4.12 Estimates of stability

Combined analysis of yield and yield components presented in Table 4 indicated the significant difference ( $p \le 0.001$ ) for G x E interaction for days to 85 % maturity, pods/plant, weight of seed/plant, 100 seed weight and grain yield. This indicated that there is a need for estimation of stability of genotypes across the environments. The mean seed yield and regression coefficient (b) of 32 genotypes were calculated but the presented are only the best 20 genotypes (Table 14). The regression coefficient (b) ranged from 0.6092 to 1.0668 showing the variation in genotypic performance across the environments.

The genotypes KT 10-1a (0.9433), KT 4g-3c (0.9299), KT 9-2d (0.9688), KT 9-1a (1.0652) and KT 7a (1.0668) had the regression coefficient (b) approximating to 1 (Table 14). These were the genotypes which produced above the overall mean yield (1.46) across all the three locations (Arusha, Mbeya and Morogoro). The genotypes Vax 3 had the lowest b value (0.6092) followed by Mshindi (0.6687), KT 2b (0.6704) and KT 11-1d

(0.6828). The genotype KT 10-1b had the b value of 1 but it produced below the average yield. Generally 62 % of genotypes had b value approximating to 1 and 38 % had the b greater than 1

Table 14: Estimates of Stability analysis parameter for seed yield of 32 genotypes grown in Morogoro, Mbeya and Arusha in 2014

Genotype	Mean	Sensitivity (b)
Vax 3	1.619	0.6092
Mshindi	1.271	0.6687
KT 2b	1.447	0.6704
KT 11-1d	1.203	0.6828
KT 6-1c	0.966	0.7119
KT 11-2d	1.180	0.7370
KT 13-2d	1.333	0.7742
KT 15a	1.369	0.8267
KT 9-2a	1.323	0.8381
KT 6-2a	1.031	0.8407
KT 9-2b	1.271	0.9203
KT 4g-3c	1.527	0.9299
KT 10-1a	1.617	0.9433
KT 7d	1.280	0.9461
KT 9-2d	1.477	0.9688
KT 6-2b	1.146	0.9737
KT 6-2c	1.429	0.9791
KT 10-1b	1.272	1.0002
KT 9-1a	1.498	1.0652
KT 7a	1.488	1.0668
Grand mean	1.46	
CV	19.8	
LSD	0.466	

#### **CHAPTER FIVE**

#### 5.0 DISCUSSION

#### **5.1** Disease Severity

Generally all the genotypes were found to be resistant to intermediate resistant to ALS as their score ranged from 1 - 4 (Van Schoonhoven and Pastor-Corrales, 1987). This observation correlates with the results of Marker Assisted Selection (MAS) using SNO2 which showed that 89 % of the genotypes had *phg-*2 gene for resistance. This shows the introgression of genes for resistance was successful. The susceptible check (Kablanketi) was found to be intermediate resistant and 11 % of the lines had no genes for resistance but were found to be resistant to intermediate, this could be disease escape. The variations in ALS reaction among locations can be due to the presence of strains that are not present in other locations and differences in altitude could have led to differences in ALS reactions. Morogoro had the lowest ALS score, suggesting that Morogoro had environment that is not conducive for many diseases. Fivawo and Nchimbi-Msolla (2012) reported the same results that there were variations in ALS reaction in the same three sites, Morogoro had the lowest overall mean ALS score followed by that at Arusha and Mbeya had a higher overall mean ALS score. The G x E interaction was not significant indicating that environment had no recognizable influence on genotype reaction to ALS.

For CBB, all the genotypes were found to be resistant, this suggested that they have the genes of CBB resistance. This was proven by MAS results which showed that 86 % of the lines had genes for resistance and it indicate that the introgression of genes for resistance was successful. The susceptible check (Kablanketi) was found to be resistant and the remaining lines (14 %) were found to be resistant to intermediate, this could be disease escape due to late planting that there was no conducive environment for the disease

progress. The highest score observed in Morogoro can be associated with presence of initial inoculum because beans were grown on the same site the previous season. Also in Morogoro there was high temperature (26 °C) during the cropping season. All these could have raised the reaction of common beans in Morogoro. Similarly Gilbertson and Maxwell (1992) reported very severe disease symptoms under high rainfall and humidity and warm temperature conditions (25 - 35 °C) with maximum development occurring around 28 °C. Additionally, factors such as heavy storms, planting of non-certified seed, equipment or irrigation water and method used for irrigation for example sprinkler contribute to enhancing disease problems.

The genotypes had different reaction to BCMV/BCMNV in Morogoro. Symptoms produced by both viral species are very similar like mosaic, stunt, chlorosis and leaf deformation but at higher temperatures systemic necrosis may be observed for BCMNV. Most of the genotypes infected with BCMV were found in Morogoro due to the high environmental temperature (Table 3) which support disease progress. Also Cadle-Davidson (2005) reported a severe mosaic, curling of the leaves, vein banding and mottled and malformed pods in susceptible genotypes at growing temperatures (26 - 28 <sup>0</sup>C). Additionally, there was a good source of inoculum from the previous beans planted on the same piece of land. Few genotypes were attacked with BCMV in Arusha and Mbeya showing that there were no favourable conditions for the outbreak of the disease. The highest temperature recordings were 19 °C and 21 °C for both Mbeya and Arusha respectively that was below the conducive temperature (26 - 28 °C). Marker assisted selection (MAS) results showed only 35 % of the 28 genotypes had BCMV resistance genes while phenotypic screening showed that 76 % of the genotypes did not show symptoms of disease across the three locations. This could be due to unfavourable environment for disease development. Resistance to different BCMV strains is controlled

by the dominant *I* gene and/or with combinations of several recessive genes (*bc*-u, *bc*-1, *bc*-12, *bc*-2, *bc*-22 and *bc*-3) (Strausbaugh *et al.*, 1999). The best combination is *I* and *bc*-3 gene where by recessive *bc*-genes control the virus multiplication in the infected tissue.

Generally, among the 28 genotypes evaluated 15 genotypes (53 %) showed to be resistant to all the three diseases (BCMV, ALS and CBB) indicating that the introgression of genes was successful. This result was encouraging due to big number of genotypes which had multiple disease resistances (MDR), the possible reason could be the use of MAS that helped to choose only those with genes of resistance for the study. Finisa and Tefera (2006) reported only 26 genotypes (13 %) and 10 genotypes (5 %) were resistant to anthracnose, angular leaf spot and common bacterial blight after evaluation of 201 genotypes in two seasons respectively but the current study has to be repeated in order to see if there will be any differences. The use of cultivars possessing multiple disease resistance provide more protection than singe disease resistance due to variability of pathogens in different environment. Additionally, it is simple for a farmer to adopt, cost effective and environment-friendly.

### 5.2 Days to 50 % Flowering and 85 % Maturity

There were variations among genotypes on days to 50 % flowering and 85 % maturity. The difference among location in number of days to 50 % flowering and those to 85 % maturity were due to effects of altitude that affects temperature. Morogoro (SUA) is located in lower altitude, Arusha is located in mid-altitude and Mbeya (Uyole) is in higher altitude. Morogoro had the highest temperature (25 °C) followed by Arusha (20 °C) and Mbeya (17 °C) that is why the plants reached 50 % flowering and 85 % maturity earlier compared to other locations. Lower temperatures result in slower growth due to the fact that photosynthesis is slowed down together with other biochemical processes. Since

photosynthesis is slowed, growth is slowed automatically. Proper plant growth is attained when the rate of photosynthesis is greater than that of respiration because the respiration consumes the photosynthetic products. Also higher temperature promotes rapid growth by facilitating the development of nodes leading to earlier flowering and maturity (Fivawo and Nchimbi-Msolla, 2012). The G x E interaction in the number of days to 85 % maturity reveals that the trait is not only genotype specific but is influenced by environment.

### 5.3 Number of Pods per Plant

The variations in the number of pods per plant among genotypes is anticipated to be due to differences in genotypes and the G x E interaction observed was due differences in adaptability and stability to the environment in which genotypes were grown. Environments on which genotypes were exposed were different in climatic conditions and soil conditions that resulted to the variations observed. Morogoro had the lowest number of pods per plant due to heavy rains that resulted to water logging at the stage of pod formation (R7) which led to abortion of many young pods and flowers. Also high temperature could have resulted into a reduction in pod and seed numbers as a result of increased abscission of flower buds, flowers and young pods. Likewise as observed by Ofir et al. (1993) common bean plants exposed to high temperature of 27 °C for 5 days at anthesis, resulted into a reduction in pod and seed numbers as a result of increased abscission of flower buds, flowers and young pods, and the failure of fertilization and seed development. These factors could also have lowered the yield of Morogoro. The best result was recorded in Mbeya, the location that had good soil and rainfall distribution compared to other locations. These conditions ensured the proper plant growth. Generally, the checks Mexico 54 and Vax 3 were the varieties maintained their rankings;

first and second in all the locations basing on number of pods per plant. Also genotypes attacked with BCMV could have led to fewer pods per plant compared to healthy plants.

### 5.4 Number of Seeds per Pod

There were significant variations among genotypes and locations except for G x E interaction. The Genotype x Environment interaction was not significant due to the fact that the trait is genotype specific and it is not affected by environment. Number of seeds per pod is one of the principal yield components in common beans. Number of seeds per pod is affected by the size of seed, the small seeded genotype have higher number of seeds per pod and vice versa. This was observed in Vax 3 which is a small seeded had larger number of seeds per pod in all the locations. Similar result was reported by Lima *et al.* (2004) where it was observed that large seed did not affect grain yield but reduce the number of seeds per pod, increase 100 seed weight mass and reduce the seed harvest index.

### 5.5 Weight of Seed per Plant

Environments affect the performance of genotypes, Morogoro had the lowest weight of seed per plant due to the heavy rains that resulted to flood at the stage of pod formation. Most of the pods had poor seeds, and for those pods which had seeds the seeds were very small due to the interference of nutrient uptake and photosynthesis during the plant growth. Moreover Morogoro had higher temperatures during reproductive stage that could have resulted in a reduction in pod and seed set due to enhanced abscission of flower buds, flowers, and pods (Ahmed *et al.*, 1992). Also number of pods determine seed weight per plant for example Mexico 54 had many pods that contributed to higher seed weight per plant. Mexico 54 has a very good genetic potential in yield.

Weight of seed per plant for Arusha was not satisfactory due to the bean flies (*Ophiomyia spp*) infestation whose feeding activities damaged plant vascular tissues thereby interfering with translocation activities of the plant. Nutrients did not move to the required sites (sinks) thereby spontaneously lowering the seed weight per plant and the variations observed within locations were due differences among genotypes.

### 5.6 100 Seed Weight

There were variations among 32 genotypes for the trait, Kablanketi had the highest 100 seed weight (large seed) and lines were grouped as medium and Vax 3 was small seeded according to Singh et al. (1991). The variation in 100 seed weight was due to genetic make-up and environments. Kablanketi and Vax 3 had the highest and lowest 100 seed weight respectively in all the three locations showing that environment did not affect the genotypes (Kablanketi and Vax 3) even if the genotype x environment was significant, they were stable by maintaining their rankings. This shows the trait is genotype specific for these two genotypes. Mbeya had the highest 100 seed weight due to the fact that there were good environmental factors that allowed good pod fill resulting into big and heavy seeds compared to Morogoro where the experiment was flooded. The same results were reported by Tryphone and Nchimbi-Msolla. (2010) that there were differences in 100 seed weight among genotypes, locations and G x E interaction for genotypes grown in Morogoro and Arusha. The weight of 100 seed weight (seed size) is important trait in bean breeding since larger seeds have higher consumer acceptance and fetch higher market prices compared to smaller seeds (Tryphone and Nchimbi-Msolla, 2010). The results shows introgression of the resistance genes into Kablanketi resulted to reducing slightly seed size. However, for some of the introgression lines, the difference between them and Kablanketi was not significant.

### 5.7 Grain Yield (t/ha)

The differences among genotypes in yield potential is the function of genotypes adaptation in different environments. Generally, lines evaluated in this experiments had better yield compared to the average yield of common beans under farmers management which is 0.7 t/ha (FAO, 2010). Breeding of these lines led to the improvement in yield. Genotypes grown in Mbeya produced higher seed yield compared to other locations due favourable environment. Morogoro had the lowest yield due to heavy rains that resulted to water logging that caused pods and seeds to rot, yellowing of the plants due to reduced soil N through volatilization and denitrification that consequently affected photosynthesis process leading to poor yield. Additionally oxygen level in the soil was reduced as a result it interfered respiration which is essential for proper plant growth.

Number of pods per plant and seeds per pod determine the grain yield, this was observed in Mexico 54 which had the highest number of pods and number of seeds per pod that resulted to higher grain yield. Also seed size can affect the final grain yield, larger seed will result to better yield while small seed will have lower grain yield. The presence of G x E interaction, indicated that environment had a major role on yield, also selection for this trait has to be done according to locations.

#### 5.8 Correlation among Variables

Positive correlations were observed between yield and days to 50 % flowering, days to 85 % maturity, number of pods per plant, number of seeds per pod and weight of seeds per plant. This indicated that breeding for one of the trait could lead to significant parallel improvement of other traits. There was no correlation between weights of 100 seeds with grain yield; this can be explained by number of seed per pod, plants from large seed produce few seeds per pod compared to small seeded. This might have led to

compensatory effect in final grain yield. Also yield was significantly negatively correlated with diseases (CBB and BCMV) for combined analysis which indicated that the increase of diseases could hinder the expression of other traits. Diseases affect general growth of plants hence lowering yield of the plants. Fivawo and Nchimbi-Msolla (2012) also reported the negative correlation between yield and ALS. Therefore it is necessary that the search for diseases genetic resistances continues and should be combined with application of proper cultural practices in order to produce appropriate common bean seed for the growers and consumers.

#### 5.9 Estimates of stability

A genotype is considered to be stable when it performs consistently across wide range of environments (Annichiarico, 2002). Genotype stability across locations is an important aspect that should be considered in crop improvement, some cultivars are well adapted to a particular environment while others are widely adapted under diverse environments (Mukandala, 2010). A suitable cultivar is that does not produce well in its area of origin but it can maintain the high yielding capacity over wide range of environments within the area of production. Finlay and Wilkinson (1963) model considered regression coefficient (b) together with high mean yield as a measure of stability. Regression coefficient (b) approximating to 1 or equal to 1 with mean yield greater than overall mean yield as a stable genotype and those genotypes with b value greater than 1 as unstable genotypes. Therefore genotypes KT 10-1a, KT 4g-3c, KT 9-2d, KT 9-1a and KT 7a had b value approximating to 1 with grain yield above the overall mean indicating wider stability and general adaptation. These genotypes may be suitable over wider range of environments (poor to good environments). Genotypes with b < 1 and high mean yield like Vax 3 could be suitable for poor environments and for small scale farmers who entirely depend on natural environments with poor soils and unreliable rainfall (Mukandala, 2010). The

genotype KT 10-1b had b = 1 but the yield was below the average. This genotype is very stable basing on b value but it is unable to exploit the high yielding environments. This shows that stability is inversely proportional to mean yield. Generally 62 % of genotypes had b approximating to 1 and 38 % had the b greater than 1 indicating the genotype are suitable for good environment

#### **CHAPTER SIX**

#### 6.0 CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusions

From this study the following can be concluded:

- i. Marker assisted selection (MAS) used to select only those with appropriate genes of resistances was successful. Most of the common bean lines had two genes and some had three genes of resistances. Lines with two genes (*phg*-2 ALS) and SAP6 (CBB) were KT 1 2b, KT 1 2c, KT 2b, KT 4b, KT 5a, KT 6 -1c, KT 6-2a, KT 6-2b, KT 6-2c, KT 6-2d, KT 7a, KT 7d, KT 9-1a, KT 9-2a, KT 9-2c, KT 9-2d, KT 10-1b, KT 10-2a, KT 10-2c, KT 10-2d, KT 11-1d, KT 11-2d, KT 13-1b, KT 13-2d, KT 15a. The lines KT 5a, KT 6 -1c, KT 7d, KT 9-1a, KT 9-2a, KT 10-2a and KT 10-2c had a combination of three genes of resistances (phg-2(ALS), SAP6 (CBB) and SW13 (BCMV). This study suggest that the introgression of ALS and CBB. However, only few lines with BCMV/BCMNV were recovered.
- ii. Performance of bean lines basing on both disease resistances and yield showed that there were ten lines that were promising. The lines were KT 13-1b, KT 4g-3c, KT 6-2d, KT 6-2c, KT 10-2d, KT 11-1a, KT 15a, KT 9-2b, KT 11-2d and KT 2b.
- iii. Genotype x Environment interaction was observed in most of the traits like days to 85 % maturity, number of pods, weight of seed (g), 100 seed weight (seed size) and yield(t/ha). The variation observed in different environment indicate importance of testing genotypes in various locations and selection of genotypes for a certain trait has to be done basing on performance of a genotype in a specific location.

- iv. Combined simple correlation analysis showed that days to 50 % flowering, days to 85 % maturity, number of pods per plant, number of seeds per pod, weight of seeds per plant and weight of 100 seeds were highly correlated with grain yield.

  This suggest that these traits have to be considered when breeding for higher grain yield.
- v. The genotypes KT 10-1a, KT 4g-3c, KT 9-2d, KT 9-1a and KT 7a had b value approximating to 1 with grain yield above the overall mean indicating wider stability and general adaptation.

#### 6.2 Recommendations

- i. Further field evaluation of the lines KT 13-1b, KT 4g-3c, KT 6-2d, KT 6-2c, KT 10-2d, KT 11-1a, KT 15a, KT 9-2b, KT 11-2d and KT 2b is needed to verify their performance and some of these lines should be recommended to be released to specific location since they had high G x E interaction.
- ii. There is a need to find another variety which is very susceptible to diseases to replace the current Kablanketi as a susceptible check because it seems Kablanketi has intermediate resistance to the diseases.
- iii. More crossing can be done to add more genes of disease resistance into these promising lines for example anthracnose or any other problematic disease in Tanzania.
- iv. Further stability analysis is needed to prove the stability of the lines evaluation of the lines KT 10-1a, KT 4g-3c, KT 9-2d, KT 9-1a and KT 7a

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#### **APPENDICES**

### **Appendix 1: Statistical models**

The statistical model for single site (1) and combined analysis (2) were used.

$$Yij = \mu + Gi + Bj + Eij \dots (1)$$

 $Y_{ij}$  is the random variable representing the response for treatment i observed in block j,  $\mu$  is the baseline mean,  $G_i$  is the i<sup>th</sup> treatment effect, Bj is the j<sup>th</sup> block effect, and  $E_{ij}$  is the random error of the observation.

$$Yijk = \mu + Li + Bj + Cij + Gk + (LG)ik + Eijk.$$
 (2)

Where: Yijk= an observation or response, $\mu$ = the experimental mean,  $G_k$ =kth effect of Genotype, Bj = the block effect, Cij= Error due to replication and location, Li= ith effect of locations, (LG)ik = the location and genotype interaction effect,  $E_{ijk}$ = Residue error.

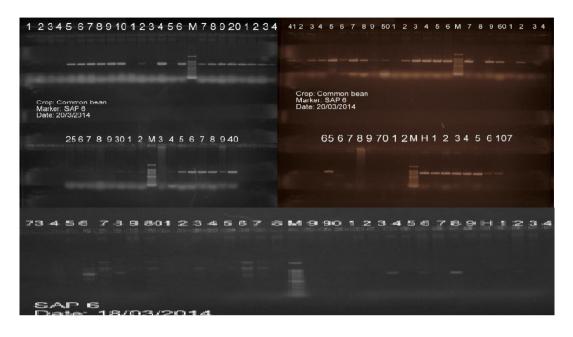
## Appendix 2: Stability analysis model

The regression model used was as follows

$$Y_{ijk} = \mu + g_i + b_{jk} + \beta_i E_j + e_{ijk}.$$
 (3)

where  $Y_{ijk}$ = is the yield of the  $k^{th}$  replicate of the  $i^{th}$ genotype in the  $j^{th}$ environment,  $\mu$  is a general mean,  $g_i$  is the main effect of  $i^{th}$  genotype,  $b_{jk}$ is the  $k^{th}$ block effect in the  $j^{th}$ environment,  $\beta_i$  is the slope of the  $i^{th}$ genotype to the environmental variable  $(E_j)$  and  $e_{ijk}$  is a random error.

**Appendix 3: Gel Pictures of SAP6** 



**Appendix 4: Gel Pictures of ROC11** 



**Appendix 5: Gel Pictures of SNO2** 



# **Appendix 6: Gel Pictures of SW13**

