# PHENOTYPIC AND MOLECULAR EVALUATION OF LINES DEVELOPED FOR MULTIPLE DISEASE RESISTANCE IN COMMON BEAN (*Phaseolus vulgaris* L.)

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## ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume serving as a key source of protein and vital micronutrients for human beings worldwide. In Tanzania common bean yield average is 1 metric tons (MT) per hectare while the potential yield is reported to be 1.5 to 3 MT per hectare. The low yield among other factors is largely contributed by occurrence of foliar diseases caused by fungi, viruses, and bacteria. In this context, breeding common beans for multiple disease resistance in adapted genotypes is proposed as the most economical and environmentally safe approach for control of these diseases. Experiments were conducted on seventeen common bean genotypes for evaluation of resistance to four major foliar diseases *viz.*, angular leaf spot, common mosaic virus disease, common bacterial blight, and anthracnose under natural infection. Phenotypic evaluation was conducted at Tanzania Agriculture Research Institute – Selian center (TARI–Selian) during the main bean growing season (March to July 2020). Experiment was laid in a randomized complete block design (RCBD) with three replications. Data were collected six weeks after planting and then after every fourteen days, three times by using the CIAT 1-9 disease rating scale. The mean disease scores were analyzed using GenStat software 15$^{th}$ version. Polymerase chain reaction (PCR) was performed using four gene specific primers to screen for specific disease resistant genes. Phenotypic results showed significant variation in disease reaction and yield ($P< 0.001$). Genotype Mex 54 had the highest yield (2888.89kg/ha) while improved genotype S-3 recorded the lowest yield (280.44kg/ha). Molecular results indicated that 17.4% of the genotypes contained all four genes while 7.9% lacked any gene for disease resistance. Positive correlation between disease severity scores and molecular marker scores were observed ($r = 0.106$; $P < 0.05$). This suggests that genotypes confirmed as resistant for both phenotypic and molecular evaluations can be recommended as promising materials for advancement and release as resistant common bean varieties for use by farmers in Tanzania.

## DECLARATION

I, Sylvia Adam Mlemba, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted at any other institution.

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## COPYRIGHT

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## DEDICATION

This work is dedicated to the highest Almighty God, my lovely husband Innocent Paulin Ritte and our son Etham Innocent Ritte.

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| | |
|---|---|
| ALS | Angular Leaf Spot |
| ANTH | Anthracnose |
| BCMNV | Bean Common Mosaic Necrotic Virus |
| BCMV | Bean Common Mosaic Virus |
| BCMVD | Bean common mosaic virus disease |
| CBB | Common Bacterial Blight |
| CIAT | Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture) |
| DMRT | Duncan multiple range test |
| DNA | Deoxyribonucleic acid |
| EDTA | Ethylenediaminetetraacetic acid |
| FAO | Food and Agriculture Organization of the United Nations |
| FAOSTAT | Food and Agriculture Organization Statistics |
| ha | Hectare |
| MDSS | Mean Disease Severity Score |
| MT | Metric tons |
| PCR | Polymerase Chain Reaction |
| QTL | Quantitative Trait Loci |
| spp. | species |
| TAE | Tris base acetic acid and EDTA |
| TMA | Tanzania Meteorological Authority |
| Xap | *Xanthomonas axonopodis pv. Phaseoli* |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background Information

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption in the world. They can be consumed as mature grain, immature seed as well as a vegetable and salads (both leaves and pods) (Broughton *et al*., 2003). It is also an important source of protein, fiber, calories, and vital micronutrients, particularly for millions of people in Latin America and eastern and southern Africa (Singh, 1999; Broughton *et al*., 2003). Frequent consumption of dry seeds of common bean combined with cereals ensures a balanced diet of essential amino acids and other nutrients that contribute to alleviating malnutrition and preventing cardiovascular disease, diabetes, and certain types of cancer (Broughton *et al*., 2003; Thompson *et al*., 2017; Viguiliouk *et al*., 2017).

Regular consumption of common beans and other pulses is now promoted by health organizations because it is reported to reduce the risk of diseases such as cancer, diabetes, or coronary heart diseases (Leterme and Munoz, 2002). This is because, common bean is low in fat and is cholesterol free. It is also an appetite suppressant because it digests slowly and causes a low sustained increase in blood sugar. Other findings show that common beans can delay the reappearance of hunger for several hours, enhancing weight-loss programs (Katun*gi et a*l., 2009). Dried beans that do not meet human food quality standards are used as feed for livestock. Post-harvest plant remains are also used as feed for domesticated animals and young tender leaves and flowers are also used as fresh vegetables in some Central and Eastern African, and in Latin America countries (Broughton *et al*., 2003).

The consumption of common bean is high in the areas with populations of low capital especially in Africa and Latin America, since they cannot afford to have protein from animal sources such as meat and fish. According to Hillocks *et al.* (2006), the common bean is reported to be a major staple food in Eastern and Southern Africa where it is recognized as the second most important source of human dietary protein and the third most important source of calories. The crop is also mostly used in mixed cropping in most of agriculture practices as it is used to improve soil fertility and weed control (Ndakidemi *et al.*, 2006).

In Africa common beans are traded by more than 100 million households (Buruchara *et al.*, 2011; FAOSTAT, 2014). Since it is high in nutrient content and with commercial potential, the common bean holds great promise for fighting hunger, increasing income and improving soil fertility in Sub Saharan Africa. It is an ideal crop for the smallholder farming systems due to its capability to fix atmospheric nitrogen (N), short maturity period (≤ 3 months), easily converted to cash to meet urgent household needs, relatively long storage and convenience of handling the harvest and its compatibility to intercrop with other crops (maize, cassava, banana, etc.) in many low-input production systems (Akibode and Maredia, 2011; FAO, 2016).

### 1.2 Problem Statement and Study Justification

Although common bean is an important food security crop in Tanzania, the United Nation Food and Agriculture Organization statistics and data from other researchers show that, the average common bean yield per hectare is 1.0 metric ton (FAOSTAT, 2018) which is low when compared to the potential yield of 1.5 to 3.0 metric tons per hectare (Hillocks *et a*l., 2006). Production of common bean in Tanzania increased from 1 114 500 tonnes in the year 2014 to 1 197 489 tonnes in the year 2019 (FAOSTAT, 2021). This increase is

largely because of the increase or expansion of the area of production and not the productivity. For example, the productivity of China (1800kg/ha) in a small portion of production 743,239ha is higher than that of Tanzania (1000kg/ha) in a production area of 1 177 400 ha (FAOSTAT, 2018). The low productivity is associated with the occurrence of many biotic and biotic constraints such as drought, low soil fertility, weeds, insect pests, use of unimproved cultivars and diseases (Chataik*a et* al., 2011; Bucheyeki and Mmbaga, 2013).

Common bean diseases caused by fungi, bacteria and viruses have been reported as the most important biological constraints hampering production and productivity of common beans in Tanzania (Mwaipop*o et a*l., 2017). Bean Common Mosaic Necrosis Virus, common bacterial blight (*Xanthomonas. phaseoli*), halo bacterial blight (*Pseudomonas syringae* pv. *phaseolicola*), angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotricum lindemuthianum*) and rust (*Uromyce sappendiculatus*) have been listed as the major diseases affecting bean production in Tanzania (Hillock*s et a*l., 2006; Tryphon*e et a*l., 2013). D*e et a*l. (2001) showed that, angular leaf spot can significantly contribute to yield reduction because the presence of the disease in common bean field results to defoliation of diseased leaves that lead to a reduction of the total leaf area which on the other hand has direct impact on the plant photosynthesis. Successful management of diseases requires a better understanding of the etiological agent involved (Rafi *et al*., 2013). Among different control measures of diseases include the use of proper fungicides, pathogen free certified seeds, cultural practices, and the use of resistant varieties. Use of disease resistant bean varieties has been suggested as the most effective measure for management of common bean diseases because it is environmentally friendly, requires low skills and it is cost effective to farmers to be applied once established (Opio *et al.*, 2001).

Four genotypes were identified in the 2004 CIAT bean project with resistance to ALS, anthracnose, and ashy stem blight (*Macrophomina phaseolina*), whereas combined resistance to rust, CBB, anthracnose and ALS were identified in some lines (CIAT, 2004). Bruchid resistance has been identified in wild *P. vulgaris* from Mexico (Van Schoonhoven and Pastor-Corrales, 1987).

Natural field conditions are characterized by the occurrence of multiple diseases that can still hinder the performance of improved common bean genotypes with a resistance to a single disease. Ongoing research have facilitated the identification of more sources of resistance to damaging diseases like CBB, ALS, BCMV, and anthracnose (Miklas and Singh, 2007; Vidigal *et al.,* 2007; Gonçalves-Vidigal *et al.,* 2011; Ddamulira *et al.,* 2015). Under these circumstances, it is necessary to leverage breeding technologies and pyramid genes into improved cultivars in order to provide resistance to more than one disease. Therefore, breeding common bean for multiple disease resistance in adapted germplasm is expected to reduce yield losses. Hence this study is focused at conducting evaluation of 17 advanced common beans genotypes for multiple disease resistance to major foliar diseases including angular leaf spot, bean common mosaic virus, common bacterial blight, and anthracnose under natural disease pressure under field conditions in Arusha, Tanzania and then confirmed by screening using molecular markers to detect the presence of specific disease resistance genes in the evaluated genotypes, in an effort to manage these diseases. The information obtained through this study will be of importance to bean breeders and farmers.

## 1.4 Objectives

### 1.4.1 Overall objective

The overall objective of this study was to evaluate common bean for multiple resistance to major foliar diseases in selected common bean lines.

### 1.4.2 Specific objectives

Specific objectives of this study were:

(i) To determine the common bean lines with multiple disease resistance to major foliar diseases of the common bean under natural disease pressure.

(ii) To determine the presence of genes for multiple disease resistance in common bean lines using molecular markers.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The Origin of Common Bean

Common bean has originated from two major gene pools which were a result of domestication. These gene pools include Mesoamerican and Andean gene pools. Mesoamerican gene pool contains seeds which are small sized compared to Andean gene pool which were originally distributed from Mexico to Columbia. Andean gene pool contains seeds which are large and were originally distributed from South Peru to Northwestern Argentina (Andes mountains) (Gepts and Debouck, 1991). The two gene pools are also different in their seed storage protein (Phaseolin), plant morphology, iso-enzymes and DNA polymorphism (Paredes and Gepts, 1995). From the two bean ancestral gene pools, cultivated common bean were further divided into races based on morphological, biochemical criteria and agro-ecological adaptation (Singh *et al.,* 1991). Wortmann *et al*. (2004) described that the common bean was introduced in coastal areas of East Africa, especially in Tanzania by the Portuguese in the 16$^{th}$ century. Further, common beans were spread in inland areas by the Arab slave traders.

### 2.2 Description and Classification

The common bean (*Phaseolus vulgaris* L.) is a major grain legume consumed worldwide for its edible seeds and pods. It is a highly polymorphic warm-season, herbaceous annual plant with two plant types; erect herbaceous bushes growing between 20-60 cm high; and twining, climbing vines that grow up to two to five m long (Smoliak *et al.*, 1990). The plant has epigeal type of germination whereby the plant is initially tap-rooted, but adventitious roots emerge soon thereafter, and dominate the tap root. The leaves grow alternately on the stems, are green or purple in color and are divided into three oval

leaflets with smooth edges. Leaves can grow 6-15cm long and 3-11cm wide. The common bean produces white, pink, lilac or purple flowers which are approximately 1 cm in diameter, and bean pods 8-20cm long and 1-1.5cm wide which contain 4-6 smooth kidney-shaped beans. The pods can range in colour from green to yellow or black to purple (N*g et a*l., 2011).

The common bean is a member of the legume family, it is a diploid species, with 2n = 22 chromosomes, classified to order Fabales and Family Fabaceae. The Genus is *Phaseolus* L. and species *Phaseolus vulgaris* L. (OECD, 2016). The genus *Phaseolus* is reported to originate from the Americas, and it comprises over 30 species of which *P. vulgaris* is the most widely grown legume, occupying nearly 90% of the cultivated area in the world (Debouck, 1999; Morales, 2006).

**2.3 Global Production of Common Bean**

The common bean considered as a “grain of hope” is produced worldwide because of its importance among the legume crops. It is cultivated in different environments from sea level to 3,000 meters above sea level (Broughton *et al*., 2003). Its production covers 28.78 million hectares with total annual production of 23.14 million tonnes, accounts for about half of the total pulse production (FAOSTAT, 2012; FAOSTAT, 2013).

The major production areas of common bean in Tanzania are the Northern zone (Kilimanjaro, Arusha, Manyara, and Tanga), Southern highlands (Mbeya, Rukwa and Iringa), Lake zone and Western regions (Kagera and Kigoma). Common beans are grown from medium to high altitude areas (Hillocks *et al.,* 2006). Tanzania ranks 6$^{th}$ among the ten largest producers of dry beans in the world (Table 1) and the largest producer in sub-Saharan Africa (FAOSTAT, 2018). It is estimated that over 80% of rural and urban poor

households in Tanzania depend on common bean as a food crop for their livelihood (Tryphone and Nchimbi-Msolla, 2010). The productivity of common bean in Tanzania is estimated to be 1000kg/ha which is still low when compared with the production potential of more than 1500kg/h (Hillocks *et al.,* 2006; FAOSTAT, 2021). More effort is needed to improve bean production at least to attain the production potential.

**Table 1: Top 10 highest common bean producing countries in the world by the year 2018**

| S/n | Area | Production (MT) | Area Harvested (Ha) | Yield (MT/Ha) |
|---|---|---|---|---|
| 1. | India | 6 220 000 | 13 545 518 | 0.5 |
| 2 | Myanmar | 4 779 927 | 2 701 865 | 1.8 |
| 3 | Brazil | 2 915 030 | 2 837 697 | 1.0 |
| 4 | United States of America | 1 700 510 | 815 850 | 2.1 |
| 5 | China | 1 324 407 | 743 239 | 1.8 |
| 6 | United Republic of Tanzania | 1 210 359 | 1 177 400 | 1.0 |
| 7 | Mexico | 1 196 156 | 1 596 224 | 0.7 |
| 8 | Uganda | 1 039 109 | 627 254 | 1.7 |
| 9 | Kenya | 765 977 | 1 170 173 | 0.7 |
| 10 | Ethiopia | 607 929 | 356 720 | 1.7 |

**Source: UN Food and Agriculture Organization (FAOSTAT) 2018**

### 2.4 Constraints of Common Bean Production

Common bean is produced worldwide and in a wide range of environments ranging from temperate, sub-tropical and tropical regions. Although the production is high in areas with average temperature of 16 to 26$^{o}$C during the growing season and the rainfall of about 300 to 600 mm throughout the crop cycle (Buruchar*a et* al., 2010). Common bean crop is not sensitive to soil type if it is reasonably fertile, well-drained and does not interfere with germination and emergence (Wortmann *et al.*, 2004). It is a short-seasoned crop and most of the varieties mature between 65 to 110 days from emergence (Graham and Ranalli, 1997). According to Hillocks (2006) the potential yield of common bean under favorable environmental conditions with best variety used is from 1500 to 3000kg/ha. However,

potential yield is not commonly attained in most bean growing areas whereas the actual yield attained can be as low as 500kg/ha. The low productivity is a result of both biotic and abiotic factors. Abiotic constraints are drought, heat, nitrogen deficiency, phosphorous deficiency and acid soil toxicities while biotic factors affecting common bean production include insect pests, diseases and weeds (Wortmann *et al.,* 2004; Beebe, 2012). Of these factors diseases are the main cause of yield losses; they can cause severe losses (20 - 100%) to yield and quality of common bean worldwide (Singh and Schwartz, 2010).

Major insect pests affecting bean production are bean stem maggot, *Ophiomyia phaseoli* and *O. spencerella.* During the seedling stage the chrysomelid beetle, *Ootheca bennigseni* and *Ophiomyia mutabilis* damage the leaves and the larvae damage the roots. Aphids (*Aphis fabae, A. craccivora*) affect beans during dry spells especially in the early stages of crop growth. Bean pod borer includes *Maruca vitrata* and *Helicoverpa armigera*. The insect pests in stored beans are bean bruchids (*Acanthoscelides obtectus* and *Zabrotes subfaciatus*) (Msolla and Misangu, 2002; Schmale *et al.*, 2002).

Diseases which affect common bean production include angular leaf spot (ALS) (*Pseudocercospora griseola*), halo blight (*Pseudomonas savastanoi pv. phaseolicola, Pseudomonas syringae* pv.*phaseolicola*), ascochyta leaf spot (*Phomaexigua var. exigua, Ascochyta phaseolorum*), leaf rust (*Uromyces appendiculaus*), anthracnose (*Colletotrichum lindemuthianum*), bean common mosaic virus (BCMV), bean common mosaic necrotic virus (BCMNV) and common bacterial blight (CBB) (*Xanthomonas axonopodis* pv. *phaseoli*) (Li *et al*., 2014). Depleted soil fertility is associated with an increase in root rot diseases caused by *Pythium* spp. and *Fusarium* spp. In sandy soils the root-knot nematodes *Meloidogyne incognita* and *Meloidogyne javanica* have also been reported to cause loss of yield of common bean (Ijani *et al.,* 2000). Other diseases are

powdery mildew (*Erysiphe polygone*), floury leaf spot (*Mycovellosiella phaseoli*), white mould (*Sclerotinia sclerotiorum*) and fusarium wilt/ yellows (*Fusarium oxysporum f. sp. Phaseoli*) (Hillocks *et al.*, 2006).

### 2.5 Major Foliar Diseases Affecting Common Bean Production in Tanzania

Major foliar diseases affecting common bean production in Tanzania includes angular leaf spot (ALS) (*Pseudocercospora griseola* (Sacc.) previously known as *Phaeoisariopsis griseola*), bean anthracnose (*Colletotrichum lindemuthianum*), bean common mosaic virus (BCMV), bean common mosaic necrotic virus (BCMNV) and common bacterial blight (CBB) (*Xanthomonas axonopodis* pv. *phaseoli*) (Hillocks *et al.*, 2006; Li *et al*., 2014). These diseases have been reported to cause yield losses of up to 100% if not well managed, depending on agro ecological zone and the cultivar used (Hillocks *et al.*, 2006).

#### 2.5.1 Common bacterial blight (CBB)

Common bacterial blight (CBB) is a notable seed borne disease of common bean, caused by the gram-negative bacterial pathogen *Xanthomonas axonopodis* pv. *phaseoli* (Xap) (Fourie, 2002; Schaad *et al.,* 2006). The disease affects foliage, pods, and seeds of common bean (Fininsa, 2003). It is a major problem of both snap beans and dry beans. Symptoms of common bacterial blight on leaves initially appear as small, water-soaked spots more evident on the underside of the leaves. With time lesions enlarge and develop into dry-brown spots. The lesions occur at the leaf margins. Coalition of the spots generalization of leaves yellowing may occur, with large dead areas of affected leaves. When the situation is severe, defoliation and killing of the premature leaves can happen. The symptoms on pods appear as lesions, covered with a yellow-colored bacterial exudate or ooze that can dry to a yellowish crusty mass (French and Muchove, 2016). Common Bacterial Blight (CBB) is a widespread problem from tropical to temperate common bean

growing environments. The disease is widely distributed in almost all bean growing areas mostly in areas with high temperature (Mkandawire *et al.,* 2004). Approximately 20 - 75% losses in common bean production are caused by CBB (Opio *et al.,* 2001; Mutlu *et al.,* 2008) and this depends on the environmental conditions and genotypes. The disease is severe under warm temperatures, high rainfall, and high humidity (Wortmann *et al.,* 1998) and its effects are most severe on non-resistant varieties. Allen and Lenne (1998) reported that each 1% increase in disease severity causes yield loss of about 10.5 - 78kg $ha^{-1}$, depending on the season and crop growth stage.

### 2.5.2 Angular leaf spot (ALS)

Angular leaf spot (ALS), is a fungal disease caused by a pathogen *Pseudocercospora griseola* (Sacc.) (Aggarwa*l et al*., 2004; Na*y et* al., 2019a) previously known as *Phaeoisariopsis griseola*. The pathogen is known for its extensive virulence diversity (Mahuku *et al.,* 2002b; Aggarwal *et al.,* 2004; Sartorato, 2004). In Latin America and Africa which are the most important production areas of dry beans in the world the disease is devastating and most recurring (Wortmann *et al.,* 1998; Stenglein *et al*., 2003; Sartorato, 2004; Crous *et al.,* 2006). Correa and Saettler (1987) and Melzer and Boland (2001) reported that ALS occurs sporadically in countries of the temperate climate zone,

including the United States and Canada. Angular Leaf Spot (ALS) can cause a yield loss of up to 80% (Schwartz *et al.,* 1981; de Jesus Junior *et al*., 2001). Symptoms of ALS in the field are angular spots on leaves and some grey dots on the undersides of the leaves. Later spots may coalesce and then defoliation can take place. Symptoms on pods include circular spots which can range from reddish brown to black in colour, this can even be

seen on the seed. Also brown and elongated lesions can be seen on stems, branches and petioles (Landera*s et al.*, 2017).

### 2.5.3 Bean common mosaic virus (BCMV)

Bean common mosaic virus disease is caused by a single- strand, positive sense, RNA Potyvirus. It is a seed borne disease transmitted by aphids (Flores-Estévez *et a*l., 2003; Singh and Schwartz, 2010). Yield losses due to BCMV and BCMNV can be as high as 100% (Damayanti *et al.*, 2008; Li *et al.*, 2014). Disease symptoms of virus and virus-like on common bean plants which can be observed in fields includes dwarfing, upward and downward leaf curl; vein banding (green and yellow); mottling; leaf distortion, vein clearing, puckering and rugosity; purpling; mosaic; mild to conspicuous yellow spots or patches; stunted growth; and necrosis on leaves (Flores-Estévez *et a*l., 2003; Mwaipop*o et* al., 2018).

### 2.5.4 Anthracnose

Bean anthracnose caused by *Colletotrichum lindemuthianum* is a seed-borne fungal disease of common bean distributed worldwide. The disease is serious under cool and humid environments (13-26°C, relative humidity above 92% and free moisture). These conditions are favorable for germination of spores and initial infection (Goodwin, 2003). The yield losses caused by this disease at favorable conditions may be up to 100 percent (Padd*er et al*., 2017; Gaudenc*ia et al.*, 2020). The loss is due to early leaf infirmity, plant death, shrunken seed and increased in the number of seeds that have been affected by having lesions on its coat (Schwartz and Pastor-Corrales, 1989).

The disease causes symptoms to appear on leaves, stems, pods and seeds (Mohammed, 2013). Such symptoms are dark brown necrotic lesions, brick red discolouration on the

lower and later upper leaf surface, brown margins around small veins, vein necrosis, wilting and bleaching of the leaflet (Alle*n et al*., 1996; Godo*y et al*., 1997; Bassanez*i et al.*, 2001). Infected stems have a dark brown eyespot with sunken cankerous center while pods develop lesions which are slightly sunken at the center and have a dark brown or purplish brown margin (Tu and Aylesworth, 1980; Alle*n et* al., 1996). Seeds infected with this fungus exhibit a brown to light chocolate-coloured spots on the seed coats.

**2.6 Breeding for Disease Resistance Cultivars and its Requirements**

Breeding for disease resistance has been reported to be the sustainable method of control of bean foliar diseases. The method has been reported to be convenient since it does not need high skill for farmers to apply it. This approach also is environmentally friendly and it minimizes the cost of production to famers once resistant variety is developed (Redinbaugh *et al*., 2004). Breeding for resistance involves the incorporation of gene(s) for resistance versus certain bean foliar disease. Genes and QTL for resistance have been identified for most common bean foliar diseases. For example, until now, six resistant genes for ALS have been reported, namely *Phg-1, Phg-2*, *Phg-3, Phg-4*, *Phg*-5 and *Phg-6* (Mahuku *et al.,* 2004; Caixeta *et al.,* 2005; Gonçalves-Vidigal *et al.,* 2011). All of these are of qualitative in nature while in addition to qualitative resistance genes, resistance to ALS was also assigned to QTL and among the QTL controlling resistance to ALS is ALS10.1 which is the major QTL that was mapped to linkage group Pv10 (Oblessuc *et al.,* 2015).

Genes for bean anthracnose also have been developed. Kelly and Vallejo (2004) reported 21 anthracnose resistance loci which were identified using the *Co* symbol which some of these alleles have been mapped in the common bean genome and are currently widely used in common bean breeding programs (Kelly and Vallejo, 2004; Vidigal *et al.,* 2007).

All genes controlling resistance are dominant genes but with the exception of the *Co-8* which is a recessive gene. The nine resistance genes *Co-2* to *Co-11* are Meso-American in origin while *Co-1, Co-12* and *Co-13* loci come from the Andean gene pool (Méndez-Vigo *et al.,* 2005; Sousa *et al.,* 2009).

For common bacterial blight, the resistance is conditioned by QTLs. Studies involving molecular markers identified 22 minor and major effect QTL distributed on all 11 linkage groups that are responsible for resistance to CBB (Tsai *et al.,* 1998; Kelly and Miklas, 1999; Miklas *et al.,* 2000; Kelly *et al.,* 2003; Miklas and Singh, 2007; Liu *et al.,* 2008). Heritability of CBB resistance in common bean is quantitatively. It can vary from low to moderately high, depending on the study and mapping populations used (Sighn and Schwartz, 2010). Some of the factors influencing expression of these QTL are genetic background, environmental conditions, and disease pressure (Miklas *et al.,* 2006). For viral diseases, the genetic control towards both viruses is assured by one dominant *I* gene and several other recessive (*bc-u, bc-1, bc-1*$^2$, *bc-2, bc-2*$^2$ and *bc-3*) genes.

Breeding for resistance involves introgression of these resistant genes into the elite variety, although breeding for resistance has been challenged by variability of the pathogens. Some pathogens that cause foliar diseases exist in many races and evolve with time whereby new races tend to emerge and lead to resistance breakdown for the already developed varieties. Ddamulir*a et a*l. (2015) reported the use of gene pyramiding technique to incorporate more than one gene which confers resistance to many races and increases the durability of the resistant variety developed.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Location and Duration of the Study

The phenotypic study was carried out at Tanzania Agriculture Research Institute (TARI) – Selian field in Arusha Tanzania. The study was conducted during the main common bean growing season (March to July 2020). This area was chosen because it is one of the hot spots for most foliar diseases of the common bean, especially fungal diseases. Molecular evaluation was carried out at Molecular Biology Laboratory of the Department of Crop Science and Horticulture at Sokoine University of Agriculture in Morogoro, Tanzania.

### 3.2 Materials

**Germplasms used and their description**

Seventeen common bean genotypes were evaluated of which twelve were germplasm bred for multiple disease resistance (ALS, CBB, BCMV and ANTH), four were resistant control genotypes used as donors for disease resistant genes and one was a susceptible control genotype. These materials were collected from the Bean Breeding Program at Sokoine University of Agriculture, Morogoro, Tanzania. The germplasms were improved using different sources of resistances as described in Table 2, Figure 1 and figure 2. Phenotypic selection was conducted based on the size and colour which resembles that of Kablanketi which is preferred by farmers so the improved genotypes would probably be adopted easily when released. Also, Selection using molecular markers was conducted at each generation. The genotypes were planted in the screen house for seed increase and stabilization. After maturity, the seeds were harvested and stored for field evaluation at TARI - Selian in Arusha (latitude $3^0$21’52.0” S, longitude $36^0$38’08.9” E).

**Table 2: Genotypes used in germplasms improvement**

| Source of resistance | Resistance gene | Diseases |
|---|---|---|
| Vax 3/Vax 4 | Major QTL | CBB |
| Vax 3/Vax 4 | *I* | BCMV |
| Mex 54 | *Phg-2* | ALS |
| G2333 | *Co-4*$^2$ | ANTH |
| Kablanket | None | Susceptible |


**Figure 1: Some of the selected common bean genotypes considering seed type**

Mex 54 x Vax 3/Vax 4

$F_1$ x Mshindi

Kablanketi x $F_1$

KT 020

KT 020/SOYA NJANO x [(Mex 54 x Vax 3) x (MCM 5001x G2333)].

KT 020 x $F_1$

⊗ $F_1$

↓

$F_7$ Lines (Generation under current study)

**Figure 2: Pedigree of the improved common bean lines used in this study**

Whereby: $F_1$ = First filial generation and ⊗ = Selfing.

## 3.3 Methods

### 3.3.1 Experimental design

Seventeen genotypes (twelve improved lines for resistance to the four mentioned diseases, four checks with known resistance genes and one susceptible line to all four diseases) were used in a field experiment. The experiment was laid in a Randomized Complete Block Design (RCBD) and three replications. Each replication was prepared in length of 8.5m and width of 3m ($93.5m^2$) with seventeen single row plots each having fifteen plants (one plant per stand). Spacing between rows were 50cm and within rows were 20cm. Separation between replications were one meter. Weed management and fertilizer application were carried out as per recommended agronomic practices for common bean production.

### 3.3.2 Phenotypic data collection and analysis

Evaluation of the symptoms and scoring of disease severity on bean leaves was conducted six weeks from planting followed by every fourteen days three times by using CIAT scale of 1-9 as explained by Van Schoonhoven and Pastor-Corales (1987) (Table 3). The observation was on the portion of leaf area affected by the disease in relation to the total leaf area, whereby one means leaf with no visible symptoms and nine represents very severe diseases or dead leaf (Figure 3). Collected data were averaged using Microsoft Excel computer program. This is because bean leaves show different rates of reaction to diseases at different stages of growth depending on the weather conditions. Then the mean disease scores were analyzed using GenStat software $15^{th}$ version.

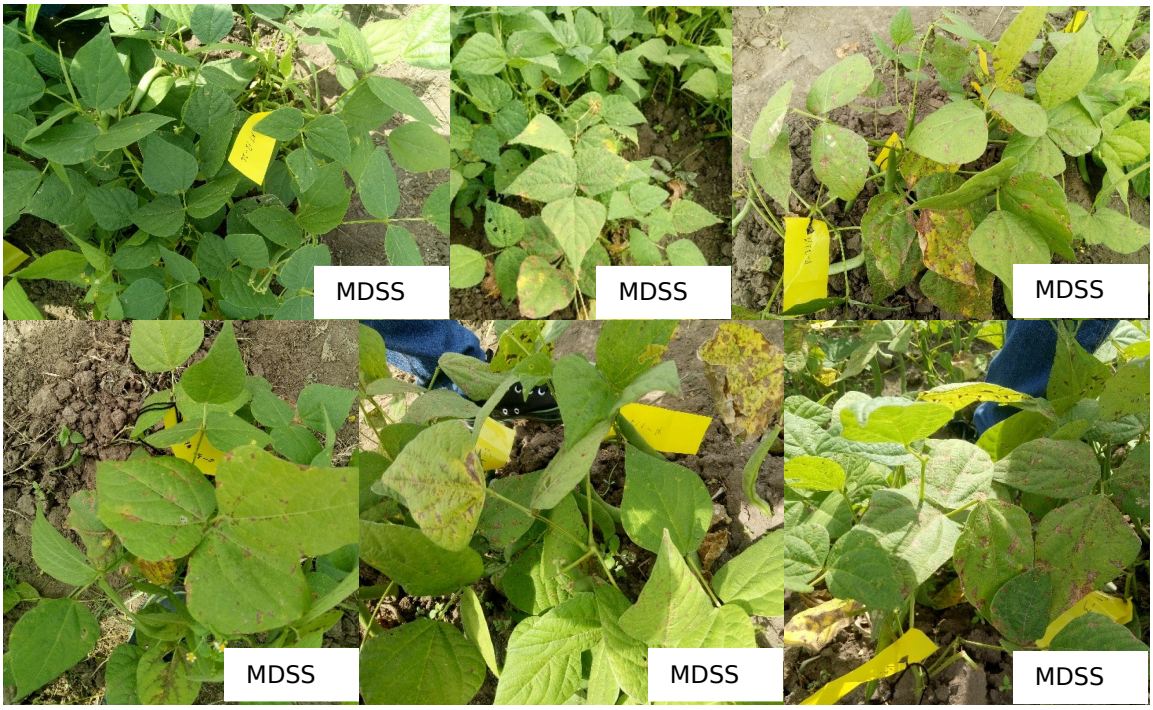


**Figure 3: Examples of how disease severity scores for ALS were determined based on the observation on the portion of leaf area affected (MDSS = Mean disease severity score)**

***Table 3: Symptom evaluation description***

| Score | Description |
|---|---|
| 1 | Leaf with no visible symptoms |
| 2 | Few isolated small lesions on mid-veins in the lower leaf surface |
| 3 | A higher frequency of small lesions on mid-veins in the lower leaf surface |
| 4 | Lesions in the mid-vein and occasionally in secondary leaf veins |
| 5 | Many small lesions scattered on mid- and secondary veins |
| 6 | Many small lesions as described in grade 5 in the lower and upper leaf surface |
| 7 | Large lesions scattered over the leaf blade |
| 8 | Many large, coalesced lesions accompanied by tissue breakdown and chlorotic or abscised leaf |
| 9 | Severely diseased or dead leaf |

**Source:** Van Schoonhoven and Pastor-Corales (1987)

### 3.3.3 Sampling method and Deoxyribonucleic Acid (DNA) extraction

Fifteen bean leaves samples were taken per each line for molecular analysis. Plastic bags labeled with the right identification number were used for sample collection. The bags were quickly placed on ice and transported to the laboratory (Molecular biology

laboratory located at the Department of Crop Science and Horticulture, Sokoine University of Agriculure, Morogoro) for DNA extraction using a protocol published by Mahuku (2004). Extracted DNA were stored for short term at 4ºC for polymerase chain reaction (PCR) analysis.

### 3.3.4 Polymerase Chain Reaction (PCR)

Polymerase chain reactions were performed with different primers specific for each disease resistant gene according to Miklas (2010) and Miller *et al.* (2018) as shown in Table 4. The PCR were performed using illustra$^{TM}$ pure Taq Ready- To- Go PCR beads containing stabilizers, bovine serum albumin, 200dNTPs, 2.5 units of pure taq DNA polymerase, 10Mm TrisHCl, 50Mm KCl and 1.5Mm $MgCl_2$. Twenty-two microliter of PCR water, 1µl of the forward primer and 1µl of reverse primer were added in each PCR tube containing beads. Then 1µl of template DNA was added in each tube making a total volume reaction of 25µl. PCR conditions for each gene primer used are described in Table 5.

**Table 4: Primers used for molecular screening, target genes and expected band size**

| No. | Disease | Primer | Target gene | Expected band size (bp) |
|---|---|---|---|---|
| 1 | CBB | SAP6 | Major QTL | 820 |
| 2 | BCMV | SW13 | *I* | 690 |
| 3 | ALS | g796 | *Phg-2* | 250 |
| 4 | ANTH | SBB14 | *Co-4²* | 1150/1050 |

**Table 5: Polymerase** CHAIN REACTION CONDITIONS FOR EACH PRIMER USED IN THE STUDY

| Primer | PCR conditions |
|---|---|
| SAP6 | 34 cycles of 10s at 94$^0$C,40s at 55$^0$C and120s at 72$^0$C; followed by 1cycle of 5minutes at 72$^0$C. |
| SW13 | 34 cycles of 10s at 94$^0$C,40s at 67$^0$C and120s at 72$^0$C; followed by 1cycle of 5minutes at 72$^0$C. |
| g796 | 34 cycles of 10s at 94$^0$C,40s at 55$^0$C and120s at 72$^0$C; followed by 1cycle of 5minutes at 72$^0$C. |
| SBB14 | 34 cycles of 10s at 94$^0$C,40s at 67$^0$C and120s at 72$^0$C; followed by 1cycle |

| | |
|---|---|
| | of 5minutes at $72^0$C. |

### 3.3.5 Electrophoresis of PCR products

Polymerase chain reaction products were separated on 1.2% agarose gel for CBB, BCMV and ANTH resistance gene and 2 % agarose gel for ALS resistance gene. This was run in parallel with 100bp DNA ladder for identification and confirmation of amplicon sizes. The gel was pre-stained with (0.5 µg/ml) ethidium bromide. Then gel electrophoresis was done at 100volts for 1 hour for Major QTL, *CO-4*$^2$ and *I* genes and 2hours for *Phg-2*, in 1X TAE buffer. These were followed by visualization on a UV trans-illuminator and the gel images were captured with a Power Shot A650IS digital camera (Canon, USA). Documentation and scoring were done according to the specific base pair of each primer by comparing with a reference molecular weight marker (100bp DNA ladder). Gel images were used to score for presence of band for resistant gene as 1 and absence of band as zero (0).

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Phenotypic Evaluation

Disease severity scores were established by the CIAT scale of 1-9 as described by Van Schoonhoven and Pastor-Corales (1987) whereby genotypes that attained the score between 1-3 were regarded as resistant and those which attained -score from 4-9 were rated as susceptible genotypes. Table 6 shows the mean disease severity scores of seventeen common bean genotypes that were evaluated for angular leaf spot, bean common mosaic virus, common bacterial blight, and anthracnose under natural disease pressure. Among evaluated genotypes, five were used as controls (G2333 for Anthracnose, Mex 54 for ALS, Mshindi for BCMV and Vax 3 for CBB). Results showed significant variation for disease reaction ($P \leq 0.001$) to all four diseases. Most of the genotypes were observed to be resistant to all diseases by attaining the mean disease severity score of $\leq 3$ except for a susceptible check Kablanketi which had a mean disease severity score of 3.6 for ALS. Generally, disease pressure was low in the season which rendered even the known susceptible landrace Kablanketi to score less for disease. This must have been culminated by weather conditions during the season which was characterized by low rainfall. Despite the low disease pressure, still the known susceptible check presented a relatively higher score showing that the developed new genotypes were improved for disease resistance.

On the other hand, results indicate that genotypes were highly resistant to anthracnose in the sense that the mean disease severity score for each genotype was 1.0. An exception was observed to Kablanketi which had a score of 1.4 (Table 6) but when it is grown in the field, it scored higher than what was observed in this study (Chilagane, 2017). Goodwin

(2003) reported that the disease is serious under cool and humid environments (13-26°C, relative humidity above 92% and free moisture) the conditions which are favorable for germination of spores and initial infection. Hence these results could be attributed to the weather conditions for the season when the experiment was conducted. There was no rain (0mm) (TMA, Arusha in 2020) in June which was the pod filling stage and critical stage for anthracnose disease development given that the environmental conditions are favorable (Figure 4). For a disease triangle to complete there must be a susceptible host to be infected, a pathogen that is virulent to a given host and favorable environment for infection establishment (Agrios, 2005). This calls for a need to repeat the study both under natural and favorable conditions and molecular screening for promising results on the status of the germplasms.

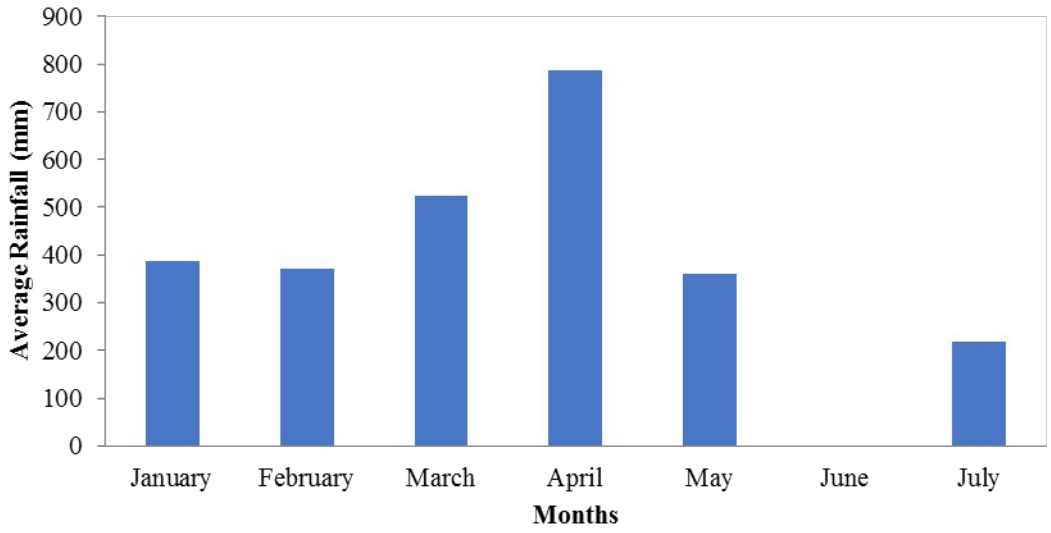


**Figure 4: A bar graph showing average monthly rainfall at TARI Selian Arusha.**

For angular leaf spot (ALS), sixteen genotypes were found to be resistant (disease severity score was ≤ 3). Kablanket was detected to be susceptible with the highest score among all the tested genotypes by attaining disease severity score of 3.6. Other genotypes with high disease severity score for ALS were, S-26, KT020-9 and KT020-6 (disease severity score of 2.70) whereas genotype KT020-10 had the lowest score (1.6). The disease severity

score of Kablanketi was 3.6 despite the fact that it is regarded as highly susceptible. This is not an expected score when Kablanketi is grown under natural disease pressure and meets suitable conditions for disease development. It is expected to score higher than it does in this study (Chilagane, 2017). These observations probably have been contributed by low disease pressure (inoculum) and by weather conditions that were comprised of low rainfall with high temperature and low relative humidity. These observations are supported by other research findings of Na*y et* al. (2019b) who reported that ALS is recurrent and severe in the tropical regions. Also, Wortmann *et al.* (1998); Mahuku *et al.* (2002a); Allorent and Savary (2005) showed that ALS is more severe in tropical and subtropical regions than in hot regions. Parrella *et al.* (2013) also reported that disease pressure in different seasons could be different.

Results for common bacterial bright (CBB) were also significantly different ($P \leq 0.001$) with disease severity score between 1.2 and 2.1. All seventeen genotypes displayed resistance under field conditions even a susceptible check Kablanketi. Genotype VAX 3 is known to possess high level of resistance to CBB resistance (Singh *et al.*, 2001), however in this study this genotype attained the mean disease severity score (1.60) which was consistent with other tested genotypes. Also, Kablanket was found to mature earlier than other genotypes (84.67 days). Perhaps this could have made the genotype to escape environmental factors that were necessary for the disease to develop. The Arusha region where the trial was set is in a highland area that is characterized by low temperature and humidity. These factors are important for a disease to develop; hence it was expected for CBB not to occur as conditions of the area were not favorable. The season in the year when the experiment was conducted was dominated by low rainfall which could be the reason for disease identification. These results are in agreement with the findings obtained

by Wortman *et al.* (1998) and Mkandawile *et al.* (2004) that the disease is endemic in areas with lowland and midaltitude areas at temperature of about 28ºC.

All seventeen genotypes depicted to be resistant to the bean common mosaic virus disease (disease severity score of 1.0). This is probably due to the fact that bean common mosaic virus is more prudent in lowland areas because of high temperature and humidity (Trujilo, 1971).

**Table 6: Mean disease severity scores under natural disease pressure in 2020 Arusha, Tanzania**

| Genotype | ALS | CBB | BCMVD | Anthracnose |
|---|---|---|---|---|
| G2333 | 1.80$^{a}$ | 1.60$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| Kablanketi | 3.60$^{c}$ | 1.90$^{ab}$ | 1.20$^{b}$ | 1.40$^{b}$ |
| KT020-1 | 2.30$^{ab}$ | 1.30$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| KT020-10 | 1.60$^{a}$ | 1.20$^{a}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| KT020-2 | 1.80$^{a}$ | 1.40$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| KT020-3 | 1.90$^{ab}$ | 1.40$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| KT020-4 | 2.20$^{ab}$ | 1.70$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| KT020-5 | 1.80$^{a}$ | 1.10$^{a}$ | 1.00$^{a}$ | 1.20$^{a}$ |
| KT020-6 | 2.70$^{bc}$ | 1.60$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| KT020-7 | 2.00$^{ab}$ | 2.10$^{bc}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| KT020-8 | 1.80$^{a}$ | 1.60$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| KT020-9 | 2.70$^{bc}$ | 1.20$^{a}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| Mex 54 | 1.70$^{a}$ | 1.80$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| Mshindi | 1.70$^{a}$ | 1.80$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| S-3 | 1.90$^{ab}$ | 1.30$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| S-26 | 2.70$^{bc}$ | 1.30$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| Vax 3 | 1.80$^{a}$ | 1.60$^{ab}$ | 1.00$^{a}$ | 1.00$^{a}$ |
| **Grand Mean** | 2.10 | 1.50 | 1.00 | 1.00 |
| **LSD** | 0.72 | 0.66 | 0.08 | 0.22 |
| **%CV** | 20.70 | 26.20 | 4.60 | 12.50 |
| **F- value** | 0.001 | 0.001 | 0.001 | 0.001 |

Means within the same column followed by the same letters are not significantly different from each other at P ≤0.05 using Duncan Multiple Range Test (DMRT). ALS = angular leaf spot disease; CBB = common bacterial blight disease; BCMVD = bean common mosaic virus disease.

## 4.2 Yield

The total average yield of common bean in Tanzania is estimated at 1000kg/ha (FAOSTAT, 2018). In this study a significant difference in yield among the studied genotypes was observed (P< 0.001). Genotype Mex 54 recorded the highest yield (2888.89kg/ha) which was within the range of potential yield of common bean (Table 7). This cultivar was used as a check for ALS resistance which is controlled by gene *Phg-2* that was earlier identified in Mesoamerican cultivar Mex 54 as a single dominant resistance locus on chromosome Pv 08 (Sartorato *et al.,* 1999). This could be the reason for its high productivity although it was grown in an area which is known to be a hotspot for ALS.

Genotypes Vax 3, G2333 and Kablanketi were also found to have high yield which is also within the potential yield range. All these four genotypes were the checks used in this study. High yields of Kablanketi (a susceptible check) probably have been contributed to the fact that it takes short time to ripe (Palilo *et al*., 2018) which could have helped it escape draught stress. The high productivity of these checks out yielded all other improved genotypes. Low soil moisture conditions caused by insufficient rains during the pod filling stage may have contributed to low yield of the improved germplasms. These findings are in agreement with those of Ntukamazin*a et al*. (2017) who reported that pod setting and seed filling stages are more sensitive to drought stress and they observed a significant reduction in number of grains per pod, 100 grain weight, and grain yield under drought condition. Similarly, Asfaw and Blair (2014) reported significant reductions in pod number per plant, seed number per pod, 100 seed weight and seed yield of common beans under drought-stressed conditions. Also, Darkwa *et al.* (2016) observed that, late flowering and pod setting stages appear to be the most sensitive stages to soil moisture stress.

Among the improved genotypes, KT020-9, KT020-3, KT020-7 and KT020-8 attained the yield of 882.44kg/ha, 841.11kg/ha, 817.11kg/ha and 715.78kg/ha respectively (Table 7).

In contrast, the improved genotype S-3 had the lowest yield (280.44kg/ha) which may be due to the fact that this genotype took a longer period to anthesis on which the average number of days taken to 50% flowering were 41.67 days. Findings of Beebe *et al.* (2008) suggests that adaptation range and physiology of cultivated common bean reflects in part its origin in the mid-altitudes with moderate temperatures, organic soils, and seasonally abundant rainfall. Being late to flower exposes the genotype to challenges like drought especially when there are insufficient rains at early growing and reproductive stages which consequently leads to low yield.

**Table 7: Growth and yield components of common bean genotypes evaluated under natural disease pressures in 2020 Arusha, Tanzania**

| Genotype | Days to 50% flowering | Days to 85% maturity | No. of pods/plant | No. of seeds/pod | 100 seed weight (g) | Yield (kg/ha) |
|---|---|---|---|---|---|---|
| G2333 | 47.67$^{ef}$ | 97.00$^{d}$ | 26.13$^{cd}$ | 6.80$^{d}$ | 28.60$^{a}$ | 2131.56$^{de}$ |
| Kablanketi | 34.00$^{a}$ | 84.67$^{ab}$ | 10.73$^{ab}$ | 4.67$^{bc}$ | 47.13$^{e}$ | 1168.89$^{bc}$ |
| KT020-1 | 36.00$^{a}$ | 92.00$^{bc}$ | 14.40$^{bc}$ | 4.93$^{bc}$ | 35.60$^{bc}$ | 373.56$^{a}$ |
| KT020-10 | 34.00$^{a}$ | 91.67$^{bc}$ | 8.67$^{ab}$ | 4.00$^{bc}$ | 32.27$^{bc}$ | 568.22$^{a}$ |
| KT020-2 | 34.67$^{a}$ | 90.33$^{bc}$ | 11.00$^{ab}$ | 4.00$^{bc}$ | 33.20$^{bc}$ | 740.44$^{a}$ |
| KT020-3 | 34.67$^{a}$ | 91.67$^{bc}$ | 11.00$^{ab}$ | 4.40$^{bc}$ | 35.83$^{bc}$ | 841.11$^{ab}$ |
| KT020-4 | 34.33$^{a}$ | 93.67$^{bc}$ | 12.67$^{ab}$ | 4.53$^{bc}$ | 34.27$^{bc}$ | 730.22$^{a}$ |
| KT020-5 | 39.00$^{ab}$ | 94.00$^{bc}$ | 9.73$^{ab}$ | 4.27$^{bc}$ | 45.67$^{d}$ | 603.33$^{a}$ |
| KT020-6 | 34.67$^{a}$ | 84.00$^{a}$ | 11.53$^{ab}$ | 3.80$^{ab}$ | 34.03$^{bc}$ | 562.44$^{a}$ |
| KT020-7 | 36.33$^{ab}$ | 92.00$^{bc}$ | 14.07$^{bc}$ | 3.67$^{ab}$ | 35.80$^{bc}$ | 817.11$^{ab}$ |
| KT020-8 | 37.33$^{ab}$ | 93.67$^{bc}$ | 13.33$^{ab}$ | 4.60$^{bc}$ | 37.87$^{d}$ | 715.78$^{a}$ |
| KT020-9 | 35.00$^{a}$ | 92.33$^{bc}$ | 10.87$^{ab}$ | 4.27$^{bc}$ | 36.43$^{bc}$ | 882.44$^{ab}$ |
| Mex 54 | 47.00d$^{e}$ | 95.00$^{bc}$ | 29.20$^{e}$ | 5.47$^{bc}$ | 46.80$^{e}$ | 2888.89$^{f}$ |
| Mshindi | 37.33$^{ab}$ | 83.67$^{a}$ | 4.73$^{a}$ | 2.67$^{a}$ | 34.57$^{bc}$ | 269.56$^{a}$ |
| S-3 | 41.67$^{bc}$ | 88.00$^{bc}$ | 5.67$^{a}$ | 3.67$^{ab}$ | 30.90$^{ab}$ | 280.44$^{a}$ |
| S-26 | 36.33$^{ab}$ | 85.00$^{ab}$ | 8.60$^{ab}$ | 3.60$^{ab}$ | 52.07$^{e}$ | 539.56$^{a}$ |
| Vax 3 | 47.33$^{ef}$ | 97.33$^{d}$ | 21.00$^{cd}$ | 6.27$^{bc}$ | 28.40$^{a}$ | 2360.00$^{e}$ |
| **Grand Mean** | 38.08 | 90.94 | 13.14 | 4.45 | 37.03 | 969.03 |
| **LSD** | 2.815 | 5.351 | 6.417 | 1.462 | 3.384 | 704.889 |
| **%CV** | 4.40 | 3.50 | 29.40 | 19.80 | 5.50 | 43.70 |
| **SE** | 1.692 | 3.218 | 3.858 | 0.879 | 2.035 | 423.828 |

Means within the same column followed by the same letters are not significantly different from one another at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT).

### 4.3 Molecular Screening Results for Identification of ALS, CBB, BCMV and Anthracnose Resistant Genes in Common Bean Genotypes

A total of 178 bean leaves samples were screened using four markers specific for genes conferring resistance to either ALS, CBB, BCMV and anthracnose. Most of the screened samples were found to have two genes of resistance, which were the Major QTL for resistance to CBB and *I* gene for BCMV resistance. The *Co-4*$^{2}$ gene for resistance to

anthracnose and *Phg-2* for ALS resistances were absent in most of the samples with absence percentages of 52.81 and 46.08 respectively (Table 8, Appendix 1).

Results of the present study showed most of the samples were having the Major QTL (88.76%) for resistance to CBB. This indicates that CBB resistance in Vax 3 is controlled by a dominant gene which tends to have many resistances than susceptible plants upon segregation. The current results are in consent with findings of other previous reports of Miklas *et al.* (2006) and Chataika *et al.* (2011) that showed resistance to CBB is quantitatively inherited with major gene effect. Bonos (2006), reported that breeding for resistance using quantitative genes involves shifting the population mean towards resistance. Also, Tryphon*e et al*. (2012) reported that control of resistance to *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) in Vax 4 is being conditioned by presence of dominant genes although it is moderately (Plate 1).

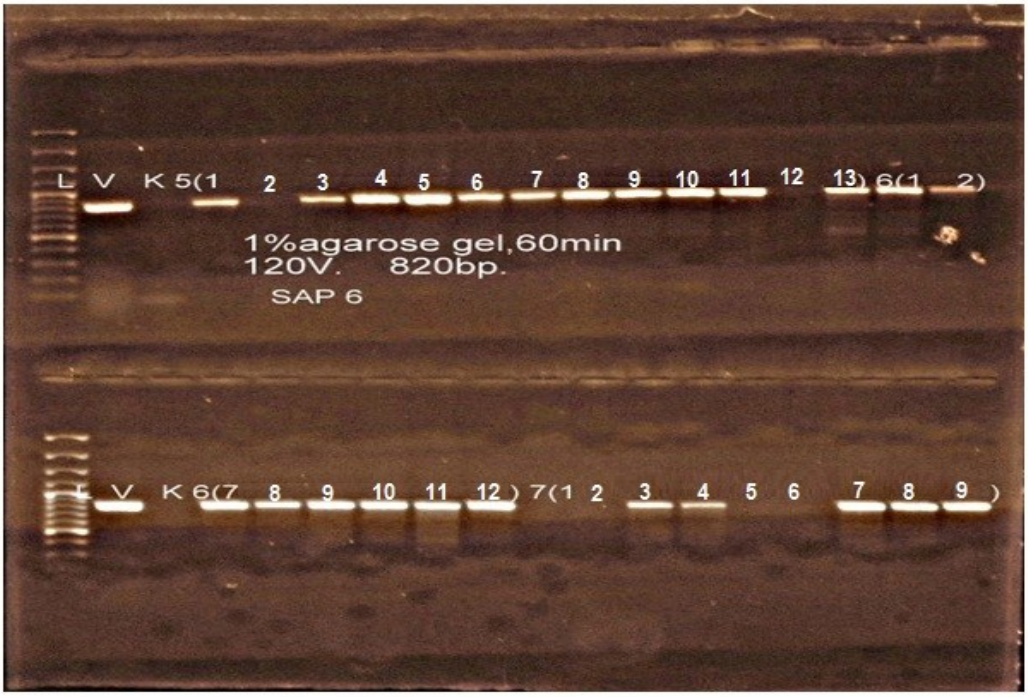


**Plate 1: PCR amplification products showing resistant and susceptible lines to CBB using primer SAP 6, where L=ladder (100bp), V = VAX 3, K = Kablanketi and 5-7 = improved line samples**

*Phg-2* gene for resistance to ALS was found in 96 samples (53.92%) (Table 8, Appendix 1). During breeding, the germplasm Mexico 54 (with Phg-2 gene) was used as a donor parent. Previous studies reported that resistance to the ALS pathogen is largely conferred by single dominant resistance genes, also referred to as loci (Na*y et al*., 2019a). Also, Mexico 54 is mentioned to be very resistant to African isolates of *P. griseola.* Small number of positive samples in this study may be attributed to other breeding factors like compatibility of the parents used, background and the effect of the environment to the genotype (G X E interaction) (Miklas *et al.,* 2006) (Plate 2).

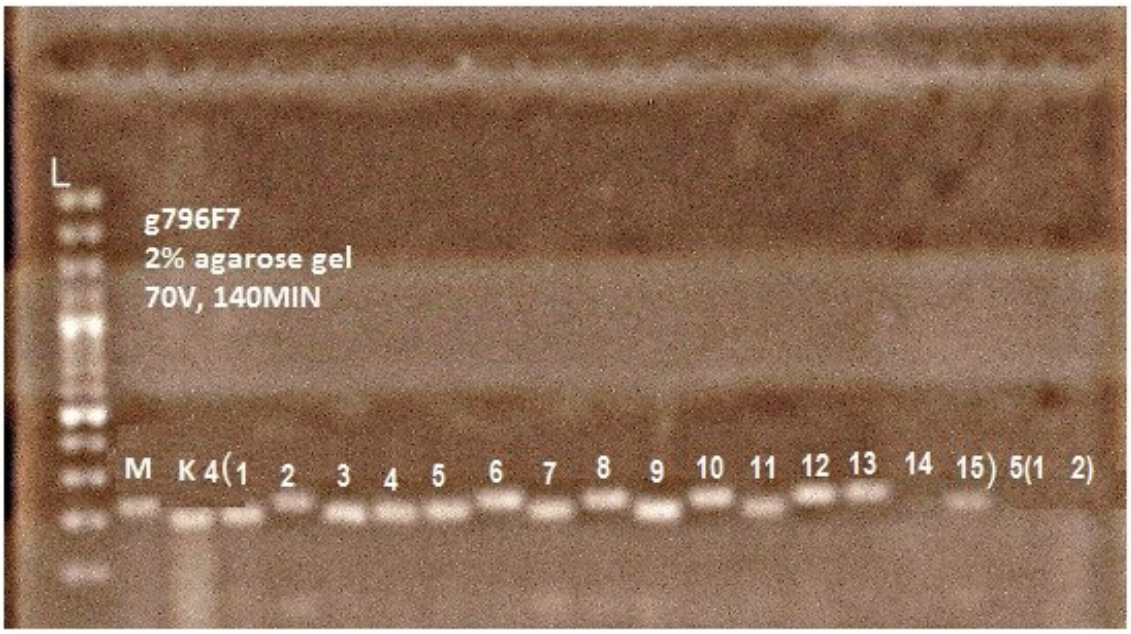


**Plate 2: PCR amplification products showing resistant and susceptible lines to ALS using primer g796, where L=ladder (100bp), M = MEX 54, K = Kablanketi and 4-5 = improved line samples.**

Eighty-four samples (47.19%) (Table 8) were found to contain *Co-4*$^2$ gene for anthracnose disease resistance. The donor parent used in this study was G2333. Previous work reported that G2333 has three resistant genes (*Co-4*$^2$, *Co-5* and *Co-7*) which offer broader resistance to a wide range of *C. lindemuthianum* races (Young *et al.,* 1998; Mahuku *et al.*, 2002b). Mpeguz*i et al*. (2020), reported that G2333 was the most resistant differential

bean cultivar affected by only 7 isolates from the Western and Southern Highland zones of Tanzania only. The author recommended the use of *Co-4, Co-5* and *Co-7* from G2333 to develop resistant varieties intended for cultivation in all other zones except Southern and Western zones because in these areas even samples with the mentioned gene were susceptible to the disease. Also, Mwalyego (1991) reported 15 separate isolates and none of them were pathogenic to G2333 bean cultivar. Hence, Mwalyego (1991) recommended that G2333 can be used as a potential donor for resistant genes in bean breeding program for the varieties to be grown in those areas in Northern, Eastern and the Lake zones. The germplasm from this breeding program developed using G2333 as a donor parent then could have other genes (Co-5 and Co-7) whereby it can be used as a source of seeds for those areas (Plate 3).

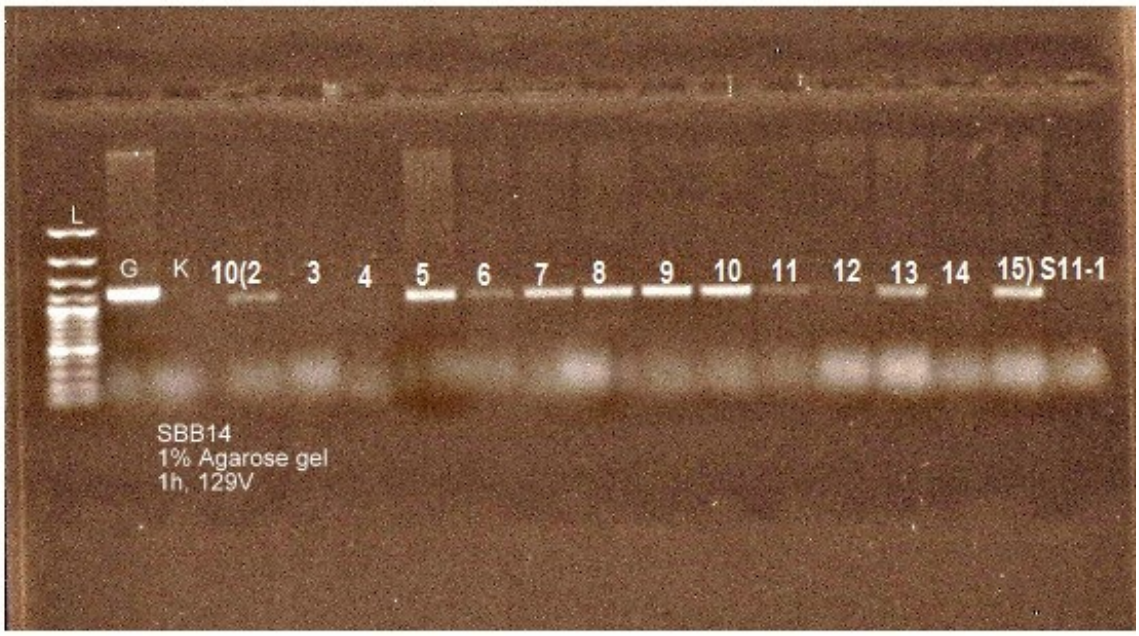


**Plate 3: PCR amplification products showing resistant and susceptible lines to anthracnose using primer SBB14, where L=ladder (100bp), G = G2333, K = Kablanketi and 10-11 = improved line samples.**

The *I* gene for resistance to BCMV was found to be present in 110 samples which was equivalent to 61.80% of the total samples (Table 8). These results are contrary to those of phenotypic screening which 100% resistance was observed. Trujilo (1971) reported that the bean common mosaic virus is more prudent in lowland areas because of high temperature and humidity, this is consistent with Arusha where this trial was carried out. The cold weather with low humidity could be the reason for the disease not to develop. Resistance to different BCMV strains was reported to be controlled by dominant *I* gene and/or with combinations of several recessive genes (bc-u, bc-1, bc-$1^2$, bc-2, bc-$2^2$ and bc-3) (Kelly *et al.,* 1995; Strausbaugh *et al.,* 1999). The results by Pase*v et* al. (2014) in identification of genes for resistance to bean common mosaic virus and bean common mosaic necrosis virus in snap bean (*Phaseolus vulgaris* L.) breeding lines using conventional and molecular methods indicated the presence of *I* gene alone or in combination with one or more recessive genes as bc-1, bc-$1^2$, bc-2 or bc-$2^2$ in lines that remained immune upon direct inoculation with NY15 viral strain that causes BCMV disease. The scar marker SW13 was recommended for reliable and rapid identification for resistance to BCMV using the *I* gene. In this study, the parent Vax 3 was used as a donor parent in breeding for resistance to BCMV. In correspondence with other studies this parent was regarded as the best choice for introgression of resistance in susceptible cultivars expecting to obtain the dominant *I* gene alone or/and with other genes (Plate 4).

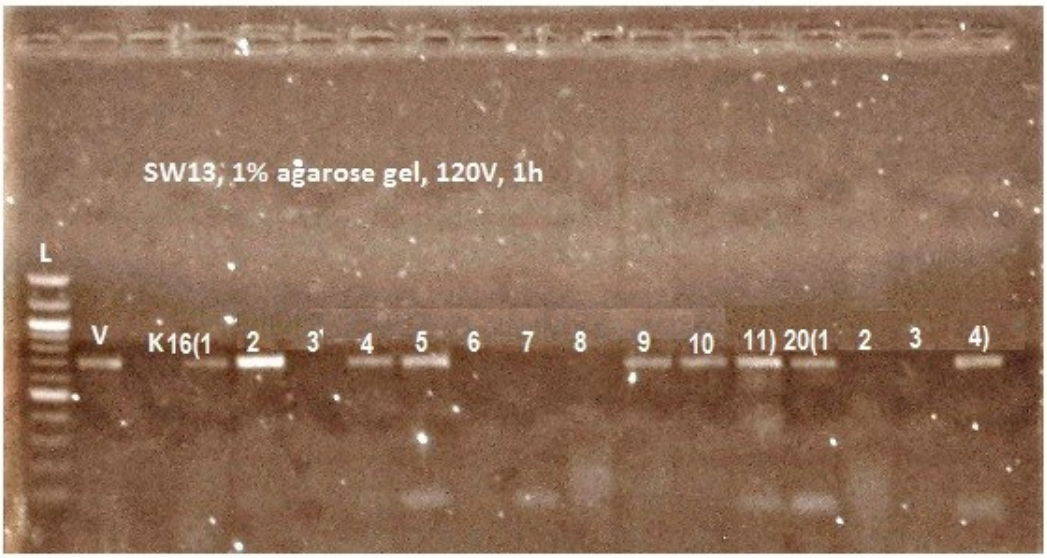


**Plate 4: PCR amplification products showing resistant and susceptible lines to BCMV using primer SW13, where L=ladder (100bp), V = VAX 3, K = Kablanketi and 16 - 20 = improved line samples**

**Table 8: Percentage of positive and negative samples when screened with gene specific markers**

| Type of genes | (+) samples | (-) Negative samples | Total |
|---|---|---|---|
| Major QTL | 158 (88.76%) | 20 (11.24%) | 178 |
| *I* gene | 110 (61.80%) | 68 (38.20%) | 178 |
| CO-4$^2$ | 84 (47.19%) | 94 (52.81%) | 178 |
| *Phg*-2 | 96 (53.92%) | 82 (46.08%) | 178 |

### 4.4 Multiple Disease Resistance

Pyramiding genes for disease resistance in a genotype is a more suitable and durable method to control multiple diseases as multiple diseases have been reported to occur in most production fields causing losses in common bean production (Singh, 2001; Valentini *et al.*, 2017; Okii *et al.*, 2018). Results showed that 33 samples (which is equal to 18.5% of the total screened samples) found to contain all four genes of resistance while 73 samples (41 %) found to have three genes of resistance. Also, 33 samples (18.5%) were found to have two genes of resistance while 27 samples (15.2%) contain a single gene for disease resistance. Though 12 samples (6.7%) found that they do not contain any gene of

resistance among the four screened genes (Figure 5). This indicates that genes for resistance to multiple disease were successfully pyramided in these improved genotypes. Marker assisted selection have been reported to be of great importance in improving common beans for multiple disease resistance (Singh *et al.*, 2001; Ter'an and Singh, 2009). This is important as resistance quickly breaks down when single resistance genes are deployed in each cultivar (Young and Kelly, 1996).

The genes for resistance to ALS (*Phg -2*) and ANTH (*Co -4*$^2$) were found to be missing in most of the genotypes. Breeding for ALS has proven to be difficult by the high pathogenic diversity and specificity of its pathogen *Pseudocercospora griseola* (Keller *et al.*, 2015). This could be the reason for the gene lacking in many tested lines.

A combination of Major QTL for CBB resistance, the *I* gene foe BCMV resistance and either *Phg -2* for ALS resistance or *Co – 4*$^2$ for ANTH resistance was observed in this study. The larger number of combinations being observed on two genes, the Major QTL and the *I* gene.

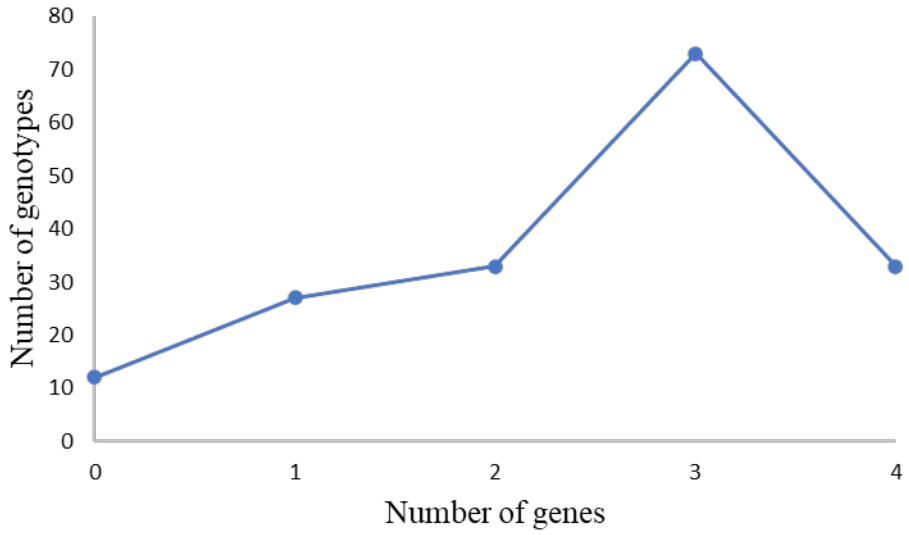


**Figure 5: Trend line chart showing relation between genotypes with multiple resistance and number of genes present.**

### 4.5 Phenotype Versus Genetic Markers Scores

Results showed that there was significant positive correlation ($r = 0.106$; $P < 0.05$), between disease severity scores and molecular marker scores (Figure 6). The correlation between disease severity scores and molecular marker scores indicate that genotypes selected as resistant with molecular marker scores would still be resistance under natural disease pressure. Phenotypic selection alone is not sufficient because some traits may fail to be expressed under certain environmental conditions. Also, in segregating population, use of markers could be the best approach as it will play a significant contribution in increasing selection efficiency without advancing the generation for gene fixation while saving time and other resources. Combination of markers and phenotypic selection has been reported to be the most effective in breeding lines that are resistant to CBB and other diseases (Miklas *et al.*, 2006). Positive correlation between molecular and phenotypic data provides strong evidence of the resistance within the studied germplasms. Genotypes confirmed as resistant at both phenotypic and molecular evaluations can be recommended as promising materials for advancement and release as resistant common bean varieties for use by farmers in Tanzania.

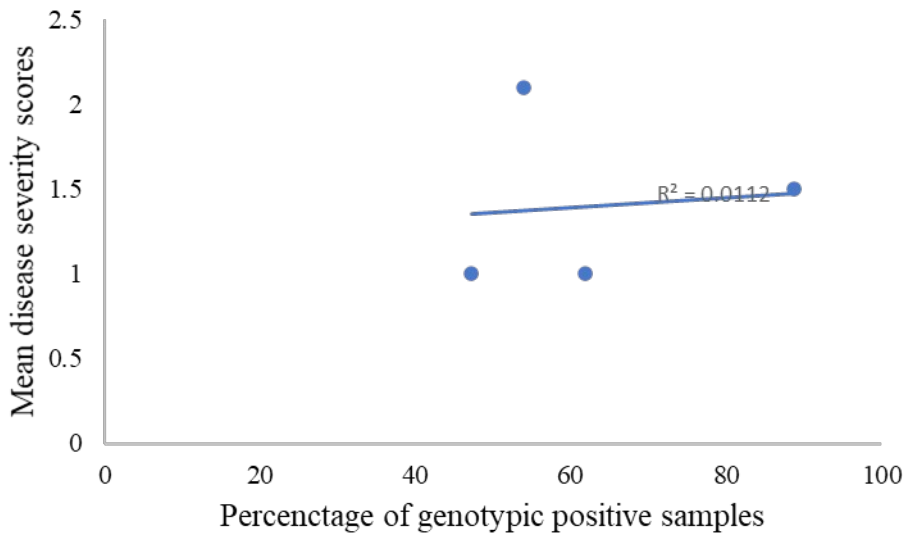

**Figure 6: Trend line chart showing correlation between mean disease severity scores and percentage of positive samples upon genotyping.**

# CHAPTER FIVE

## 5.0 CONCLUSION AND RECOMMENDATIONS

### 5.1 Conclusion

This research investigated phenotypic and molecular characteristics of improved common bean germplasm with the aim of contributing to common bean production through breeding for disease resistant varieties.

From this study, a large proportion of lines screened have shown to be improved for multiple disease resistance and they contain multiple genes for disease resistance (ALS, CBB, BCMV and Anthracnose). Some improved genotypes (KT020-9, KT020-3, KT020-7 and KT020-8) among tested also presented reasonably high yields which are recommended for further screening under high disease pressure.

Evidence from this study suggests that breeding common bean for multiple disease resistance could be one of the strategies for increasing common bean yields especially under high disease pressure where the susceptible varieties will suffer high reduction of yields due to multiple diseases. Also, positive correlation between molecular and phenotypic data shows that use of markers for selection can be used to save time and resources and plants can be screened at early stages. This work contributes to the bean breeding program efforts as it enabled the identification of important phenotypic and genotypic features of the studied genotypes that could then be used in the genetic improvement of common bean genotypes against damaging diseases like ALS, CBB, BCMV and anthracnose.

## 5.2 Recommendations

This study was conducted in a season which had minimal rainfall. This could have contributed to the low disease pressure as explained in disease triangle (presence of susceptible host, virulent pathogen and conducive environment for disease development) which requires all required factors to be available. Hence, it is recommended that,

i. This experiment should be repeated in a season with abundant rainfall that will make the environment favorable for disease perpetuation. Also, it should be repeated in different hot spot locations.
ii. In addition to that, it is also suggested that these materials may be further screened under screenhouse with artificial inoculations using specific virulent strains of the pathogen in order to reconfirm the phenotypic responses of these genotypes to the four foliar diseases i.e., ALS, CBB, BCMV and anthracnose.
iii. Though there is a positive correlation between phenotypic and molecular data in this study still it is recommended that phenotypic evaluation when combined with molecular evaluation needs to be conducted to generate the best results on genotype selection.
iv. Also, it is recommended that, depending on the area of the production and the prevalent disease in that area, varieties with less than the four genes can still be advanced and selected to suit different production environments depending on the prevailing condition in an area and the disease occurrence.

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# APPENDICES

## Appendix 1: Scores of each line after screening with specific molecular markers

| SAMPLE ID | PRIMERS USED | | | | NUMBER OF GENES |
|---|---|---|---|---|---|
| | SAP 6 | SW13 | SBB14 | G796 | |
| 1-1 | 0 | 0 | 0 | 0 | 0 |
| 1-2 | 1 | 0 | 0 | 0 | 1 |
| 1-3 | 1 | 0 | 0 | 0 | 1 |
| 1-4 | 1 | 0 | 0 | 1 | 2 |
| 1-5 | 1 | 0 | 1 | 1 | 3 |
| 1-6 | 1 | 0 | 0 | 0 | 1 |
| 1-7 | 1 | 0 | 0 | 0 | 1 |
| 1-8 | 1 | 0 | 1 | 0 | 2 |
| 1-9 | 0 | 0 | 0 | 0 | 0 |
| 1-10 | 1 | 0 | 1 | 1 | 3 |
| 1-11 | 0 | 0 | 0 | 0 | 0 |
| 1-12 | 1 | 0 | 1 | 1 | 3 |
| 1-13 | 1 | 0 | 0 | 0 | 1 |
| 1-14 | 0 | 0 | 0 | 0 | 0 |
| 1-15 | 1 | 0 | 0 | 0 | 1 |
| 2-1 | 1 | 1 | 0 | 1 | 3 |
| 2-2 | 1 | 1 | 0 | 1 | 3 |
| 2-3 | 1 | 0 | 0 | 1 | 2 |
| 2-4 | 1 | 1 | 0 | 1* | 3 |
| 2-5 | 1 | 0 | 0 | 1 | 2 |
| 2-6 | 1 | 1 | 0 | 0 | 2 |
| 2-7 | 1 | 1 | 1 | 1 | 4 |
| 2-8 | 1 | 1 | 0 | 0 | 2 |
| 2-9 | 1 | 1 | 0 | 1 | 3 |
| 2-10 | 1 | 1 | 1 | 1* | 4 |
| 2-11 | 1 | 1 | 1 | 1 | 4 |
| 2-12 | 1 | 1 | 0 | 1* | 3 |
| 2-13 | 1 | 0 | 0 | 0 | 1 |
| 2-14 | 1 | 0 | 0 | 0 | 1 |
| 2-15 | 1 | 0 | 0 | 0 | 1 |
| 3-1 | 1 | 0 | 0 | 0 | 1 |
| 3-2 | 1 | 1 | 0 | 1 | 3 |
| 3-3 | 1 | 1 | 0 | 1 | 3 |
| 3-4 | 0 | 1 | 0 | 1 | 2 |
| 3-5 | 1 | 1 | 0 | 1 | 3 |
| 3-6 | 1 | 0 | 0 | 1 | 2 |

| | | | | | |
|---|---|---|---|---|---|
| 3-7 | 1 | 1 | 0 | 1 | 3 |
| 3-8 | 1 | 1 | 1 | 1 | 3 |
| 3-9 | 1 | 1 | 0 | 1 | 3 |
| 3-10 | 1 | 1 | 0 | 1 | 3 |
| 3-11 | 1 | 1 | 0 | 1 | 3 |
| 3-12 | 0 | 0 | 0 | 1 | 1 |
| 3-13 | 1 | 1 | 0 | 1 | 3 |
| 3-14 | 1 | 1 | 0 | 1 | 3 |
| 3-15 | 0 | 1 | 0 | 1 | 2 |
| 4-1 | 1 | 1 | 1 | 0 | 3 |
| 4-2 | 1 | 1 | 1 | 1 | 4 |
| 4-3 | 1 | 1 | 1 | 0 | 3 |
| 4-4 | 1 | 1 | 1 | 0 | 3 |
| 4-5 | 1 | 1 | 1 | 0 | 3 |
| 4-6 | 1 | 1 | 1 | 1 | 4 |
| 4-7 | 1 | 1 | 1 | 0 | 3 |
| 4-8 | 1 | 1 | 1 | 1 | 4 |
| 4-9 | 1 | 1 | 1 | 0 | 3 |
| 4-10 | 1 | 1 | 1 | 1 | 4 |
| 4-11 | 1 | 1 | 1 | 0 | 3 |
| 4-12 | 1 | 1 | 1 | 1 | 4 |
| 4-13 | 1 | 1 | 1 | 1 | 4 |
| 4-14 | 1 | 1 | 1 | 0 | 3 |
| 4-15 | 1 | 0 | 1 | 0 | 2 |
| 5-1 | 1 | 0 | 0 | 1 | 2 |
| 5-2 | 0 | 0 | 0 | 0 | 0 |
| 5-3 | 1 | 0 | 1 | 1 | 3 |
| 5-4 | 1 | 0 | 1 | 0 | 2 |
| 5-5 | 1 | 1 | 0 | 0 | 2 |
| 5-6 | 1 | 1 | 1 | 0 | 3 |
| 5-7 | 1 | 1 | 1 | 1 | 4 |
| 5-8 | 1 | 1 | 1 | 1 | 4 |
| 5-9 | 1 | 1 | 1 | 0 | 3 |
| 5-10 | 1 | 1 | 1 | 1 | 4 |
| 5-11 | 1 | 1 | 0 | 1 | 3 |
| 5-12 | 0 | 0 | 0 | 0 | 0 |
| 5-13 | 1 | 1 | 0 | 1 | 3 |
| 5-14 | 1 | 0 | 1 | 1 | 3 |
| 5-15 | 0 | 1 | 0 | 1 | 2 |
| 6-1 | 1 | 1 | 1 | 0 | 3 |
| 6-2 | 1 | 0 | 1 | 0 | 2 |
| 6-3 | 1 | 1 | 0 | 1 | 3 |
| 6-4 | 1 | 0 | 0 | 1 | 2 |
| 6-5 | 1 | 1 | 0 | 1 | 3 |
| 6-6 | 1 | 0 | 0 | 0 | 1 |
| 6-7 | 1 | 1 | 1 | 0 | 3 |
| 6-8 | 1 | 1 | 1 | 0 | 3 |

| | | | | | |
|---|---|---|---|---|---|
| 6-9 | 1 | 1 | 1 | 0 | 3 |
| 6-10 | 1 | 1 | 0 | 1 | 3 |
| 6-11 | 1 | 1 | 0 | 1 | 3 |
| 6-12 | 1 | 1 | 0 | 1 | 3 |
| 6-13 | 1 | 1 | 1 | 0 | 3 |
| 6-14 | 1 | 1 | 1 | 0 | 3 |
| 6-15 | 1 | 0 | 1 | 0 | 2 |
| 7-1 | 0 | 0 | 0 | 0 | 0 |
| 7-2 | 0 | 0 | 0 | 0 | 0 |
| 7-3 | 1 | 1 | 0 | 1 | 3 |
| 7-4 | 1 | 1 | 1 | 0 | 3 |
| 7-5 | 1 | 1 | 1 | 0 | 3 |
| 7-6 | 1 | 0 | 0 | 0 | 1 |
| 7-7 | 0 | 0 | 0 | 0 | 0 |
| 7-8 | 1 | 1 | 1 | 0 | 3 |
| 7-9 | 1 | 1 | 1 | 0 | 3 |
| 7-10 | 1 | 1 | 1 | 0 | 3 |
| 7-11 | 1 | 1 | 0 | 0 | 2 |
| 7-12 | 1 | 0 | 0 | 0 | 1 |
| 7-13 | 1 | 0 | 1 | 0 | 2 |
| 7-14 | 0 | 0 | 1 | 0 | 1 |
| 7-15 | 1 | 0 | 0 | 1 | 2 |
| 8-1 | 1 | 0 | 0 | 1 | 2 |
| 8-2 | 1 | 1 | 0 | 1 | 3 |
| 8-3 | 1 | 1 | 0 | 1 | 3 |
| 8-4 | 1 | 1 | 1 | 1 | 4 |
| 8-5 | 1 | 1 | 1 | 1 | 4 |
| 8-6 | 1 | 1 | 0 | 1 | 3 |
| 8-7 | 1 | 1 | 1 | 1 | 4 |
| 8-8 | 1 | 1 | 1 | 1 | 4 |
| 8-9 | 1 | 1 | 1 | 1 | 4 |
| 8-10 | 1 | 1 | 1 | 1 | 4 |
| 8-11 | 1 | 1 | 1 | 1 | 4 |
| 8-12 | 1 | 1 | 0 | 1 | 3 |
| 8-13 | 1 | 0 | 0 | 1 | 2 |
| 8-14 | 1 | 1 | 1 | 1 | 4 |
| 8-15 | 1 | 1 | 0 | 1 | 3 |
| 9-1 | 1 | 1 | 1 | 1 | 4 |
| 9-2 | 1 | 1 | 0 | 0 | 2 |
| 9-3 | 1 | 0 | 0 | 0 | 1 |
| 9-4 | 1 | 1 | 1 | 1* | 4 |
| 9-5 | 1 | 1 | 1 | 1* | 4 |
| 9-6 | 1 | 0 | 0 | 1* | 2 |
| 9-7 | 1 | 1 | 0 | 0 | 2 |
| 9-8 | 1 | 1 | 1 | 0 | 3 |
| 9-9 | 1 | 0 | 1 | 0 | 2 |
| 9-10 | 1 | 1 | 1 | 1 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| 9-11 | 1 | 1 | 1 | 0 | 3 |
| 9-12 | 1 | 0 | 0 | 0 | 1 |
| 9-13 | 1 | 1 | 1 | 1* | 4 |
| 9-14 | 1 | 1 | 1 | 1 | 4 |
| 9-15 | 1 | 0 | 1 | 0 | 2 |
| 10-1 | 1 | 0 | 1 | 0 | 2 |
| 10-2 | 1 | 1 | 1 | 0 | 3 |
| 10-3 | 1 | 0 | 0 | 0 | 1 |
| 10-4 | 1 | 1 | 0 | 1 | 3 |
| 10-5 | 1 | 0 | 1 | 0 | 2 |
| 10-6 | 1 | 1 | 1 | 1* | 4 |
| 10-7 | 1 | 1 | 1 | 0 | 3 |
| 10-8 | 1 | 1 | 1 | 0 | 3 |
| 10-9 | 1 | 1 | 1 | 1* | 4 |
| 10-10 | 1 | 1 | 1 | 0 | 3 |
| 10-11 | 1 | 1 | 1 | 1 | 4 |
| 10-12 | 1 | 0 | 1 | 1 | 3 |
| 10-13 | 1 | 0 | 1 | 0 | 2 |
| 10-14 | 1 | 1 | 1 | 0 | 3 |
| 10-15 | 1 | 1 | 1 | 0 | 3 |
| S11-1 | 1 | 0 | 0 | 0 | 1 |
| S11-2 | 1 | 1 | 1 | 1 | 4 |
| S11-3 | 1 | 1 | 1 | 1 | 4 |
| S11-4 | 1 | 1 | 1 | 1 | 4 |
| S11-5 | 1 | 1 | 0 | 1 | 3 |
| S11-6 | 1 | 1 | 0 | 1 | 3 |
| S11-7 | 1 | 1 | 0 | 1 | 3 |
| S11-8 | 1 | 1 | 0 | 1 | 3 |
| S11-9 | 1 | 1 | 0 | 1 | 3 |
| S11-10 | 1 | 1 | 0 | 1 | 3 |
| S11-11 | 1 | 1 | 1 | 1 | 4 |
| S11-12 | 1 | 1 | 0 | 1 | 3 |
| S11-13 | 1 | 1 | 0 | 1 | 3 |
| S11-14 | 1 | 1 | 0 | 1 | 3 |
| S11-15 | 1 | 1 | 0 | 1 | 3 |
| S19-1 | 1 | 0 | 0 | 1 | 2 |
| S19-2 | 1 | 0 | 0 | 0 | 1 |
| S19-3 | 1 | 0 | 0 | 0 | 1 |
| S19-4 | 1 | 0 | 0 | 0 | 1 |
| S19-5 | 1 | 0 | 0 | 1 | 1 |
| S19-6 | 1 | 0 | 1 | 1 | 3 |
| S19-7 | 1 | 0 | 0 | 0 | 1 |
| S19-8 | 0 | 0 | 1 | 0 | 1 |
| S19-9 | 0 | 0 | 1 | 0 | 1 |
| S19-10 | 0 | 0 | 0 | 0 | 0 |
| S19-11 | 0 | 0 | 0 | 0 | 0 |
| S19-12 | 0 | 0 | 0 | 0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| S19-13 | 0 | 0 | 1 | 1 | 2 |