INHERITANCE AND SELECTION FOR RESISTANCE TO ANGULAR LEAF SPOT (*Phaeoisariopsis griseola* (Sacc)) AND COMMON BACTERIAL BLIGHT (*Xanthomonas campestris* pv *phaseoli)* IN YELLOW BEANS

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DECLARATION

I certify that, to the best of my knowledge, the material contained in this thesis has never been submitted for a degree in any university, and contains no material previously published or written by another person, except where due reference to such published works have been acknowledged.

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

I dedicate this work to my late parents Mr and Mrs Muimui Muimanenwa, who encouraged me early in life to walk on this trying path called 'education.'

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LIST OF ABBREVIATIONS

ALS	Angular leaf spot
ANOVA	Analysis of variance
BC_1P_1	First backcross to recurrent (susceptible) parent, P_1
BC_1P_2	First backcross to donor parent, P_2
BLDA	Bean leaf dextrose agar medium
CBB	Common bacterial blight
CGIAR	Consultative Group on International Agricultural Research
CIAT	Centro Internacional de Agricultura Tropical
DAP	Di-ammonium phosphate
df	degree of freedom
DRC	Democratic Republic of Congo
F_1	First generation from a cross
F_2	Second generation of a cross
P_1	First parent (Recurrent parent)
P_2	Second parent (Donor parent)
Pg	*Phaeoisariopsis griseola*
PABRA	Pan African Bean Research Alliance
SABRN	Southern African Bean Research Network
Xc	*Xanthomonas campestris*

ABSTRACT

Angular leaf spot (*Phaeoisariopsis griseola)* and common bacterial blight (*Xanthomonas campestris* pv *phaseoli)* are two major diseases of common bean (*Phaseolus vulgaris* L.). In Africa, the two diseases account for losses of 604,600 tons per year. High levels of cultivar resistance to the two diseases would minimise yield losses, reduce production costs and facilitate the production and distribution of pathogen-free seed. The objectives of this study were; 1) To determine the inheritance of resistance to angular leaf spot (in Mexico 54) 2) Determine the inheritance of resistance to common bacterial blight in Wilk 2 and VAX 6, and 3) to incorporate resistance to the two diseases into two susceptible but popular Zambian landraces, Lusaka Yellow and Pembela.

The two susceptible bean genotypes were crossed with three other cultivars resistant to angular leaf spot and common bacterial blight. Six crosses were made: two between the angular leaf spot resistant parent and the susceptible landraces, and four with common bacterial blight resistant parents. F_1, F_2 and backcrosses to both parents were generated in bi-parental crosses. The progenies and the parents were evaluated for resistance in the screen house and field at Kabete Field Station, University of Nairobi. These were inoculated with *Phaeoisariopsis griseola* isolate 63-55 for angular leaf spot and *Xanthomonas campestris* pv *phaseoli* to determine the inheritance of the two diseases. Lusaka Yellow showed susceptible reaction to *Phaeoisariopsis griseola* isolate 63-55, confirming its susceptibility. All plants of Mexico 54 were resistant to the same isolate. All the F_1 were resistant to angular leafspot indicating that resistance was dominant. There was no significant deviation from the expected 3:1 ratio for resistance to susceptible in the F_2 population, confirming that resistance to angular leaf spot was monogenic and dominant. The backcross to Lusaka Yellow showed a 1:1 segregation ratio while the backcrosses to Mexico 54 were all resistant. Inoculation of Pembela with isolate 63-55 showed that all plants were susceptible. In the cross Pembela x Mexico 54, all the F_1 plants were resistant. F_2 progenies segregated in a 3:1 ratio for resistant to susceptible. Backcross to Pembela gave a 1:1 ratio for resistant to susceptible. All backcrosses to Mexico 54 were resistant. The results confirmed that resistance to angular leaf spot in Mexico 54 is controlled by a single dominant gene.

Inheritance to common bacterial blight was found to be dominant. All the plants of Wilk 2 and VAX 6 were resistant. Those of Lusaka Yellow and Pembela were susceptible. All the F_1 in the four crosses for common bacterial blight were resistant. The F_2 segregated in the 3:1 ratio for resistant to susceptible. Backcross to susceptible parents showed a 1:1 ratio for resistant to susceptible. Backcrosses to resistant parents were all resistant. Both additive and non additive gene effects were important sources of variability in plant height, number of pods per plant, pod length, number of pods per pod and grain yield.

CHAPTER ONE

1.0 Introduction

Common bean (*Phaseolus vulgaris*) is the best known and most widely grown species of the genus *Phaseolus*. Beans are grown for their immature edible pods and for the dry ripe seeds, and to a lesser extent for green-shelled beans. Leaves are also eaten in some parts of the tropics. In Eastern and Southern Africa, common bean is recognised as the second most important source of human dietary protein after animal protein and a third most important source of calories (Pachico, 1993). Beans are rich in amino-acids lysine and tryptophan (Purseglove, 1974). These complement the amino acid methionine, which is in low quantities in maize protein – zein, found in maize, so that food with protein of a high biological value is achieved.

In Africa, annual bean production is estimated at 4 million hectares (ha) grown mostly by small scale farmers (Wortmann *et al.,* 1998). Most of this is in Eastern, Central and Southern Africa. Major seed classes grown include red mottled, large red kidney, small red, yellow, navy, purples, black and sugars (Appendix 1) (Wortmann *et al.,* 1998). These classes are grown in different areas, depending on local preferences and market demand.

Navy beans are usually grown for the canning industry. Navy beans are small whites that usually yield well under low-input conditions. They account for about 9% of total African production (Wortmann *et al.,* 1998), but are in high demand from the canning industry and in urban areas, where they are popular for their taste and relatively short cooking time. Commercial and small farm production of navy beans for export to canning markets has long been the dominant pattern in the Rift Valley and Hararghe areas of Ethiopia. Larger scale production is also found in South Africa and Zimbabwe. In other countries of eastern and southern Africa, they are grown on a small scale for the local industries. In east and central Africa alone, area under navy beans is estimated at about 310,000 ha, mostly in Ethiopia, Kenya, Sudan, Uganda and Tanzania.

Brown and yellow are other important seed types (Appendix 11) that account for about 11% of the production in Eastern and Southern Africa (Wortmann *et al.,* 1998). Brown and yellows are often grown in mixtures with other seed types (Wortmann *et al.,* 1998). These seed types are especially important in Angola, Zambia and Democratic Republic of Congo (DRC). They are also grown in a belt of countries extending from south-west Uganda to Mozambique

Yellow beans are an important class in Zambia, where they are common in most of the mixtures found in the market (Mulila -Mitti *et. al.,* 1989). Yellow beans are preferred for their high price and short cooking time. Some of the widely grown yellow beans include Pembela, Lusaka Yellow, Tabora. These cultivars fetch high prices and are comparable to Kabulangeti and white types. However, yellow bean cultivars are susceptible to diseases and as a result are low yielding. To increase production farmers tend to have two crops in a season (especially in Mbala area of Zambia). Breeding yellow bean cultivars with resistance to major diseases would increase their productivity and improve returns for farmers

Principal constraints to bean production in Africa include low soil fertility, periodic water stress, insect pests and diseases (Allen and Edje, 1990; Allen *et al.,* 1989). Major bean diseases are caused by fungi, bacteria and viruses. The most important bean diseases include angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*), bean common mosaic (caused by a virus) and root rots. Root rots are caused by several plant pathogenic fungi, major ones being *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp. and, *Sclerothium rolfsii* (Buruchara, 1993). Appendix 10 shows the losses caused by the major diseases of beans in Africa. The most important elements limiting soil fertility in Africa are nitrogen, phosphorous, aluminium toxicity and acidity related complexes.

1.2 Justification

Yield losses in beans due to diseases and soil related problems make it essential that cost effective management strategies be developed and used. Although fungicides can effectively control common bean diseases, the crop is mainly grown by small-scale farmers, who rarely use chemicals because they are expensive and uneconomical. Cultural methods such as rotation and use of clean seed can contribute to disease control, but land scarcity due to rapidly increasing human population make it difficult to practice crop rotation. Pathogen-free seed can be obtained by growing the crop in the dry season, when conditions are unfavourable for disease development. However this requires using irrigation, which is very expensive for the small-scale farmers. Moreover, some purchased seed is not always clean. Therefore, the strategy most likely to be effective in the management of bean diseases is an integrated disease management. This strategy should have a strong component of disease resistant varieties, that are environmentally friendly, safe to use and easy to adopt.

The current focus is to develop varieties that have multiple-constraint resistance. Therefore, breeding programmes aim at pyramiding or accumulating several resistance sources in a variety as a way of breeding broad and durable resistance in common bean in general, and in preferred bean classes in particular.

Currently Africa is a net importer of beans and indications are that it may take considerable time period to be self sufficient in dry beans. Therefore the strategy of developing cultivars that are tolerant to major biotic and abiotic stresses is aimed at increasing production and reducing the deficits. To realize this goal, it is important to identify sources of resistance to the diseases, determine their mode of inheritance and deploy the resistance genes in order to improve the preferred but low yielding types in these market classes.

However, currently preferred varieties of yellow beans are susceptible to diseases and lack tolerance to low P and low N. The most important diseases that adversely affect yellow beans are angular leaf spot and common bacterial blight. Little work has been done to breed yellow bean with resistance to angular leaf spot and common bacterial blight.

1.3 Objectives

The overall objective of this study was to contribute to the development of high yielding marketable yellow bean varieties with tolerance to angular leaf spot and common bacterial blight for production in east, central and southern Africa.

1.3.1 Specific objectives

1. To determine the inheritance of resistance to angular leaf spot (in Mexico 54)
2. To determine the inheritance of resistance to common bacterial blight (in Wilk 2 and VAX 6)
3. To develop and evaluate segregating populations of yellow beans with resistance to angular leaf spot and common bacterial blight.
4. To determine inheritance of some quantitative traits in segregating populations on yellow beans

1.4 Hypothesis

Resistance to angular leafspot found in Mexico 54 of the Mesoamerican gene pool is heritable and can be transferred to the Andean gene pool. Additionally, resistance to common bacterial blight found in VAX 6 and Wilk 2 is heritable and can be transferred to Andean gene pool.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of common bean

The common bean is the world's most important food legume, accounting for about 57% of the world's food legume production (CGIAR, 2001). The crop is a major staple in the diets, especially for the poor in sub - Saharan Africa. It provides an inexpensive source of protein for consumers and is often referred to as the "poor man's meat" (CIAT, 2001). It is characterised as a near - perfect food because of it's high protein content and generous amounts of iron, folic acid, complex carbohydrates and other essentials (Kornegay *et al.*, 1996). The crop is grown for its immature edible pods and for the ripe and dry seed (Purseglove, 1974). The leaves are edible and are also used as a pot herb in some parts of the tropics (CGIAR, 2001).

In Europe, the United States and other temperate countries, beans are grown mainly for the green immature pods, eaten as a vegetable and are also canned and frozen. Whole dried beans are also cooked with tomato sauce and canned and are usually referred to as baked beans. In eastern and southern Africa, beans, with 22.1% average protein content, rank as the second most important source of human dietary protein and the third most important source of calories (Pachico, 1993). In these areas, consumption of the crop exceeds 50 kg/ person per year. In Rwanda, beans provide 65% of dietary protein (Kornegay *et al.*, 1996). In Uganda, beans provide about 25% of the total calories and 40% of the protein intake. Therefore, it plays an important role in preventing malnutrition, a problem that would be highly prevalent in many parts of Africa where the basic diet is starchy food.

In addition to human nutrition, beans provide income for the small holder farmer through sale of surplus produce. Analysts estimate global production to be 18 million metric tonnes annually with a market value of US $10.7billion (CGIAR, 2001). Within the context of export diversification initiative, beans have gained a major dominance in terms of tonnage and monetary value of exports. Beans are also an attractive crop for farmers because of it's adaptability to different cropping systems and short growing cycle (CGIAR, 2001).

2.2 Growth requirements and production levels

Common bean does well in areas of medium rainfall from the tropics to the temperate regions. An annual total rainfall of about 600 to 650 mm is considered ideal. They can be grown on most soil types, from light sands to heavy clays and also on peat soils. However, they are sensitive to high concentration of manganese, aluminium and boron (Purseglove, 1974). Excessive rain causes flower drop and increase the incidence of disease. Moderate rain is required for flowering and pod setting period. High temperatures during the flowering stage lead to abscission of flowers and a low pod set resulting in low yields (Wortmann and Allen 1994). In Africa, production is concentrated in the cool highlands of central and tropical eastern Africa, where beans are the most important pulse crop (CIAT, 1989). However, beans are also grown as a winter irrigated crop in North Africa and parts of southern Africa. Within the highland areas, the production environment is diverse; the altitude ranges from 800 to 2300 m above sea level (CIAT, 1989). Beans are killed by frost. Day temperatures of below 20° C will delay maturity and cause empty mature pods to develop. Dry weather is required for the harvesting and shelling of beans.

In Africa annual production is estimated at 4 million hectares (ha) grown mostly by small scale farmers. Most of this is in eastern, central and southern Africa. The average yields by small scale farmers in Africa are below 600 kg ha^{-1}. Different seed classes are grown such as red mottled, large red kidney, small reds, yellows, navy, purples, black etc. (Wortmann *et al.,* 1998). These classes are grown in different areas, depending on the local preferences and market demand.

2.3 Bean gene pools and their characteristics

Common bean belong to two gene pools namely, Mesoamerican and Andean (Singh 1989). The Mesoamerican gene pool is characterised by small to medium seed size of all seed colours and growth habits. Leaf size and internode length are small, intermediate or large. The group is often characterised by an ovate, cordate or hastate terminal leaflet of the trifoliate leaves and large, broad cordate or lanceolate bracteoles. Pods are 8-15cm long, slender, fibrous or parchmented, and easy to thresh. They

contain six to eight seeds. On the other hand the Andean gene pool is mostly of growth habit I through to III. The gene pool is characterised by medium (25-40 g/100 seeds) and large seeds (>40 g/100 seeds) of often kidney or cylindrical shapes, which vary greatly in colour. Leaves are often large with hastate ovate or rhombohedric central trifoliolates, with long, dense straight hairs. Dry pods are fibrous, hard, medium to long (10-20 cm), leathery and posses four to six seeds.

Bean genotypes are of different characteristics. The major market classes in eastern, central and southern Africa are calima (red mottled), reds (large and kidney shaped) yellow and tans, white (large and medium), cream and purples, which all belong to the Andean race (Singh, 1989). Small reds, navy (small white) and blacks belong to the Mesoamerican race (Singh, 1989). Within the *P. vulgaris* species there are many seed types that differ in size, shape and colour. Within each market class there are different cultivars and the seeds of these differ very little from one another. However, considerable differences may occur in adaptability, growth habit, disease resistance and many other characteristics.

2.4 Bean production constraints in East, Central and Southern Africa

Principal agronomic constraints of bean production in eastern, central and southern Africa include soil fertility, periodic water stress, insect pests and diseases (Allen and Edje, 1990, Allen *et al.*, 1989). Bean diseases include those caused by fungi, bacteria and viruses. Among the most important bean diseases are angular leaf spot (*Phaeoisariopsis griseola* L.), anthracnose (*Colletotrichum lindemuthianum)*, rust (*Uromyces appendiculatus*), common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*), bean common mosaic and root rots. Root rots are caused by several plant pathogenic fungi, major ones being *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp. and *Sclerotium* spp. (Buruchara, 1993).

The most important soil fertility related elements that limit bean production in Africa are low nitrogen, low phosphorous, aluminium toxicity and acidity related complexes. Highly acidic soils, with pH as low as 4.2, are found in the bean-producing areas of Mbala district of northern Zambia, in the Usambara Mountains near Lushoto in Tanzania and in

the Nile-Zaire Crest of Rwanda (CIAT, 1989). In most bean growing areas of Africa, the crop is predominantly grown by small scale farmers and the yield are below 700 kg ha^{-1}, which is far below the potential of 2000 kg ha^{-1} and above, of the crop. These genotypes have continued to be used because they have other good characteristics that are preferred by the farmers. For this reason improvement of these genotypes is of paramount importance.

2.5 Bean Production in Zambia

Beans are an important source of protein for both urban and rural population of Zambia. Most of the beans in Zambia are grown in Region II and III, while Region I (Table 2.1) tends to be too hot for the crop, but could be grown under irrigation during the relatively cooler periods of the year (June-September).

Beans are predominantly used as dry grain. However, shelled green seeds, green pods and tender leaves, are also boiled and used as relish. Dry beans are usually boiled. Beans yields are very low. The national average is between 400 - 600 kg ha^{-1}. In most cases, the low yields are due to biotic and abiotic stresses (Greenberg *et al.*, 1986), while low yielding genotypes are also a major contributing factor to these low yields. Major biotic stresses include angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*), bean common mosaic (caused by a virus) and Ascochyta blight (*Phoma exigua var. diversispora).* Insect pests include bean stem maggot (*Ophiomyia phaseoli* and *O.spencerella*), bean foliage beetle *(Ootheca mutabilis*, aphids *(Aphis fabae)* and bruchids (*Zabrotes subfasciatus*). Abiotic stresses in Zambia include low N and P, aluminium toxicity, soil acidity and droughts in some areas.

In Zambia beans are predominantly grown in mixtures and intercropped with maize, sorghum, cassava and other crops (Mulila *et. al.*, 1989). In northern part of the country the common mixture is Mbala local (yellow, white and brown mixtures). Most popular mixtures have large, round white and yellow seeds with or without red or purple speckling and large pink oval seeds with red mottling. Black and small red types are not

popular (Mulila *et. al.*, 1989). These mixtures can later be separated into yellow, white and purple to fetch premium prices. The black grain type is never sold separately but always as a small component of a mixture. Lately, the yellow beans have become so popular that some local merchants buy them from Tanzania and export to Botswana. This trend could be attributed to the increased number of professionals in Botswana who are coming from bean consuming countries of southern and eastern Africa. Botswana is not traditionally a heavy bean consuming nation.

Small-scale farmers mainly grow the bean crop in Zambia. Most of the produce is sold on the informal market, suggesting that the reported production of 15,000 t per year is underestimate. An estimated 32000 ha (Wortmann *et al.*, 1998) are under bean production in Zambia, most of which is in the higher altitude, cooler and high rainfall zone (Region III) of Northern, North-western and Luapula provinces, followed by the medium rainfall warm zone (Region II) in Central, Lusaka and Eastern provinces. The drier and lower altitude western and southern provinces are probably too hot for bean production. Table 2.1 gives climatic characteristics of the different Agro-ecological zones (AEZ) in Zambia

Table 2.1: Altitude, rainfall and main crops in major bean growing areas of Zambia

Province	AEZ*	Altitude (masl)	Rainfall (mm)	Main crops grown
Northern	III	1200-1700	1000-1500	Beans, coffee,
Luapula	III	1200-1700	1000-1500	Finger millet,
North western	III	1200-1700	1000-1500	Maize, wheat
Copper belt	III	1200-1700	1000-1500	Cassava
Central	II	1000-1120	900-1000	Beans, maize,
Lusaka	II	1000-1200	900-1000	Groundnuts
Eastern	II	1000-1200	900-1000	

*AEZ- Agro-ecological zone

Source: Mulila *et al.*, 1989

In relative terms, bean production in Zambia has been low compared to neighbouring countries in the region such as Malawi and Tanzania. Average national bean production figures are less than 15,000 metric tonnes with over 60% being produced in the northern province of Zambia (Table 2.1). To make up for the bean grain demand deficit, Zambia has been importing beans from neighbouring countries. In 1997, the country imported 162 MT of beans from Malawi and in 1998, 1108 MT worth US$11,200 were imported from Tanzania (CSO, 1998). The tonnage of beans imported into the country informally from neighbouring countries especially Malawi and Tanzania is far much higher than the reflected official trade figures. This could be attributed to limited scope of monitoring seed exchange among farmers especially in the high bean producing areas in the border area; a fact that could be substantiated by the diversity of local bean varieties in Zambia.

Time series data on the production of bean grain in Zambia is characterised by instability. According to statistics obtained from the crop forecasting surveys conducted by the Central Statistics Office, over a five year period, the area planted to beans has been fluctuating while the average yield per unit area has almost remained low and somehow stable

The average yield per unit area reflected in the computations is so low probably because the data includes marginal areas not suitable for producing beans. However, data indicates that there is some marginal increase in the production of beans over the last five years, although both the area planted and the quantities of bean grain produced have been increasing. The seasons registering the increase coincide with the time when most improved varieties of beans were released by the Food Legumes Research Team in the country; making it more rational for one to assume that the rise in production was due to extensive promotion and dissemination of new technologies.

The crop enjoys a relatively higher urban market price compared to rural areas

In terms of income to the growers of beans, socio-economic studies so far conducted suggest that beans are increasingly becoming an important cash crop and that it is no

longer grown by women alone as per tradition but also by men. According to crop production statistics compiled by the Central Statistics Office in Zambia recently, about 472,757 households are growing mixed beans of which 386,563 (the majority) are male (CSO, 2003).

Although the promotion of food legume crops of which beans is part has received considerable attention from stakeholders over the recent past in Zambia, past agricultural policies were not very favourable. History contends that the first two and half decades following independence in Zambia were characterised by agricultural policies that were squarely focussed on maize promotion. Large-scale marketing support coupled with extensive fertiliser and input subsidies induced farmers to devote ever-larger area to maize production (Zulu, 2000) at the expense of crop diversification. Consequently when decades of large-scale maize subsidies came to an abrupt end with the change of government in 1991, farmers diversified out of maize production and reduced fertiliser use by over two-thirds as availability diminished and input prices escalated (CSO, 2003). It is in this context that present agricultural policies lays emphasis on crop diversification to include low input crops of the likes of food legumes which have proven to be more appropriate for resource poor small scale farmers.

2.5.1 Agronomic practices and cropping systems

Beans are predominantly grown by small-scale farmers in association with maize, sorghum, sweet potatoes and cassava, also by commercial farmers, mostly for seed production under improved management, including sole cropping. Time of planting varies according to location but is usually between December and end of February. The main bean crop is usually grown during the rainy season, but a dry season crop may be grown, especially in 'dambos' (depressed areas with high soil moisture content) from June to Sept. Most farmers do not apply fertilizer to their bean crop and the few who do, rarely use more than 30 kg ha^{-1}. Plant population in small scale farmers' fields are usually well below the recommended rates. Farmers usually weed their bean crop at least once during the growing season.

2.5.2 Bean Improvement in Zambia

Bean research in Zambia started in the mid 1950s at Lunzuwa research station, Mbala in the Northern province (Mulila *et al.*, 1989). Then the grain legume breeder, mainly responsible for groundnuts, also did some agronomic work on beans at Mount Makulu research station. Between 1966-1976 extensive varietal screening, breeding and agronomic work in Northern, Luapula and Copperbelt provinces resulted in the release of Misamfu speckled sugar, Misamfu stringless, Mexican 142 and Nanzinde (Mulila *et al.*, 1989). Mr. D. Roose, a virologist in the Belgian Cooperative Project at Mt. Makulu during 1977-1983 evaluated a number of bean lines from CIAT, and resulted in the release of BAT 85 and BAT 331. In 1985, Carioca was released through the efforts of Mr. Roose and the Grain Legumes Research Team (GLRT).

The adoption of carioca has been hampered by the rather small size. While the farmers were impressed with the yields of carioca, they were sceptical about the marketability of the variety in competition with the locally preferred large seeded types. In 1989, a bean hybridization program was initiated, which among other objectives, aimed at improving the yield level and stability, tolerance to major diseases and conformity to consumer preference of adapted, large seeded types.

2.6. Angular leaf spot

2.6.1. Distribution and economic importance

Angular leaf spot is primarily a disease of the tropics and subtropics where it is widespread in *Phaseolus*-bean producing areas. In Africa, angular leaf spot is considered to be the most widely spread and economically important disease of beans (Wortmann 1994). Wortmann and Allen (1994) reported that angular leaf spot was the most important Pan-African constraint to bean production. Angular leaf spot is an important bean disease which causes economic damage to beans in most parts of Africa. Wortmann and Allen (1994) reported that yield losses of 50-60% have been demonstrated on-farm in Democratic Republic of Congo (DRC). Hagedorn and Wade (1974) reported yield losses of about 50% in the U.S. Losses of 40-80% were reported in Colombia by Schwartz *et al.*, (1981). The disease significantly reduced the number of seeds per pod, as well as seed weight, premature defoliation, shrivelled pods and shrunken seeds (Santos - Filho *et al.*, 1978). Yield losses can reach 80% under severe conditions of infection (Schwartz *et al.*, 1981). Furthermore, yield losses of 384,200 tons per year due to the disease in Africa alone have been reported (Wortmann *et al.*, 1998).

2.6.2. Conditions favouring infection

Infection and disease development can occur over a wide range of temperature, 16-28°C with optimum of 24°C (Cardona and Walker, 1957). Fluctuating weather conditions (temperature, relative humidity, and sunlight) usually favour disease development (Correa-Victoria *et al* 1989). Environmental conditions are important for disease transmission to occur. Conditions favourable for the spread of the disease are air currents, splashing water and high humidity (Correa-Victoria, 1984; Saettler and Correa, 1988).

2.6.3. Causal Organism and transmission

Angular leaf spot is caused by the fungus *Phaeoisariopsis griseola*. Angular leaf spot is primarily a disease of the tropics and subtropics where it is widespread in *Phaseolus*-bean producing areas. The angular leaf spot pathogen has been reported to to have a high degree of pathogenic variability (Sartorato, 2002; Pastor Corrales et al., 1998). Barrows and Cardona (1958) pointed out that the epiphytotic of angular leaf spot usually result from lack of crop rotation, using overhead irrigation to obtain four crops a year, thus

providing suitable moisture for infection. Similarly, Wallace (1952) and later (Allen *et al.*, 1996) found that prolonged rains favoured multiplication of the disease. Humidity and moisture are essential for optimal infection. Cardona- Alverez (1956) and Allen *et al.*, (1996) found that the main source of inoculum was infected debris from the previous season. It could also be seed borne (Allen *et al.*, 1996). Dhingra and Kushalappa (1980) and Saettler and Corea (1988) reported infection percentages up to 9% in bean seeds from plants showing heavy pod attack. Only seeds located underneath pod lesions located at the pod suture were found to be infected, suggesting that seed infection takes place through the helium (Dhingra and Kushalappa, 1980; Sengooba and Mukiibi, 1986). There is high pathogen diversity resulting in varieties exhibiting different reactions at different locations. In this study a highly virulent isolate, Pg 63-55 was used in this study.

2.6.4. Symptomatology

Angular leaf spot occurs on all aerial plant parts. Lesions may appear on the primary leaves, but usually do not become prevalent on subsequent foliage until late flowering or early pod set (Allen *et al.*, 1996) Spots originating on the lower leaf surface are delimited by the veins and veinlets and develop into grey lesions, which later turn light-brown. Lesions may be surrounded by a chlorotic halo but they lack a coloured border. The striking angularity of the spots is a diagnostic feature of *P. griseola*. The lesions may be so numerous as to cause premature defoliation (Allen *et al.* 1996).

Leaves on primary the lesions are round and usually larger than the spots on trifoliate leaves and may develop concentric rings. Initially the leaf spot are grey, but later become dark brown. On trifoliates they have an angular shape and are surrounded by a chlorotic halo. Under humid conditions, leaf lesions become covered with small black columns of hyphae, which bear the spores of the fungus (Allen *et al.* 1996). The older leaves of adult plants usually show more severe symptoms. Lesions may cover large areas of the leaf, leading to premature defoliation. Lesions on pods are less frequent than on leaves. On pods the lesions appear as oval or circular spots superficial at first, with reddish brown centres surrounded by dark coloured borders (Allen *et al.* 1996). The spots vary in size and, ultimately, may become so crowded that they coalesce and

occupy the width of the pod. Infected pods will have poorly developed or shrivelled seeds. Lesions on stems and petiole are brown and elongated.

2.6.5. Management Practices

The recommended methods for the control of angular leaf spot can be grouped into cultural, biological, chemical, host plant resistance and integrated.

Angular leaf spot is believed to be spread from infected straw and volunteer plants grown out of season. Barros *et al* (1958) suggested that crop rotation, possibly with two years between bean crops to allow for decomposition of plant residues, and restricting growing to one time of the year. Wallace (1952) suggested that sowing should not be spread over a long period and debris from the previous season should be destroyed. Use of clean seeds is also an effective method of control, since the pathogen is also seed borne, (Grogan and Kimble, 1967; Karanja *et al* 1994). The use of clean seed as a means of controlling diseases is not easy for small scale farmers as they always recycle or buy from their neighbours who could be selling diseased seed.

Chemical control of the angular leaf spot is possible by seed treatment or foliar spray. A number of chemicals have been found to be effective in control of angular leaf spot. These include zineb (zinc ethylene 1,2 - bisdithiocarbamate), which appear to be more effective. Other fungicides, which have proved effective, include benomyl, captafol, captan, carbendazim, thiophanate-methyl, triforine and ziram (Ploper, 1980; Srivastava and Gupta, 1994). Hidalgo and Araya (1993) recommended spraying at growth stages R5 (before flowering) and R7 (pod formation).

The effectiveness of cultural and chemical control methods is limited due to the high production costs of using chemical control, the ability of the pathogen to survive in plant debris for a long period of time and land availability for crop rotation. In view of this, the development and introduction of resistant varieties combined with other control practices, is regarded as the most effective and relatively cheap means of reducing disease incidence and consequently the yield losses.

Integrated disease management (IDM) uses the philosophy of managing the pest population rather than eliminating it. This implies that the elimination of the problem is general and long term with minimum harmful side effects. IDM is a combination of methods chosen to supplement natural control and give the maximum long-term reliability with the cheapest and least objectionable protection. In beans this method is usually the combination of cultural, chemical and biological methods in controlling the diseases.

Biological control is the use of biological organisms, for example; viruses, bacteria or fungi for the control of pathogens, insect pests or weeds. This method is probably almost as old as the history of agriculture. Biological control of bean diseases has not been very widely tested and used by bean farmers.

2.7 Common bacterial blight

2.7.1. Distribution and economic importance

Common bacterial blight is distributed world wide (Mukunya *et al.,* 1981). The disease is most prevalent at low to mid-altitudes under warm conditions (CIAT 1996). In Africa it has been reported as being of high importance in Malawi, Kenya, Ethiopia Uganda, Burundi, Zambia, Lesotho, D.R. Congo (Wortmann, *et al* 1998). In 1967, common bacterial blight damaged at least 75% of Michigan's 260,000 hectares of navy beans with 10-20% yield reduction (Focus on Michigan's bean Industry, 1971). In two years of field trials, Wallen and Jackson (1975) reported a 38% yield loss in Ontario, Canada, due to common bacterial blight. Yield losses estimated at 25% and 45% have been obtained by natural and artificial infections respectively, in Colombia (Yoshii *et al.,* 1971a). Losses of 26-62% have been recorded in Uganda, where it is estimated that each 1% increase in blight severity causes yield loses of 10.5 – 78 kg ha^{-1} depending on the season and crop growth stage (Allen *et al* 1996). In sub-Saharan Africa common bacterial blight causes losses of about 220,400 metric tons per year alone (Appendix 10) (Wortmann *et al.,* 1998).

2.7.2. Conditions favouring infection

Common bacterial blight is most prevalent at low to mid altitudes under warm conditions. It causes greater damage to plants at 28°C than at lower temperatures. The pathogen grows

optimally in vitro from 28 to 32°C. Growth declines gradually as temperature is lowered and stops at 16°C. In general, however, common bacterial blight epidemics are favoured by high temperature and humidity (Sutton and Wallen, 1970).

2.7.3. Causal organism and transmission

Common bacterial blight is a bacterial disease caused by *Xanthomonas campestris* pv. *Phaseoli,* also known as *Xanthomonas campestris* pv. *phaseoli* (Vauterin *et al.* 1995). Common bacterial blight of beans is seed borne. Infected seed is important for long distance and local dissemination of the bacteria (Zaumeyer and Thomas 1957). Seeds are the primary source of inoculum for common bacterial blight of beans (Sutton and Wallen, 1960). Water is a potential agent in the spread of common bacterial blight. Water contaminated with common bacterial blight if used for surface irrigation on beans will spread the bacteria (Steadman *et al.*, 1955). Rain splash and wind can transfer inoculum from infected plants to healthy plants. The spread from plant to plant is achieved through the slime exudation from infected plants.

2.7.4. Symptoms

Leaf symptoms initially appear as water-soaked spots, which enlarge and frequently coalesce with adjacent lesions. Infected tissues appear flaccid and lesions are often encircled by a narrow zone of lemon-yellow tissue (Allen *et al.* 1996). Pod lesions appear as water-soaked spots, which may enlarge and become dark, red and slightly sunken. If infection occurs during pod and seed development, infected seed may rot or shrivel. Symptoms on seed manifest as butter-yellow spots on white or light coloured seeds (Saettler and Perry, 1972; Allen *et al.* 1996). but not easily seen on medium to dark-coloured seeds. Seedlings that develop from severely infected seed may have damaged growing tips, become stunted, or are killed (snakehead) (Zaumeyer and Thomas, 1957). The bacteria attack aerial parts of the plant, including leaves, petioles, pods and seeds.

2.7.5. Management practices

Recommended control measures for common bacterial blight of beans include cultural methods, chemical and use of resistant varieties.

Cultural practices are important in controlling common blight. Eliminating weeds, volunteer beans and other potential hosts of *X. campestris* pv. *phaseoli* will reduce disease incidence. Good weed control will not only remove potential sources of epiphytotic *X. campestris* pv. *phaseoli* populations, but will also improve aeration around the crop so that the plants dry faster, thus reducing the chances for bacterial spread and infection. *X. campestris* pv. *phaseoli* is readily spread by water (Allen *et al.* 1996). Walking or working in the field while plants are wet will splash the bacteria and create wounds. Plants should be allowed to dry before allowing workers or machinery to enter. Eliminating infected plant debris is very important, particularly in tropical regions (Saettler, 1991). A rotation of at least two years between bean crops will give time for the *X. campestris* pv. *phaseoli* population to decline in the debris. Deep ploughing will also encourage the breakdown of infected plant debris and reduce the population of *X. campestris* pv. *phaseoli* (Gilbertson and Hagedorn, 1990). Another option is to burn the crop debris to eliminate infected plant material. The incidence of *X. campestris* pv. *phaseoli* can also be reduced if beans are grown with maize rather than in a monoculture (Van Rheenen *et al.*, 1981; (Allen *et al.* 1996). Maize crop appears to provide a physical barrier to the movement of *X. campestris* pv. *phaseoli* between bean plants.

Chemical control may reduce leaf infection but usually has little improvement on yield. Copper compounds may be used (Weller and Saettler, 1976). Foliar antibiotic treatment can provide some control but is undesirable because it can result in antibiotic-resistant mutants of *X. campestris* pv. *phaseoli*.

There are no reports of high resistance to *X. campestris* pv. *phaseoli* in *P. vulgaris*. However, many lines of *P. vulgaris* show some resistance to *X. campestris* pv. *phaseoli*. Such varieties may be planted if available. Increased resistance can be developed by selecting for horizontal rather than vertical resistance (Garcia-Espinosa, 1997). Partial resistance to *X. campestris* pv. *phaseoli* in *P. vulgaris* has been linked to delayed flowering under long photoperiods *P. acutifolius* is highly resistant to *X. campestris* pv. *phaseoli*. Partial resistance has been transferred from this genotype to *P. vulgaris* (Goodwin *et al.*, 1995). There are also several other reports of resistance transferred

from *P. acutifolius* to *P. vulgaris* (Thomas and Waines, 1984; Park *et al.*, 1998; Yu *et al.*, 1998). Resistance in *P. acutifolius* is controlled by one or two dominant genes and is related to the hypersensitive response (Zapata, 1998; Urrea *et al.*, 1999). In addition, crosses between *P. coccineus* and *P. vulgaris* also showed resistance to *X. campestris* pv. *phaseoli* (Zapata *et al.*, 1985; Yu *et al.*, 1998). A number of *P. vulgaris* lines with varying levels of resistance to *X. campestris pv. phaseoli* have been registered (Miklas *et al.*, 1999).

The effectiveness of cultural and chemical control methods is limited due to the high production costs of using chemical control. In view of this, the development and introduction of resistant varieties combined with other control practices, is regarded as the most effective and relatively cheap means of reducing disease incidence and consequently reduce yield losses.

2.8 Breeding for disease resistance

The first study of genetics of disease resistance was that by Biffen in 1905 (cited by Singh,1995). He reported the inheritance of resistance to leaf rust of wheat variety Rivet in crosses with susceptible varieties. In F_2 there were 3 susceptible to 1 resistant plant, indicating that the resistance was controlled by a single recessive gene. Subsequently, several other studies showed that resistance to various diseases is monogenically determined, but cases of duplicate, complementary and other interactions have been reported (Singh, 1995).

To put disease resistance in its proper place in breeding strategy then, we note that; a little resistance to several diseases is usually necessary because extreme susceptibility to what is normally thought of as a minor disease can kill an otherwise excellent variety and high resistance approaching immunity, is good to have if it can be got without compromising other characteristics, but the example of many successful varieties shows us that moderate resistance wisely used can be agriculturally very satisfactory.

2.9 Breeding for resistance to angular leaf spot and common bacterial blight

Breeding for resistance to angular leaf spot (ALS) has been done by several people in the region. CIAT –ALS nursery was distributed in the region (eastern and southern Africa) in the mid 80s and these were evaluated for resistance to angular leaf spot. In Zambia out of the tested 627 lines, six were found to be resistance to angular leaf spot; sixty were found to be moderately resistant (Mulila *et al.*, 1989).

2.9.1 Sources and mode of resistance to angular leaf spot

Diverse sources of resistance to angular leaf spot in bean genotypes have been reported (Correa *et al.*, 1989; Beebe and Pastor Corrales, 1991). Examples of resistant cultivars include A 75, A 140, A 152, A 175, A 229, BAT 76, BAT 431, BAT 1432, BAT 1458 and G5686 (CIAT,1984). Regagnin *et al.*, (2005) found angular leaf spot resistance in AND 277, Cornell 49-242 was found to be resistant to Angular leaf spot (Nietsche *et al.*, 2000), while Mahuku *et al*,.(2004) found resistance in G 10474. CIAT (2003) reported resistance in Mexico 54. Sources of resistance reported from Africa include GLP 24, GLP X-92, GLP - 806 and GLP 77(CIAT 1984). Other source include CAL 143. Resistance sources have also been identified in the secondary and tertiary *Phaseolus* gene pools namely wild and weedy *P. vulgaris*, *P. coccineus* and *P. polyanthus*.

Only a few studies of inheritance have been conducted for angular leaf spot and the results are not in agreement. Santos-Filho *et al.* (1976) reported that resistance in Coraota 260 is controlled by a single recessive gene. However, resistance was dominant in a few crosses. Resistance to specific isolates of *P.griseola* has been reported to be simply inherited and molecular markers have been identified for some these resistance genes (Mahuku *et al.*, 2004), Ferreira *et al.*, 2000; Miklas *et al.*, 2005). Cardona-Alvarez (1962) reported that resistance was controlled by a single dominant gene. It is possible that several disease-resistance mechanisms to *P. griseola* in beans exist, thus the different results.

2.9.2 Sources and mode of resistance to common bacterial blight

Sources of resistance to common bacterial blight have been reported in tepary bean, *P acutifolius* (Schuster *et al.,* 1983). Singh and Muñoz (1999) reported resistance breeding to common bacterial blight through gene pyramiding. Exceptional lines resistant to common bacterial blight developed at CIAT, found to be performing very well included VAX 3, VAX 4, VAX 6, XAN 159. Moreover VAX 3, VAX 4 and VAX 6 possess levels of CBB resistance that are as high as those found in *P. acutifolius* accessions (Singh and Muñoz, 1999). This suggests that the most sound strategy for breeding for resistance is to pyramid resistance genes from several different *Phaseolus* species. Deidré (2002) found that Wilk 2 was resistant to CBB in most areas it was tested and used it in the improvement programme.

Silva *et al.* (1989) reported that inheritance of resistance to common bacterial blight in the trifoliate leaf and plant canopy was controlled by a single major gene. Yonghe Bai *et al.* (1996) reported that genetic studies indicate that resistance to common bacterial blight is different depending on the source of resistance and may be determined by both major and minor genes (McClory, 1985)

2.10 Breeding methods

Breeding for resistance to diseases has utilized the following methods; pure-line selection, pedigree, bulk and backcross methods (Simmonds, 1979).

Backcrossing method is based on repeated backcrossing of the F_1 and the subsequent generations to the recurrent parent (Singh 1983). In this method the hybrid and the progenies in the subsequent generations are repeatedly backcrossed to one of the parents. As a result, the genotype of the backcross progeny becomes increasingly similar to that of the parents to which it is backcrossed. Hayward, *et al.* (1993) mentions backcrossing (BC) as a breeding method used to transfer useful genes from a genetic stock (population, inbred line, individual plants, varieties and wild plants) called the donor to a breeding material inbred line or population which is desired to improve, the recurrent parent. The method consists of crossing the donor and the recurrent parent to make F_1 generation followed by one or more backcrosses to the recurrent parent. Types

of genes that can be transferred using backcrossing include single dominant genes, single recessive genes or polygenes underlying a quantitative trait (Singh 1983). In the backcross method, the hybrid and the progenies in the subsequent generations are repeatedly backcrossed to one of their parents. The objective of the backcross is to improve one or two specific defects of a variety, which is adapted to an area and has other desirable characteristics.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Experimental sites

This study was conducted in greenhouse and field conditions. Generation of study populations and evaluation for resistance were done in the greenhouse at Kabete Field Station, College of Agriculture and Veterinary Sciences, University of Nairobi, Kenya. Field experiments were conducted at Kabete Field station found at latitude 11° 4' 20" S and longitude 36° 45' E. The altitude is 1820 metres above sea level. Kabete has an average annual rainfall of 1046 mm. Mean maximum temperatures is 23° C and a minimum of 12° C. Dominant soils are well drained, very deep dark reddish brown friable clays.

3.2 Plant Materials and Plant Culture

Two Zambian varieties, Lusaka Yellow and Pembela, which are widely grown and preferred, but susceptible to angular leaf spot and common bacterial blight, were used as females. The source of resistance for angular leaf spot was Mexico 54. Resistant parents to common bacterial blight were Wilk 2 and VAX 6. Characteristics of these parental lines are shown in Table 3.1.

3.3 Generation of Experimental populations

The seeds of the parental lines were sown in the screen house in rows that were 60 cm apart and 15 cm within row spacing. Planting was done in a staggered manner to synchronize days to flowering. Di-ammonium Phosphate (DAP - 18% N and 46 % P_2O_5) fertiliser was applied at planting time at the rate of 150 kg ha^{-1}. Weeding and irrigation was done whenever required.

Table 3.1: Some characteristics of parental lines used in the study

Genotype	Origin	Seed colour	Growth habit [a]	Seed size	Days to flowering	Reaction to ALS [b]	Reaction to CBB [b]
Mexico 54	CIAT	Pink	III	Medium	43	resistant	moderate
Wilk 2	CIAT	White	I	Small	35	resistant	resistant
VAX 6	CIAT	Dark red	II	Small	35	resistant	resistant
Lusaka Yellow[c]	Zambia	Yellow	II	Medium	41	susceptible	susceptible
Pembela[c]	Zambia	Yellow	II	Medium	33	susceptible	susceptible

[a] I -determinate; II -indeterminate erect; III -indeterminate semi-prostate and IV -indeterminate prostate.

[b] ALS = Angular leaf spot; CBB = Common bacterial blight

[c] Land race

The two susceptible parents, Lusaka Yellow and Pembela, are local landraces in Zambia, while Wilk 2 and VAX 6 are CIAT lines obtained from the Dry Bean Programme of the Agricultural Research Council of South Africa. Seed of Mexico 54 was obtained from the Regional Bean Programme at Kabete, University of Nairobi.

Mexico 54 is an angular leaf spot resistant variety developed at CIAT. It has been tested widely in Africa and showed resistance to most pathotypes. Mexico 54 is an indeterminate prostrate, semi-climber (type III). It has medium sized seeds that are pink in colour.

Wilk 2 is a common bacterial bight resistant line resulting from the work at Cornell University and CIAT in the 1980s. It is strongly believed that it has common bacterial blight resistance from three species: *Phaseolus vulgaris*, *Phaseolus coccineus* and *Phaseolus acutifolius*, including XAN 159 or its sisters (Singh and Muñoz, 1999). Wilk 2 is a determinate upright type (Type I). It has small white seeds.

VAX 6 is a common bacterial blight resistant line developed at CIAT from interspecific hybridisation of *Phaseolus vulgaris* and *Phaseolus acutifolius* and gene pyramiding.

VAX 6 is an indeterminate upright type (Type II), with small red seeds. The line possesses levels of common bacterial bight resistance that are as high as those found in *Phaseolus acutifolius* accessions (Singh and Muñoz, 1999).

Lusaka Yellow is a local landrace variety grown in all bean growing regions of Zambia. The variety is susceptible to angular leaf spot and common bacterial blight. Lusaka Yellow has an indeterminate upright type (Type II) with medium seed size which are yellow. It fetches premium prices in local markets in Zambia due to its colour and seed size.

Pembela is a Zambian local variety which is also grown in most bean growing areas of the country. Pembela is preferred for its colour and size, and cooks fast hence the name 'Pembela' meaning 'wait', because it cooks within a short time. It is susceptible to angular leaf spot and common bacterial blight. It is an indeterminate upright type (Type II) with medium size, yellow seeds.

3.3.1 Crosses

The six crosses made were:

1. Lusaka Yellow X Mexico 54
2. Pembela X Mexico 54
3. Lusaka Yellow X Wilk 2
4. Lusaka Yellow X VAX 6
5. Pembela X Wilk 2
6. Pembela X VAX 6

Crosses 1 and 2 were used to study inheritance of resistance to angular leaf spot; 3 - 6 were for common bacterial blight studies.

3.3.2 Emasculation and pollination

Emasculation and pollination was done during the early morning hours (before 11:00hrs) and in the evening (after 17:00hrs). This ensured that desiccation of the freshly pollinated stigma was avoided. Buds, which were plump, showing colour and would open the following day were chosen as the female flowers. Using a fine tipped forceps, the standard

petal was opened by inserting the point of the forceps into the suture and pushing from side to side. The wings were carefully removed with the forceps to expose the coiled keel. A small incision was made near the base of the keel with the point of forceps and the upper half of the keel was grasped and carefully peeled up and back to expose the anthers and the stigma (Tumwesigye, 1988). The anthers were carefully examined to find out if they had dehisced and shed pollen. If they had, the flower was not used because self-pollination may have occurred. If the anthers had not shed pollen, all the stamens were removed carefully.

After emasculation of the female flower, pollination was done immediately using the rubbing or hooking method (CIAT, 1987; Buishand, 1956). Freshly opened flowers were chosen from donor parents to provide pollen. The wings were removed using the forceps and half the coiled keel removed by peeling up and back. In the hooking method the stigma bearing loosely attached pollen was pulled and hooked on the stigma of the female flower. After hooking, the standard petal was carefully closed enclosing the female stigma with the hooked -on male contents. After crossing a cotton thread and a tag labelled with the pedigree of the cross was tied loosely on the flower stalk.

At maturity the pods were harvested together with their identification tags. These were sun- dried and threshed to give F_1 seed. Part of the F_1 seed from each cross was sown in the screen house to produce F_2 seeds and also backcrossed to both parents.
The experimental materials consisted of six basic populations, namely P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2. The progeny derived from backcrossing F_1 to the female parent was designated BC_1P_1 and those from the backcrossing to the male parent as BC_1P_2.

3.4 Pathogen culture and inoculum preparation

3.4.1 Pathogen isolation and culturing

Isolation of angular leaf spot was made from lesions of naturally infected bean leaf showing fungal sporulation. In case of non-sporulating lesions, the fungus was induced to sporulate by incubating the infected tissues in moist chambers. Small pieces of infected tissues were surface sterilised with 3 % (v/v) sodium hypochlorite for 5 minutes

and rinsed in 3 changes of sterile distilled water. The sterilised tissues were plated on BLDA and incubated for 14 days and purified on fresh media.

Common bacterial infected leaves were collected from Kabete Field Station and Nyahururu area. The pathogen was prepared by cutting off infected portion of the leaves into small portions. The portions were put in 2.5 % sodium hypochlorite solution for 2-3 minutes. They were then washed in 3 changes of sterile distilled water. The cut leaf pieces were then placed into Mcarteney bottles containing small amount of sterile water and macerated using a sterile glass rod. Then a loop-full of bacterial extract was picked and streak on nutrient agar and incubated at 27 ^{0}C for 48 hours.

Three weeks old bean seedlings which were planted in inoculation chambers were watered to give high humidity 24 hours before inoculation. Using a half litre Baygon atomizer (Bayer E.A), the plants were inoculated mainly on the abaxial side of the first primary and trifoliolate simple leaves at a distance of 10-15 cm until run off.
Control plants were sprayed with sterile distilled water. The plants were maintained in the chambers until maturity and examined for disease symptom development

3.4.2 Multiplication of Inoculum and Inoculation

Bean leaf decoction agar medium (BLDA) (Karanja *et al.* 1994) was used to culture angular leaf spot pathogen *P. griseola.* 100 g of freshly collected bean leaves were weighed and crushed in a blender with small amount of distilled water. The mixture was filtered through a double layer of cheese cloth and sterile water was added to make up 1 litre. 20 g of glucose and 20g of agar were then added and the pH of the mixture adjusted to 6.8. The mixture was sterilized in an autoclave at 121^{0}C at 15 psi for 15 minutes and approximately 20 ml was dispensed into sterile petri dishes. The inoculum was harvested by flooding the petri dishes with the fungal growth with sterile distilled water and using a glass slide the growth was scraped carefully. The suspension was filtered through a triple layer of muslin cloth. The number of conidia in a millilitre of water was determined using a haemocytometer. The suspension was adjusted up to the required concentration of 2x10^{6} conidia per ml.

The common bacterial blight inoculum was prepared by flooding the petri dishes containing the bacterial growth with sterile distilled water. Using a bent glass rod the bacteria was scraped off. The bacterial population was then adjusted to $3x10^9$ colony forming unit (CFUs) per millilitre by the plate count method

Three weeks old bean seedlings, which were planted in inoculation chambers, were watered to give high humidity 24 hours before inoculation. Using a half litre Baygon atomizer (Bayer E.A), the plants were inoculated mainly on the abaxial side of the first trifoliate and primary simple leaves at a distance of 10-15 cm until run off.

A turbid common bacterial suspension with a density of 10^6 bacteria cfu of sterile distilled water was used as inoculum to infect the plants. Plant leaves were artificially injured using a multiple needle puncher. This was done to aid the bacteria entry into the plant system. The inoculum was applied to the leaves at 40 to 50 psi pressures at a 10-15 centimetres distance from nozzle to leaf. A repeated inoculation was carried out at the early reproductive stage. Only one repeated inoculation was done. Control plants were sprayed with sterile distilled water. The plants were maintained in the chambers for the whole growing period and examined for disease symptom development.

3.5 Inheritance of resistance to angular leaf spot and common bacterial blight

3.5.1 Greenhouse experiment

This experiment was conducted in the greenhouse to determine the inheritance of resistance to angular leaf spot and common bacterial blight. In this experiment, three to four seeds of parents and their F_1, F_2 and backcross progenies for each cross were sown in plastic pots containing sterilized medium composed of soil, manure, sand, and ballast in the ratio 2:1:1:1:1, respectively. Three to four seeds of each parent and F_1 were planted in three pots per replication. The BC_1P_1 and BC_1P_2 were planted into 14 pots per replication. F_2 progenies were planted in 20 pots per replication. Seedlings were allowed to grow in chambers constructed to provide a conducive environment for disease development. The experimental design was a randomised block design with

three replications. Sisal ropes were used to support the plants that showed some climbing tendency. The plants were kept weed free and watered whenever necessary.

3.5.2 Field Experiment

The parents (P_1 and P_2), F_1, F_2 and the backcross generations (BC_1P_1 and BC_1P_2) were grown in a randomised complete design with three replications at Kabete field station, University of Nairobi. The design was chosen due to the fact that the field was relatively small and uniform. The parents and F_1 were sown in one row each per replication. Four rows for the F_2 generations and four for the backcrosses, in each replication were planted. The rows were 3 m long and 50 cm apart. The distance between plants was 15 cm. A total of 60 plants for each of P_1, P_2 and F_1 generations and 360 plants for the F_2, $BC_1P_1$and BC_1P_2 generations were planted. Sticks of about 2 m long were used to support the climbing genotypes. Data collected from the field experiment for quantitatively inherited traits were included, days to flowering and days to maturity, which is the number of days from planting to the date when 50% of the plants in a plot had flowered and were physiologically mature, respectively. Plant height was recorded as the length from the base of the plant at soil level to the tip. Average plant height of five randomly selected plants in the plot was obtained. The number of pods per plant was calculated by taking the total number of pods from 5 randomly selected plants in a plot. Pod length was measured from the base to the tip of the pod. The total length of the pods from the 10 randomly selected plants was averaged to give the pod length. The total number of seeds from the five randomly selected plants in a plot was divided by the total number of pods to estimate the number of seeds per pod. A random sample of 100 seeds was weighed to determine the 100 seed weight for each progeny. Seeds from all the plants in a plot were weighed and the total weight averaged to give the seed yield per plant that was then multiplied by the number of plants in one hectare to give seed yield per hectare.

3.6 Disease Assessment

Disease assessment started 15 days after inoculation for angular leaf spot and 30 days for common bacterial blight when symptoms had appeared. The assessment was based on the CIAT (1987) 1-9 disease severity scale (Table 3.2). Two assessments were done in the screen house for both angular leaf spot and common bacterial blight. Each plant was assessed by scoring three trifoliate leaves starting from the base. A mean score was calculated for each plant and this was used to determine the level of reaction to the pathogen

Table 3.2 General scale used to evaluate the reaction of bean germplasm to fungal and bacterial pathogens (van Schoonhoven, 1987)

Rating	Category	Description	Comments
1 2 3	Resistant	No visible symptoms or very light symptoms (5-10%)	Germplasm useful as parent or commercial variety.
4 5 6	Intermediate	Visible and conspicuous symptoms resulting only in limited economic damage (10-60%)	Germplasm can be used as commercial variety or source of resistance to diseases
7 8 9	Susceptible	Severe to very severe symptoms causing considerable yield losses or plant death(60-100%)	Germplasm in most cases not useful as parents or commercial varieties

Plants with scores of 1 to 3 were considered to be resistant, 3-6 as intermediate and those with scores ranging from 6 to 9 were rated susceptible.

3.7 Data Analysis

Genstat statistical package (Genstat 6.1) was used to analyse the data. Chi-square method was used to test significance of observed segregation ratios. Analysis of variance (ANOVA) was used to estimate genetic variances for quantitative traits. The disease severity data was subjected to qualitative genetic analysis using a chi- square to compare the Mendelian segregation of observed to hypothetical ratios. The hypothesis is that observed ratio did not differ from the expected monohybrid or dihybrid ratios, or test cross. Heterosis for each of the traits was calculated from the mid-parent and better parent values and defined as follows;

$$\% \text{ Heterosis} = \frac{F_1 - MP}{MP} \times 100$$

Where F_1 = Hybrid mean
MP = mid parent value

Variance components were calculated using the following formulae

$VP_1 = E$

$VP_2 = E$

$VF_1 = E$

$VBC_1 (F_1XP_1) = 1/4A + 1/4D + E$

$VBC_2 (F_2XP_2) = 1/4A + 1/4D + E$

$VF_2 (F_1XF_1) = 1/2A + 1/4D + E$

W here;

P_1 - Parent 1

P_2 - Parent 2

F_1 - Resultant progeny

F_2 - Progeny of cross F_1xF_1

BC_1 - F_1xP_1

$BC_2 - F_1xP_2$

Heritability was calculated as follows
$h^2 = VA/VP$

CHAPTER FOUR: RESULTS

4.1. Qualitative traits

4.1.1 Inheritance of resistance to angular leaf spot

4.1.1.1 Symptoms

Angular leaf spot symptoms appeared on the susceptible parents and progenies 24 days after inoculation. The development of the disease on susceptible lines was after within 5 days of inoculation progressing from rating 4 to 9 in 3 weeks. This was more so on Lusaka Yellow (Plate 4.1) than on Pembela. However, disease development did not differ among the susceptible progenies.

No disease was observed on Mexico 54 (Plate 4.2). Progenies in F_2 segregated for both susceptible and resistance (Plate 4.3)

Plate 4.1 Lusaka Yellow (P_1) showing angular leaf spot symptoms on leaves.

Plate 4.2 Mexico 54 showed resistant reaction after inoculation with *P. griseola* isolate 63-55.

Plate 4.3 Lusaka Yellow x Mexico 54 F_2 plants segregating for resistant (R) and susceptibility (S) to angular leaf spot.

Pembela showed susceptible symptoms (Plate 4.4

Plate 4.4: Pembela (P_1) showing angular leaf spot symptoms on leaves.

4.1.1.2 Lusaka yellow x Mexico 54

All the plants of Lusaka Yellow were susceptible while those of Mexico 54 were resistant. All the F_1 plants showed resistant reactions to *P. griseola* isolate 63-55. Isolate 63-55 was found to be more virulent than other isolates tested hence the decision to use it. The backcross to Lusaka Yellow showed 1:1 segregation ratio while the backcross to Mexican 54 had all the progenies resistant. The observed number of plants resistant and susceptible to angular leaf spot in P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 progenies of the crosses Lusaka Yellow and Mexico 54 are shown in Table 4.1.

Table 4.1: Reaction of Lusaka Yellow and Mexico 54, their F_1, F_2, $BC_1P_1$and BC_1P_2 progenies to inoculation with isolate 63-55 of *Phaeoisariopsis. griseola*

Parent/cross	Generation	Number of plants			X^2	Pr
		Resi-stant	Susce-ptible	Expected Ratio		
Lusaka Yellow (LY)	P_1	0	25			
Mexico 54	P_2	27	0			
LY x Mexico 54	F_1	37	0			
LY x Mexico 54	F_2	145	44	3:1	0.25	0.5-0.7
(LY x Mexico 54) x LY	BC_1P_1	63	56	1:1	0.54	0.3-0.5
(LY x Mexico 54) x Mexico 54	BC_2P_2	115	2	1: 0	0.0	1.00

4.1.1.3 Pembela x Mexico 54

All Pembela plants were susceptible to the angular leaf spot isolate *Pg* 63-55 used in the study, while all Mexico 54 plants were resistant (Table 4.1). The F_1 were all resistant while F_2, segregated in the 3:1 ratio for resistance to susceptible. Backcrosses of the F_1 to the susceptible parent produced progeny that showed a 1 resistant: 1 susceptible ratio. However backcross progeny to the resistant parent were all resistant.

Table 4.2: Reaction of Pembela and Mexico 54, their F_1, F_2, $BC_1P_1$and BC_1P_2 to inoculation with isolate 63-55 of *P. griseola*

Parent/cross	Number of plants: Generation	Resistant	Susceptible	Exp. Ratio	X^2	Pr
Pembela	P_1	0	29			
Mexico 54	P_2	30	0			
Pembela x Mexico 54	F_1	30	0			
Pembela x Mexico 54	F_2	120	40	3:1	0.13	0.5-0.7
(Pembela x Mexico 54) x Pembela	BC_1	58	64	1:1	1.21	0.2-0.3
(Pembela x Mexico 54) x Mexico 54	BC_2	95	2	1:0	0.00	1.00

4.2 Inheritance of Quantitative traits

4.2.1. Lusaka Yellow x Mexico 54

Duration to flowering: Lusaka Yellow flowered 41 days after planting. Mexico 54 flowered in 44 days. The mean for the F_1 was 41 days with a range of 40 to 44. Mean days to flowering for F_2 plants were 43 with a range of 41 to 44. The backcrosses to Lusaka Yellow flowered in 43 days with a range of 41 to 46 days. Plants for the backcross to Mexico 54 flowered in 42 days (range 41 to 44).

Duration to maturity. Lusaka Yellow and Mexico 54 matured in 85 and 89 days, respectively. F_1 plants reached maturity in 85 days (range 83-86 days). F_2 plants started maturing in 83 days and latest to mature was after 89 days with a mean of 87. Backcrosses to Lusaka Yellow were maturing on average after 87 days with a range of 84-88 days. In the backcross to Mexico 54 the plants were on average maturing in 88 days with range of 86-89 days.

Plant height. Plant height in this cross was influenced by Mexico 54 which is a type III. The mean plant height of Lusaka Yellow was 91 cm but varied from 80 to 110 cm. Mexico 54 mean height was 151 cm with a range of 135 -175 cm. F_1 ranged from 120 – 155 cm with a mean height of 138 cm. The mean plant height F_2 was 142 from a range of 70 – 220 cm. Plant height for the Lusaka Yellow backcross progeny varied from 120 to 140 cm with an average of 130 cm. Backcross to Mexico 54 had a mean height of 154 cm with a range from 125 -180 cm. The degree of dominance for this trait was 0.74.

Pods per plant. Lusaka Yellow had the lowest mean number of pods/ plant at 13 with a range of 6-23. Mexico 54 had a range of 13-38 with a mean of 24 pods/ plant. F_1 range was from 20-46 giving a mean of 28 pods/ plant, which was 17% better than the better parent. Backcross to Lusaka Yellow and to Mexico 54 had means of 28 and 29 from the ranges of 16-42 and 17-64 pods/ plant respectively. In the F_2 progeny the mean number of pods per plant was 20 with a range of 2-85. The degree of dominance was 0.70 with additive genetic differences of 61%.

Pod length: Mean pod length for Lusaka Yellow and Mexico 54 were 13 and 11 cm respectively. The range for Lusaka Yellow was 9-15 and for Mexico 54 was 7.5-14cm. For the F_1, mean pod length of 10cm was from the range of 5.4-14cm. F_2 pod length ranged from 4.2 – 17 cm with a mean of 11. Backcross to Lusaka Yellow and to Mexico 54 both had mean pod length of 11 with ranges of 8-13 and 6-14 respectively. Pod length showed a degree of dominance of 31%.

Seeds per pod: The number of seeds/ pod between parents and the progenies was similar. Lusaka Yellow, Mexico 54 and backcross to Mexico 54 (BC_1P_2) all had a mean number of seeds/pod of 5 and range was from 2-7. F_1 and backcross to Lusaka Yellow (BC_1P_1) had a mean of 4 ranging from 2-6 seeds/ pod. F_2 had a range from 1-7 seed/ pod and a mean of 4. The degree of dominance for this trait was 0.28.

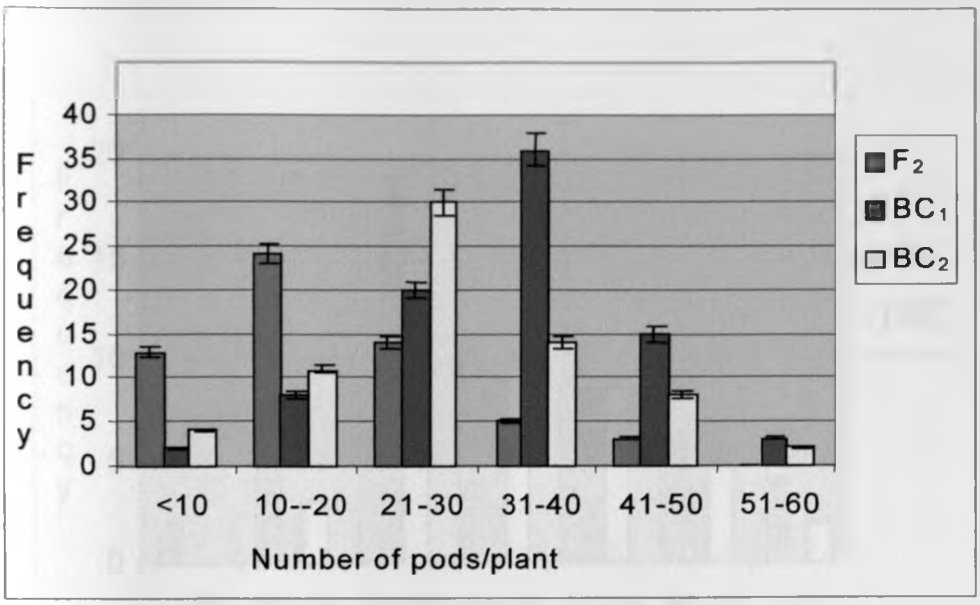
Seed size: The 100 seed weight for Lusaka yellow was 38 g. Mexico 54 had 100 seed weight of 40 g. The F_1 seed size was better than that of both parents. It weighed 41g/ 100 seeds. F_2 seed size ranged from 15-60 g/ 100 seeds with a mean of 38g. Backcross to Lusaka Yellow had range of 33-49 and a mean of 38g/ 100 seeds. The backcross to Mexico 54 seed size ranged from 30-51 g/ 100 seeds with mean of 41g. Seed size of the F_1 was better than the mid parent value by 6%. This trait showed an additive genetic difference of 65% with a degree of dominance of .073.

Grain Yield: Lusaka Yellow and Mexico 54 had yields of 2534 and 2216 kg ha^{-1} respectively. The F_1 for this cross had a better yield compared to both parents. The

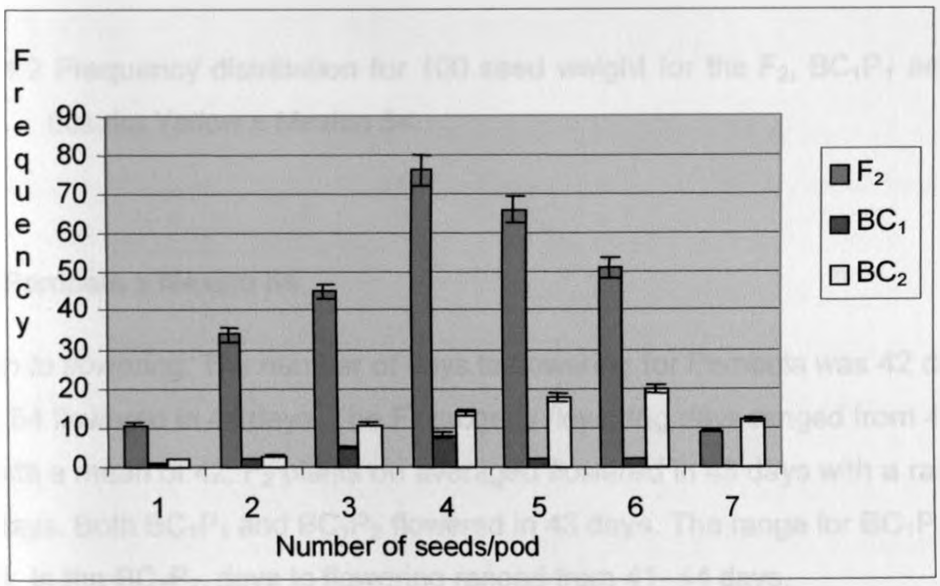
mean yield for F_1 was 2618 kg ha^{-1}. This was about 10 % above mid parent value. The F_2 had a mean yield of 2318.7 kg ha^{-1}. The backcross to Lusaka Yellow had mean yield of 1932.1 kg ha^{-1}. The mean yield for the backcross to Mexico 54 was 2745.6 kg ha^{-1}. The trait showed about 57% due additive genetic differences while the degree of dominance was 0.45.

Table 4.3: Means for the selected traits in P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 of the cross Lusaka Yellow x Mexico 54

Generation	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds /pod	100 Seed weight (g)	Grain yield kg ha^{-1}
P_1	41	85	91	13	13	5	38	2534
P2	44	89	151	24	11	5	40	2216
F_1	41	85	138	28	10	4	41	2618
BC_1P_1	43	87	130	28	11	4	38	1932
BC_1P_2	42	88	154	29	11	5	41	2746
F_2	43	87	142	20	11	4	38	2319
CV (%)	2.2	1.7	13.5	33.8	5.9	9.0	15.9	16.6
SE	0.54	0.836	10.13	4.16	0.375	0.224	3.71	227.4
LSD(0.05)	1.605	2.485	30.10	12.37	1.114	0.666	NS	675.6



(a)



(b)

Figure 4.1. Frequency distribution for number of pods per plant (a) and number of seeds per pod (b) in the F_2, BC_1P_1 and BC_1P_2 of Lusaka Yellow x Mexico 54.

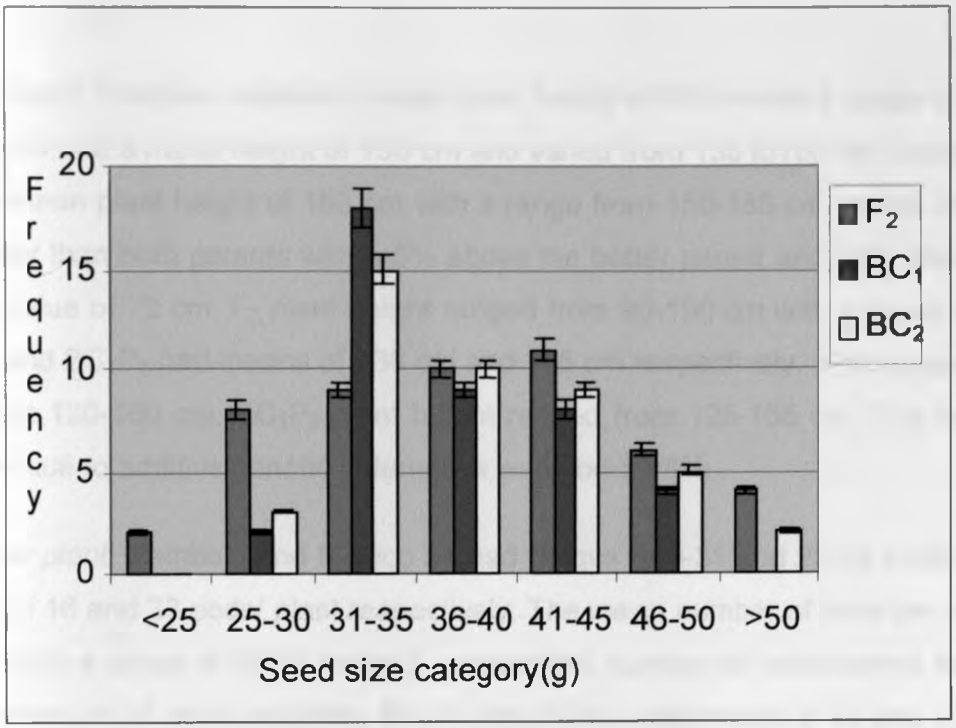


Figure 4.2 Frequency distribution for 100 seed weight for the F_2, BC_1P_1 and BC_1P_2 of Lusaka Yellow x Mexico 54.

4.1.2.2 Pembela x Mexico 54

Duration to flowering. The number of days to flowering for Pembela was 42 days. Mexico 54 flowered in 44 days. The F_1 progeny flowering days ranged from 41- 44 days, with a mean of 42. F_2 plants on averaged flowered in 43 days with a range from 41-45 days. Both BC_1P_1 and BC_1P_2 flowered in 43 days. The range for BC_1P_1 was 42-44 days. In the BC_1P_2, days to flowering ranged from 41–44 days.

Duration to maturity. The number days to maturity for Pembela were 85. Mexico 54 took 89 days to reach physiological maturity. F_1 progeny matured on average in 90 days with a range of 88-91 days. On average the F_2 plants were maturing in 88 days. The maturity for the F_2 ranged from 86-91 days. BC_1P_1 was maturing in 87 days with range from 86-89 days. BC_1P_2 plants were reaching maturity in 89 days (range 87 – 90days).

Plant Height. Pembela attained a mean plant height of 59 cm with a range of 50-82 cm. Mexico 54 had a mean height of 156 cm and varied from 135 to180 cm (mean 156 cm). F_1 had mean plant height of 163 cm with a range from 150-185 cm. In this cross the F_1 was taller than both parents with a 5% above the better parent and 52% above the mid parent value of 72 cm. F_2 plant height ranged from 90-190 cm with a mean of 137 cm. BC_1P_1 and BC_1P_2 had means of 138 cm and 145 cm respectively. The range for BC_1P_1 was from 120-160 cm. BC_1P_2 plant height ranged from 125-165 cm. The variability in this trait due to additive genetic differences was about 48%.

Pods per plant: Pembela and Mexico 54 had ranges of 6-31 and 25-54 pods/ plant with means of 16 and 33 pods/ plant respectively. The mean number of pods per plant for F_1 was 41 with a range of 32-56. In the F_2 generation, number of pods ranged from 2 to 68 with a mean of 23 pods per plant. BC_1P_1 and BC_1P_2 had means of 25 and 27 pods per plant with ranges of 10-48 and 11-54 respectively.

Pod length; Pembela had short pods (mean of 9.5 cm) as compared to Mexico 54 which had a mean pod length of 12 cm from the range of 8 to 14 cm. F_1 mean pod length was 10 cm with a range of 7 to 13 cm. The range for the F_2 was from 3.5 to 13 cm with a mean pod length of 10 cm compared to F_1 which had a mean pod length of 10 cm with a range of 7-13. BC_1P_1 and BC_1P_2 had ranges of 6-12 and 5.5-13.5 with means of 9 and 11 cm respectively. The BC_1P_1 had shorter pod length compared to the BC_1P_2

Seeds per pod. The mean number of seeds per pod for Pembela was 3 (range 1-5). Mexico 54 had a mean seeds per pods of 5 with a range of 2-7. A mean of 5 seeds (range 2-6) per pod for the F_1 was recorded. The range for the F_2 was from 1-8 seeds per pod with a mean of 5. BC_1P_1had a mean of 3 with a range of 1-7 seeds per pod. BC_1P_2 mean number of seeds per pod was 5 (range 2-7).

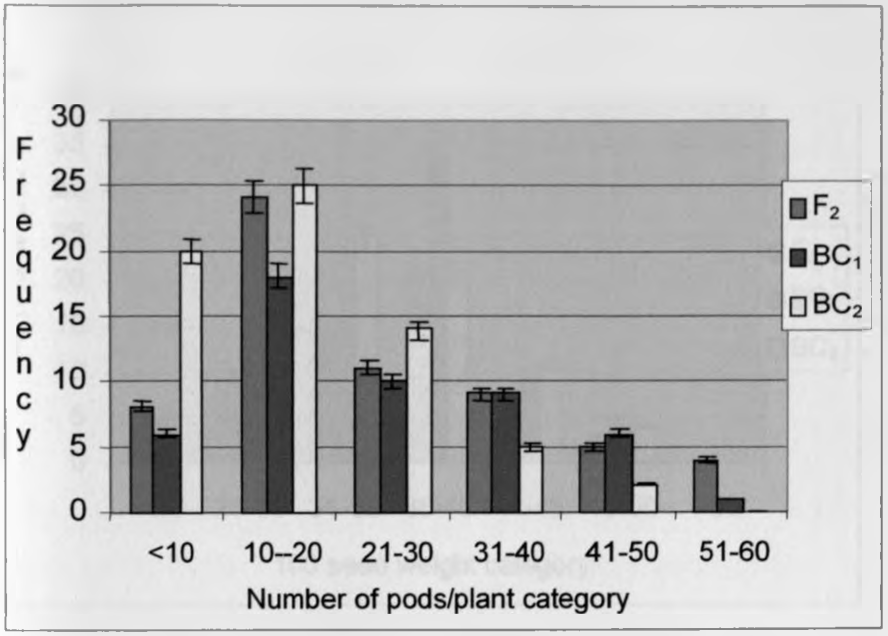
Seed size: Pembela had slightly larger seeds compared to Mexico 54. Mean 100-seed mass was 37 g for Pembela and 33 g for Mexico 54. However seed sizes in the two parents were within the medium category in the CIAT classification (Schoonhoven and

Pastor-Corrales, 1987)]. The mean 100 seed weight for F_1 was 40 g. F_1 had larger seeds on overall compared to the other progenies and parents. An increase of 16% in seed size above the better parent and 21% above the mid parent value was observed in the F_1. In the F_2 the mean 100 seed weight ranged from 15 to 55 g/ 100 seeds, with an average of 35 g. For both BC_1P_1 and BC_1P_2 the mean was 36 g with ranges of 23-48 and 28-51 g/ 100 seeds respectively.

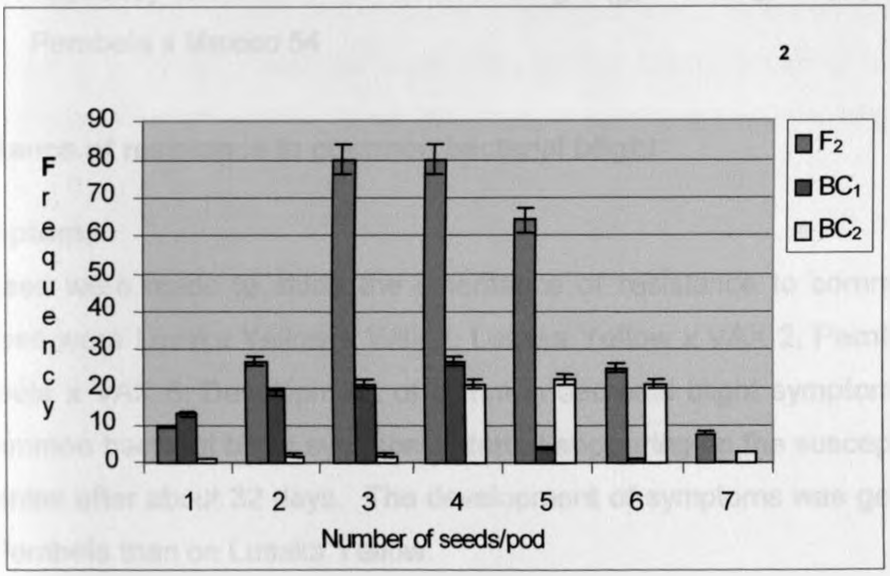
Grain yield: Pembela had a mean yield of 2671 kg ha^{-1}. Mexico 54 on average yielded 2686 kg ha^{-1} A mean yield of 2976 kg ha^{-1} was recorded for the F_1. A heterosis of 11% was observed above the better parent value. F_2 on average yielded 2572 kg ha^{-1}. BC_1P_1 yielded 3580 kg ha^{-1} and BC_1P_2 had a mean yield of 2899 kg ha^{-1}.

Table 4.4: Means for the selected traits in P_1, P_2, F_1, F_2 and backcrosses of the cross Pembela x Mexico 54

Generation	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds /pod	100 Seed weight (g)	Grain yield kg ha^{-1}
P_1	42	85	59	16	9.5	3	37	2670.6
P_2	44	89	156	33	12	5	33	2686.1
F_1	42	90	163	41	10	4	43	2977.5
BC_1P_1	43	87	138	25	9	3	36	3580.3
BC_1P_2	43	89	145	27	11	5	36	2899.3
F_2	43	88	137	23	10	4	35	2571.5
CV (%)	1.7	2.0	11.6	40.6	7.6	12.4	12.3	14.1
SE	0.423	1.022	8.87	6.05	0.439	0.284	2.60	226.5
LSD(0.05)	1.251	3.024	26.26	17.9	1.30	0.841	7.711	670.6



(a)



(b)

Figure 4.3 Frequency distribution for number of pods per plant (a) and number of seeds per pod (b) in the F_2, BC_1P_1 and BC_1P_2 of Pembela x Mexico 54

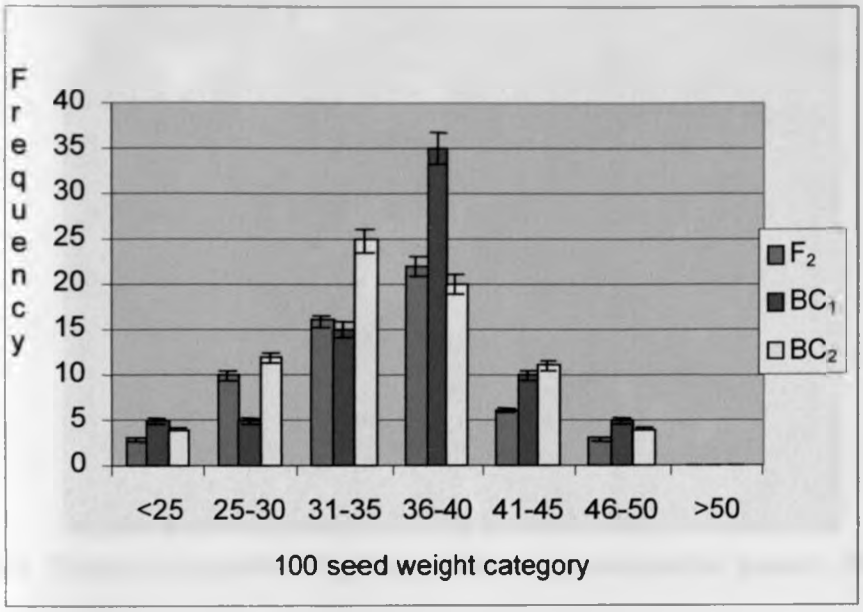


Figure 4.4 Frequency distribution for 100 seed weight (g) in the F_2, BC_1P_1 and BC_1P_2 of Pembela x Mexico 54

4.2 Inheritance of resistance to common bacterial blight

4.2.1 Symptoms

Four crosses were made to study the inheritance of resistance to common bacterial blight. These were Lusaka Yellow x Wilk 2, Lusaka Yellow x VAX 2, Pembela x Wilk 2 and Pembela x VAX 6. Development of common bacterial blight symptoms was slow. Typical common bacterial blight symptoms started appearing on the susceptible parents and progenies after about 32 days. The development of symptoms was generally more rapid on Pembela than on Lusaka Yellow.

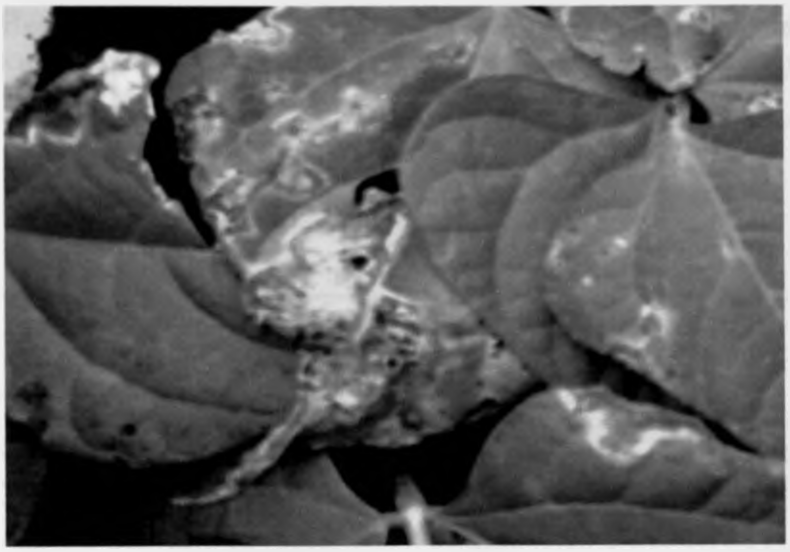


Plate 4.5. Common bacterial blight symptoms on susceptible parent, Pembela.

4.2.1 Lusaka Yellow x Wilk 2

All Lusaka Yellow plants showed susceptible reaction to common bacterial blight. Wilk 2 was resistant to common bacterial blight with all the plants showing some level of resistance (1-3). All the F_1 plants were resistant to common bacterial blight. F_2 plants segregated in a 3:1 ratio for resistant to susceptible. This indicated that resistance in Wilk 2 could be controlled by a major gene. The results in BC_1P_1 were 50% resistant and 50% susceptible. All the plants in the BC_1P_2 were resistant (Table 4.3). This is in agreement with the work of Deidré (2002) that Wilk 2 has high level of resistance to *Xanthomonas campestris* pv *phaseoli*

Table 4.5: Reaction of Lusaka Yellow and Wilk 2, their F_1, F_2, backcrosses to inoculation with an isolate of *Xanthomonas campestris* pv *phaseoli.*

Parent/cross	Generation	Number of plants: Resistant	Number of plants: Susceptible	Exp. Ratio	X^2	Pr
Lusaka Yellow	P_1	0	30			
Wilk 2	P_2	30	0			
Lusaka Yellow x Wilk 2	F_1	29	0			
Lusaka Yellow x Wilk 2	F_2	143	41	3:1	0.72	0.3-0.5
(Lusaka Yellow x Wilk 2) x Lusaka Yellow	BC1	66	58	1:1	0.52	0.3-0.5
(Lusaka Yellow x Wilk 2) x Wilk 2	BC_2	130	4	1:0	0.0	1.00

4.2.2 Lusaka Yellow x VAX 6

All VAX 6 plants showed resistant reaction after inoculation with *X. campestris* pv *phaseoli.* All Lusaka Yellow plants were susceptible. The symptoms appeared as water soaked spots on the lower part of the leaves. The lesions were later surrounded with a narrow yellowish zone which turned brown. All the F_1 plants were resistant. F_2 plants segregated in a 3:1 ratio for resistant to susceptible. Backcross to Lusaka Yellow segregated in the ratio of 1:1. All the plants in the backcross to VAX 6 were resistant (Table 4.4). This is in line with the work of Singh and Munoz (1999) which showed that VAX 6 has high levels of resistance to common bacterial blight.

Table 4.6: Reaction of Lusaka Yellow and VAX 6, their F_1, F_2, BC_1P_1 and BC_1P_2 to inoculation with an isolate of *Xanthomonas campestris* pv *phaseoli*.

Parent/cross	Gene ration	Number of plants		Exp. Ratio	X^2	Pr
		Resi stant	Susce ptible			
Lusaka Yellow	P_1	0	28			
VAX 6	P_2	29	0			
Lusaka Yellow x VAX 6	F_1	30	0			
Lusaka Yellow x VAX 6	F_2	160	43	3:1	1.68	0.1-0.2
(Lusaka Yellow x VAX 6) x Lusaka Yellow	BC_1	63	71	1:1	0.48	0.3-0.5
(Lusaka Yellow x VAX 6) x VAX 6	BC_2	145	0	1:0	0.0	1.00

4.2.3 Pembela x Wilk 2

Pembela plants were all susceptible to common bacterial blight isolate used in the study. Wilk 2 plants were all resistant. All the F_1 plants were resistant. F_2 plants segregated in the 3:1 ratio for resistant and susceptible. Half of the backcross to the susceptible parent was resistant with the other half showing susceptible reaction to the inoculum, representing a 1:1 ratio (Table 4.5). In the backcross to the resistant parent all the plants showed resistant reaction. This indicated high levels of resistance in Wilk 2 as was reported by Deidré (2002).

Table 4.7: Reaction of Pembela and Wilk 2, their F_1, F_2, BC_1P_1 and BC_1P_2 to inoculation with an isolate of *Xanthomonas campestris* pv *phaseoli*.

Parent/cross	Gene ration	Number of plants: Resi stant	Susce ptible	Exp. Ratio	X^2	Probability
Pembela	P_1	0	30			
Wilk 2	P_2	30	0			
Pembela x Wilk 2	F_1	29	0			
Pembela x Wilk 2	F_2	145	44	3:1	1.94	0.1-0.2
(Pembela x Wilk 2) x Pembela	BC_1	66	56	1:1	0.83	0.2-0.5
(Pembela x Wilk 2) x Wilk 2	BC_2	122	0	1:0	0.0	1.00

4.2.4. Pembela x VAX 6

All Pembela plants showed susceptible reaction after inoculation with *X. campestris* pv *phaseoli*. VAX 6 plants were all resistant (Table 4.6). In F_1 all the plants showed resistant reaction to the inoculum. F_2 plants segregated in the 3:1 ratio for resistant to susceptible. Back cross to the susceptible parent segregated in the 1:1 ratio. The plants in the backcross to the resistant parent showed resistance to the inoculum. This is in line with the work of Singh and Munoz (1999) which indicated high levels of resistance to common bacterial blight in the VAX lines including VAX 6. Moreover, this is in line with Deidré (2002) findings of high levels of resistance to common bacterial blight in VAX 6.

Table 4.8: Reaction of Pembela and VAX 6, their F_1, F_2, BC_1P_1 and BC_1P_2 to inoculation with isolate of *Xanthomonas campestris* pv *phaseoli*.

Parent/cross	Gene ration	Number of plants: Resi stant	Susce ptible	Exp. Ratio	X^2	Probability
Pembela	P_1	0	25			
VAX 6	P_2	27	0			
Pembela x VAX 6	F_1	37	0			
Pembela x VAX 6	F_2	145	44	3:1	0.25	0.5-0.7
(Pembela x VAX 6) x Pembela	BC_1	63	56	1:1	0.82	0.3-0.5
(Pembela x VAX 6) x VAX 6	BC_2	116	0	1:0	0.0	1.00

4.2.2 Quantitative traits

4.2.2.1. Lusaka Yellow x Wilk 2

Duration to flowering: Lusaka Yellow flowered in 43 days (Table 4.8). Wilk 2 flowered in 41 days. Mean duration to 50% flowering among the F_1 progeny was 43 days. The F_2 plants flowered in 42 days. Backcross to Lusaka Yellow took 43 days and so was the backcross to Wilk 2. Dominance accounted for about 86%.

Duration to maturity: Lusaka Yellow matured in 87 days. Wilk 2 took 84 days. F_1 and F_2 matured in 84 and 85 days respectively. Both backcross to Lusaka Yellow and Wilk 2 matured in 86 days. The range for the F_1 was between 83-86 days. F_2 days to maturity ranged from 82-87. Results showed that duration to maturity was controlled by additive genes. 71% of the variability in duration to maturity was due to additive genetic effects. Dominance accounted for 4%.

Plant height Lusaka Yellow had a mean plant height of 88 cm with very little variation.. Wilk 2 plants were shorter. They had a mean height of 29 cm but varied from 27 to 31 cm.

F_1 had taller plants with mean height of 117cm with a range from 100 to127cm. More variations in plant height were observed in the backcrosses and F_2 progenies. In F_2, plant height varied from 40 to 130 cm with a mean of 79 cm (Table 4.8). Backcross

progeny to Lusaka Yellow had a range from 85-120cm and a mean of 97cm (Table 4.8). Backcross to Wilk 2 had a mean plant height of 59cm (range 45-75cm).

Pods per plant. Wilk 2 had more pods per plant than Lusaka Yellow. Lusaka Yellow had an average of 27 pods per plant and a range of 20 to 48 pods. In contrast, Wilk 2 had an average of 34 pods per plant and a range of 24 to 50 pods. F_1 had a range of 24-50 giving a mean number of pods/ pant of 36. The number of pods /plant for the F_1 was better than the better parent by 6%. Backcross to Lusaka Yellow had mean of 30 pods per plant (range of 16-52). Backcross to Wilk 2 had a mean number of pods per plant of 40 (range 19-69). F_2 had a range of 2-67 resulting in a mean number of pods per plant of 24.

Pod length: Lusaka Yellow had longer pods than Wilk 2 but there was considerable variability within the two parental lines. Lusaka Yellow had a mean pod length of 13 cm (range 9-16). Wilk 2 had a mean pod length of 11cm from the range of 7-14.5. F_1 pod length mean was 13 (range 9.5-15). F_2 had ranges of 7.5 – 16 giving a mean of 12 cm (Table 4.8). The mean pod length for the BC_1P_1 was 13 cm and varied from 10-15cm. Among the backcrosses to Wilk 2, pod length ranged from 6.3 – 19 cm with mean of 12 cm (Table 4.8). Degree of dominance was 0.71.

Seeds per pod: Lusaka Yellow had more seeds in a pod than Wilk 2. This could be attributed to its longer pods. Lusaka Yellow had an average of 5 seeds per pod. Wilk 2 had 4 seeds per pod. In the F_1 the mean number of seeds per pod was 5. The range for F_1 was from 2-7 seeds per pod. In the F_2 the range was 1-9 seeds per pod with a mean of 5. Progeny of the backcross to Wilk 2 had 2 to 7 seeds per pod, with a mean of 5 seeds in a pod. Backcross progeny to Lusaka Yellow had 3 to 8 seeds/pod, with a mean of 5. The F_1 was better than mid parent value by 8%.

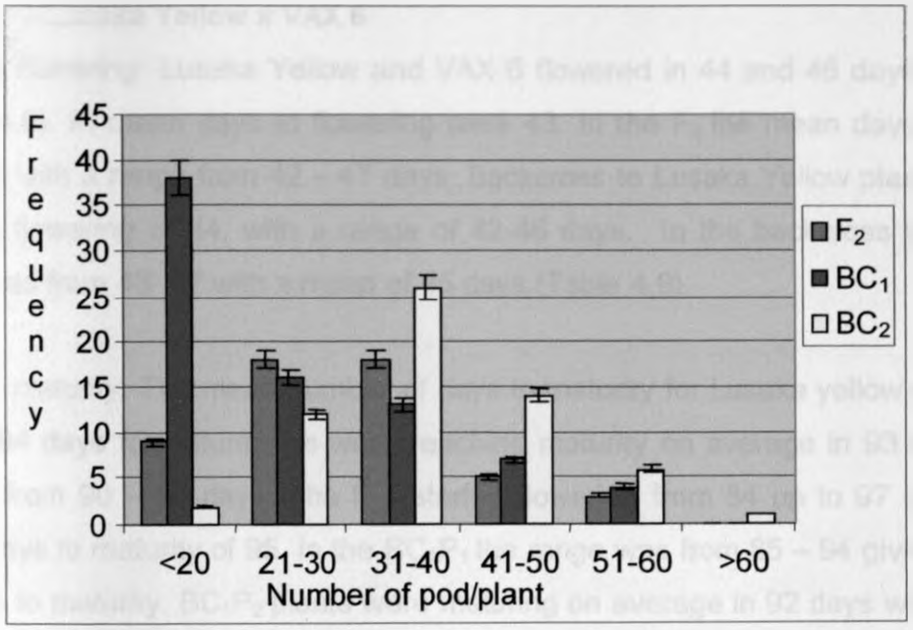
Seed size: Lusaka Yellow had larger seeds than Wilk 2. The mean 100 seed weight for Lusaka Yellow and Wilk 2 were 38 g and 30 g, respectively (Table 4.8). F_1 progeny showed heterosis for 100-seed mass. F_1 plants had a mean 100 seed mass of 40 g, an improvement over both parents. The improvement was by 17% above the mid parent

value. F_2 had a wider range of seed sizes. 100-seed mass varied from 16 to 50g with a mean of 33g (Table 4.8). This could be attributed to segregation of genes controlling seed size. The mean 100 seed weight for the backcrosses to Lusaka Yellow was 38 g with a range of 25 – 47g. Backcross to Wilk 2 had a range of 24-43 g / 100 seeds with a mean of 34g (Table 4.8). Genetic analyses showed that seed size was largely controlled by additive genes (Table.4.10). More than 52% of the variability was due to additive gene action.

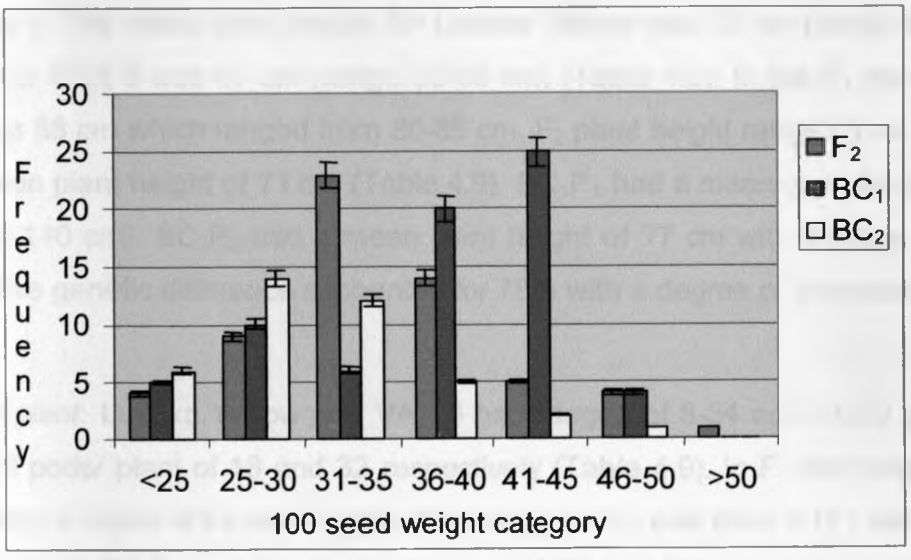
Grain Yield: Wilk 2 yielded more than Lusaka Yellow. The mean yield for the Lusaka Yellow was 2576 kg ha^{-1}. Wilk 2 had a yield of 3046 kg ha^{-1}. However, yield of the F_1 progeny showed no heterosis for grain yield. The mean yield for the F_1 was 2649 kg ha^{-1} (Table 4.8). The mean yield for the F_2 was 2701 kg ha^{-1}. Yield per plant in the F_2 generation varied from 10 to 32 g/plant. The backcross to Lusaka Yellow had a mean yield of 2916 kg ha^{-1}. The backcross to Wilk 2 gave a mean yield of 2948.5 kg ha^{-1}. Yield per plant basis in the BC_1P_1 ranged from 18 to 22 g/plant. In the backcross to Wilk 2 (BC_1P_2) the yield range was between 16 and 22 g /plant. The degree of dominance for this trait was 0.36 and effects due to additive genetic differences accounted for about 55%.

Table 4.9: Means for the selected traits in P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 of the cross Lusaka Yellow x Wilk 2, at Kabete, 2004.

Generation	Days to flowering	Days to maturity	Plant height (cm)	Pods/ plant	Pod length (cm)	Seeds /pod	100 Seed weight (g)	Grain yield kg ha^{-1}
P_1	43	87	88	27	13	5	38	2576.5
P_2	41	84	29	34	11	4	30	3045.8
F_1	43	84	117	36	13	5	40	2649.3
BC_1P_1	43	86	97	30	13	5	38	2916.0
BC_1P_2	43	86	59	40	12	5	34	2948.5
F_2	42	85	79	24	12	5	33	2700.7
CV (%)	2.1	1.8	16.2	27.9	14.1	6.8	13.7	19.0
SE	0.508	0.872	7.48	4.40	0.969	0.183	2.834	304.6
LSD(0.05)	1.503	2.582	22.15	13.02	NS	0.5413	8.388	NS



(a)



(b)

Figure 4.5 Frequency distribution for number of pods per plant (a) and 100 seed weight (b) in the F_2, BC_1P_1 and BC_1P_2 of Lusaka Yellow x Wilk 2

Lusaka Yellow x VAX 6

Days to flowering: Lusaka Yellow and VAX 6 flowered in 44 and 46 days respectively (Table 4.9). F_1 mean days to flowering were 43. In the F_2 the mean days to flowering were 45 with a range from 42 – 47 days. Backcross to Lusaka Yellow plants had mean days to flowering of 44, with a range of 42-46 days. In the backcross to VAX 6 the range was from 43- 47 with a mean of 45 days (Table 4.9).

Days to maturity: The mean number of days to maturity for Lusaka yellow was 85. VAX 6 took 94 days to mature. F_1s were reaching maturity on average in 93 days but this ranged from 90 – 95 days. The F_2s started flowering from 84 up to 97 days giving a mean days to maturity of 95. In the BC_1P_1 the range was from 85 – 94 giving a mean of 91 days to maturity. BC_1P_2 plants were maturing on average in 92 days with a range of 86 – 96 days.

Plant height: The mean plant height for Lusaka Yellow was 72 cm (range 65 – 95 cm) and that for VAX 6 was 47 cm (range 35-60 cm) (Table 4.9). In the F_1 the mean plant height was 88 cm which ranged from 80-85 cm. F_2 plant height ranged from 25 -125 cm with a mean plant height of 71 cm (Table 4.9). BC_1P_1 had a mean plant height of 85 cm (range 65-110 cm). BC_1P_2 had a mean plant height of 77 cm with a range from 50-95 cm. Additive genetic difference accounted for 75% with a degree of dominance of 0.50.

Pods per plant: Lusaka Yellow and VAX 6 had ranges of 8-34 and 21-50 giving mean number of pods/ plant of 18 and 32 respectively (Table 4.9). In F_1 the range was from 47-65 giving a mean of 54 pods /plant. The range for F_2 was from 7-111 with a mean of 32 pods/ plant. BC_1P_1 and BC_1P_2 had means of 52 and 37 pods/ plant with ranges of 39-75 and 18-50 respectively. Degree of dominance for this trait was 0.72.

Pod length: The mean pod length for Lusaka Yellow was 14 cm, with a range of 7.5-15.5 cm. The mean pod length for VAX 6 was 10 cm (range 7-13.5). F_1 pod length ranged from 8-12 cm resulting in a mean of 10 cm. F_2 mean pod length was 10 cm with a range of 5 -13.5. BC_1P_1 had a mean pod length of 10 cm and a range of 7.5 – 12.5.

BC_1P_2 had a mean pod length of 10 cm with a range of 8 -15 cm. The degree dominance was 0.76. Additive genetic differences accounted for 39%.

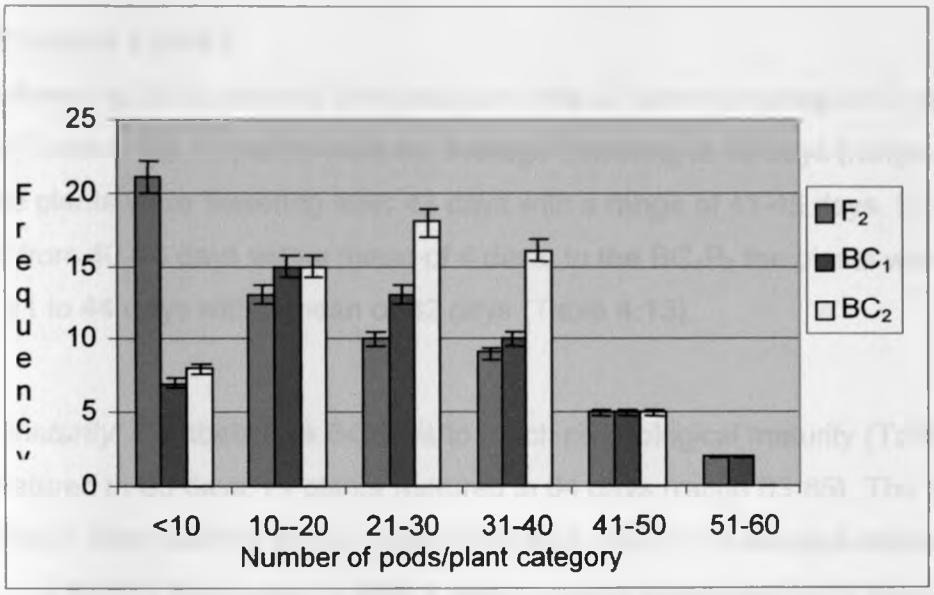
Seeds per pod: The number of seeds per pod in Lusaka Yellow ranged from 1-7 and had mean of 6. VAX 6 had a mean number of seeds per pod of 5 with a range of 3-8. In the F_1 the range was from 3-6 with a mean of 5. From a range of 1-8 seeds per pod the mean for F_2 was 4. Backcrosses to Lusaka Yellow and VAX 6 had both means of 5 with similar ranges of 2-7 seeds per pod.

Seed size: Lusaka Yellow had a mean 100 seed weight of 38 g (Table 4.9). VAX 6 had the smallest mean 100 seed weight of 22 g compared to Lusaka Yellow and their progenies. F_1 mean 100 seed weight was 35 g. The range for the F_2 was from 17 g to 49 g with a mean of 32 g/ 100 seed weight. Ranges in BC_1P_1 and BC_1P_2 were 25 to 46 and 21 to 43 g/ 100 seed weight with means of 35 and 29 g respectively (Table 4.9).

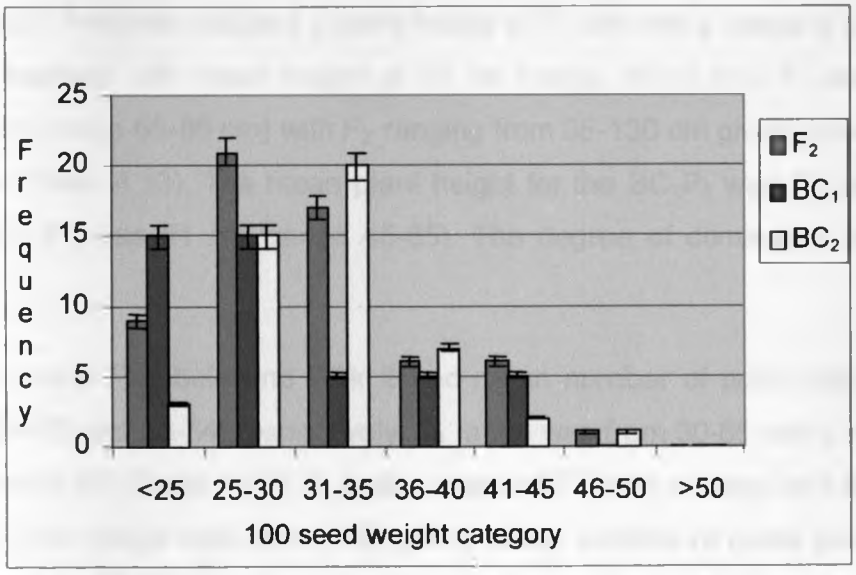
Grain yield: Grain yield for Lusaka Yellow and VAX 6 were 2321 and 2351 kg ha^{-1} respectively (Table 4.9). The yield for the F_1 was better compared to both parents, with a mean yield of 2451 kg ha^{-1}. The range of yield per plant in the F_2 was from 19 – 31 g giving mean yield of 3291 kg ha^{-1}. BC_1P_1 had a mean yield of 2688 kg ha^{-1} with yield per plant ranging from 18- 22 g (mean 20 g). BC_1P_2 yield per plant ranged from 16-22 g (mean 19 g) giving a mean yield of 2519.8 kg ha^{-1}. The degree of dominance for this trait was 0.43.

Table 4.10: Means for the selected traits in P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 of the cross Lusaka Yellow x VAX 6.

Generation	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 Seed weight (g)	Grain yield kg ha^{-1}
P_1	44	85	72	18	14	6	39	2321
P_2	46	94	47	32	10	5	22	2352
F_1	43	93	88	54	11	5	35	2451
BC_1P_1	44	91	85	52	10	5	35	2688
BC_1P_2	45	92	77	37	11	5	29	2520
F_2	45	95	71	32	10	4	32	3291
CV (%)	1.3	2.2	23.3	41.1	6.0	10.6	12.7	14.3
SE	0.33	1.186	9.67	8.69	0.355	0.2874	2.364	234.8
LSD(0.05)	0.989	3.524	28.74	25.81	1.054	0.8539	7.024	697.6



(a)



(b)

Figure 4.6 Frequency distribution for number of pods per plant (a) and 100 seed weight (b) in the F_2, BC_1P_1 and BC_1P_2 of Lusaka Yellow x VAX 6

Pembela x Wilk 2

Days to flowering: Both parents (Pembela and Wilk 2) were flowering in 42 days after planting (Table 4.13). F_1 plants were on average flowering in 43 days (range 42-44). In the F_2 the plants were flowering after 43 days with a range of 41-45 days. BC_1P_1 plants flowered from 40-44 days with a mean of 4 days. In the BC_1P_2 the plants were flowering as from 41 to 44 days with a mean of 42 days (Table 4.13).

Days to maturity: Pembela took 84 days to reach physiological maturity (Table 4.13). Wilk 2 matured in 86 days. F_1 plants matured in 84 days (range 83-85). The F_2 plants in 82 to 87 days after planting with a mean of 85 days. BC_1P_1 on average matured in 86 days (range 85-87). Backcross to Wilk 2 was on average matured in 85 days after planting with a range of 83-86 days.

Plant height: Pembela attained a plant height of 71 cm with a range of 55-85 cm. Wilk 2 was the shortest with mean height of 26 cm (range 20-35 cm). F_1 mean plant height was 74 cm (range 65-85 cm) with F_2 ranging from 35-130 cm giving a mean plant height of 77 cm (Table 4.13). The mean plant height for the BC_1P_1 was 70 cm (range 55-95) and for BC_1P_2 was 61 cm (range 45-85). The degree of dominance for this trait was 0.77.

Pods per plant: Pembela and Wilk 2 had mean number of pods/ plant of 33 and 34 (ranges 24-65 and 23-54) respectively. F_1 range was from 30-65 with a mean number of pods/ plant of 43 (Table 4.13). F_2 had a mean of 27 from a range of 3-85 pods/plant. In the BC_1P_1 the range was from 4-48 giving mean number of pods/ plant of 30. BC_1P_2 had a range of 32-67 giving mean number of pods/ plant of 45. This trait had a degree of dominance of 0.49

Pod length: Pod length for Pembela on average was 10 cm (range 7-13.5). Wilk 2 had a range of 7.5-13.5 cm with a mean of 11 cm. F_1, F_2 and BC_1P_2 all had means of 11cm but differed in the range of the pod length. The range for F_1 was 7.5-13.5 with that of F_2 ranging from 4-15cm. BC_1P_1 had a mean pod length of 10cm with the range of 8-13.5 cm. BC_1P_2 had a range of 8.5-15cm. The degree of dominance for the trait was 0.74

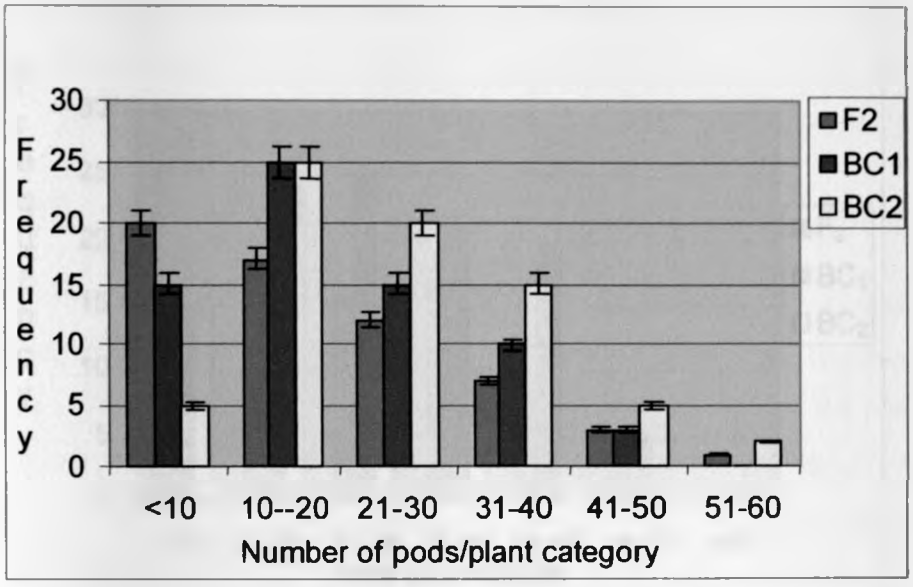
Seeds per pod: The average number of seeds per pod for Pembela was 4 with a range of 2 – 6 seeds per pod. A range of 2-7 seeds was recorded for Wilk 2 with a mean of 5. The F_1 number of seeds per pod ranged from 2-6 with a mean of 4 seeds. A mean of 4 seeds per pod was recorded for F_2 with a range of 1-7. The number of seeds per pod for BC_1P_1 ranged from 2-6 with a mean of 4 (Table 4.13). BC_1P_2 had a mean of 5 seeds per pod with a range of 3-7. The additive genetic difference was about 0.43 while the degree of dominance for this trait was 0.27.

Seed size: The 100 seed weight means for Pembela and Wilk 2 were 40 and 28 g respectively (Table 4.13). F_1 100 seed weight on average was 39 g (Table 4.13).The average 100 seed weight for the F_2 was 35g with a range of 20-57 g. The mean 100 seed weight for BC_1P_1 was 39 g (range 25-47 g). BC_1P_2 100 seed weight ranged from 20-40 g with a mean of 31g.

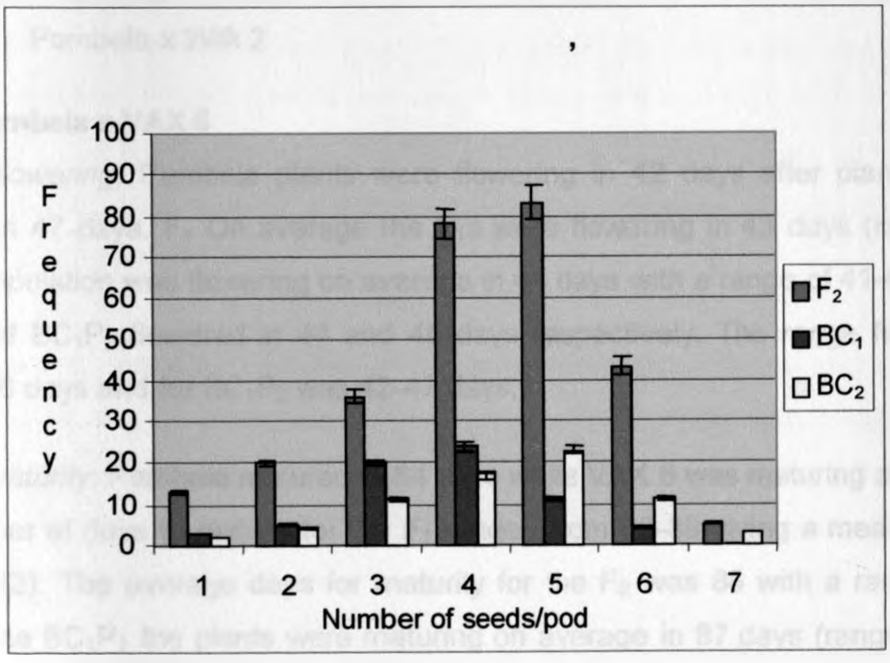
Grain yield: Pembela and Wilk 2 had mean yields of 2212 and 2659 kg ha^{-1} respectively. Heterosis was observed in this cross with better yield in the F_1 compared to the parents. An average yield of 3081 kg ha^{-1} was recorded for the F_1. Heterosis of 27% was observed above the mid parent value and 16% above the better parent value. F_2 mean yield was 2917 kg ha^{-1} (Table 4.13). The mean yield for the backcross to Pembela was 3059 kg ha^{-1} (Table 4.11). In the BC_1P_2 the mean yield was 2605 kg ha^{-1}. Effects due to additive genetic differences were 58% while the degree of dominance was 0.44.

Table 4.11: Means for the selected traits in P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 of the cross Pembela x Wilk 2.

Generation	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 Seed weight (g)	Grain yield kg ha^{-1}
P_1	42	84	71	33	10	4	40	2212
P_2	42	87	26	34	11	5	28	2659
F_1	43	84	74	43	11	4	39	3081
BC_1P_1	41	86	70	30	10	4	39	3059
BC_1P_2	42	85	61	45	11	5	31	2605
F_2	43	85	77	27	11	4	35	2917
CV (%)	2.3	1.8	17.2	31.1	5.2	11.8	15.8	13.5
SE	0.566	0.891	6.44	5.36	0.704	0.2897	3.28	218.3
LSD(0.05)	1.675	2.638	19.06	15.86	2.085	0.857	9.70	646.2



(a)



(b)

Figure 4.7 Frequency distribution for number of pods per plant (a) and number of seeds per pod (b) in the F_2, BC_1P_1 and BC_1P_2 of Pembela x Wilk2

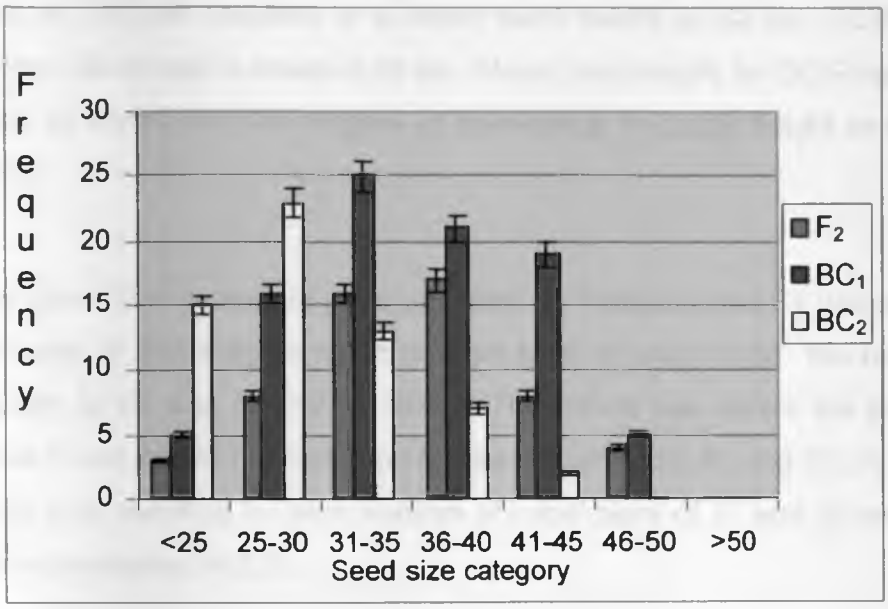


Figure 4.8 Frequency distribution for 100 seed weight in the F_2, BC_1P_1 and BC_1P_2 of Pembela x Wilk 2

4.2.1.5 Pembela x VAX 6

Days to flowering: Pembela plants were flowering in 42 days after planting. VAX 6 flowered in 47 days. F_1 On average the F_1s were flowering in 43 days (range 42-45). The F2 population was flowering on average in 44 days with a range of 41-46 days. The BC_1P_1 and BC_1P_2 flowered in 44 and 45 days respectively. The range for $BC_1P_1$was from 42-46 days and for BC_1P_2 was 42-47 days.

Days to maturity: Pembela matured in 84 days while VAX 6 was maturing after 87 days. The number of days to mature for the F_1 ranged from 86-89 giving a mean of 87 days (Table 4.12). The average days for maturity for the F_2 was 86 with a range of 63-89 days. In the BC_1P_1 the plants were maturing on average in 87 days (range 85-88). On average BC_1P_2 was maturing in 86 days (range 84-88 days).

Plant height: Pembela and VAX 6 had plant height ranges of 50-75 and 35-55 cm with means of 63 and 44 cm respectively (Table 4.12). The range for F_1 was from 55-85 with a mean of 69 cm. The F_2 range was wide with the minimum plant height of 30 cm and a

maximum of 120 cm resulting in a mean plant height of 62 cm. BC_1P_1 plant height ranged from 55-85 with a mean of 68 cm. Mean plant height for BC_1P_2 was 58 cm from the range of 40-80 cm. The degree of dominance for plant height in this cross was about 0.65.

Pods per plant: The number of pods per plant for Pembela was 23 (range 14-35). VAX 6 had a range of 16-35 with a mean number of pods/ plant of 28. The mean number of pods / plant for F_1 was 50 (range 38-65). The hybrid was above the better parent by 78% while it was above the mid parent value by 95%. BC_1P_1 and BC_1P_2 had ranges of 27-70 and 8-50 resulting in mean number of pods/ plant of 37 and 29 respectively. The trait had a dominance of 0.70.

Pod length: Pembela and VAX 6 both had mean pod length of 10 cm with ranges of 7.5-13.5 and 7-11 cm respectively (Table 4.12). In the F_1 the pod length range was 7-12 cm giving a mean of 10 cm. F_2 had pod length range of 5 to 13 cm with a mean of 9 cm (Table 4.12). Both BC_1P_1 and BC_1P_2 had similar means and ranges of 10 cm and 6-12 cm (Table 4.12). The degree of dominance for this trait was 0.36 and 55% was due to additive genetic differences.

Seeds per pod: Pembela and VAX 6 had mean number of seeds/ pod of 4 and 6 with ranges of 2-6 and 2-8 respectively. The range for F_1 was 2-6 with a mean of 5 (Table 4.12). F_2 had a range of 1-7 with a mean of 4. The BC_1P_1 range was 2-7 with a mean of 4 seeds/ pod. The range in the BC_1P_2 was from 1 to7 with a mean of 5. This trait had a degree of dominance of 0.61 and an additive genetic difference of 39%.

Seed size: Pembela and VAX 6 had 100 seed weight of 38 and 20 g respectively. F_1 seed size on average was 30g. The range in the F_2 was from 12 to 51g/ 100 seeds with a mean of 28g (Table 4.12). The means for BC_1P_1 and BC_1P_2 were 35 and 27g with ranges of 28-43 and 21-36 g/ 100 seeds respectively.

Grain yield: Pembela and VAX 6 gave yields of 2546 and 2555 kg ha^{-1} respectively (Table 4.12). A heterosis of 8 % above the mid-parent value was observed in this cross. The mean yield for F_1 was 2749 kg ha^{-1}. The average yield for the F_2 was 2790 kg ha^{-1}. The mean yield for the $BC_1P_1$was 2906 kg ha^{-1}. BC_1P_2 had a mean yield of 3065 kg ha^{-1}. On single plant yield basis the range for F_2 was 17 – 27 g/ plant for BC_1P_1 was 19-25 g/plant and 20 – 26 g/plant for BC_1P_2 The degree of dominance was 0.56.

Table 4.12: Means for the selected traits in P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 of the cross Pembela x VAX 6.

Generation	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 Seed weight (g)	Grain yield kg ha^{-1}
P_1	42	84	63	23	10	4	38	2546
P_2	47	87	44	28	10	6	20	2555
F_1	43	87	69	50	10	5	30	2748
BC_1P_1	44	87	68	37	10	4	35	2906
BC_1P_2	45	86	58	29	10	5	27	3065
F_2	44	86	62	28	9	4	28	2790
CV (%)	1.3	1.5	15.4	26.2	6.4	8.4	13.3	12.2
SE	0.345	0.726	5.38	4.51	0.355	0.2118	2.231	195.8
LSD(0.05)	1.021	2.149	15.93	13.35	NS	0.627	6.607	579.5

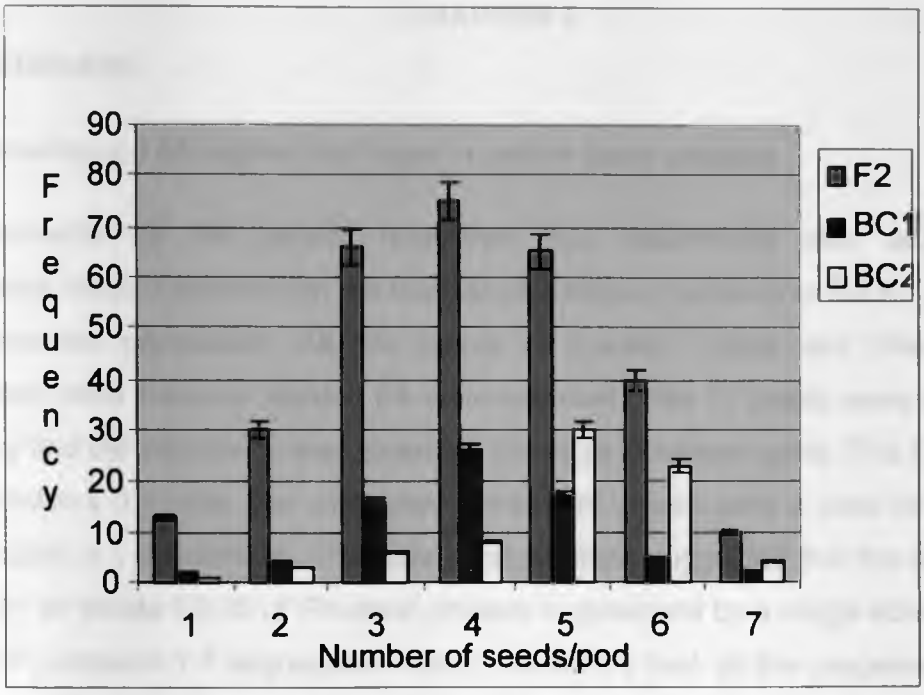


Figure 4.9. Frequency distribution for number of seeds per pod in the F_2, BC_1P_1 and BC_1P_2 of Pembela x VAX 6

CHAPTER 5

5.0 DISCUSSION

5.1 Inheritance of angular leaf spot in yellow bean crosses

Characterisation of the genetic resistance was determined after analysing the segregating ratios obtained from the disease phenotypic reactions of the F_2 populations and backcross generation. All the plants of Lusaka Yellow and Pembela were susceptible while those of Mexico 54 were resistant. The F_1 plants were all resistant indicating that the resistance was governed by single dominant gene. The F_2 progenies segregated in a 3:1 ratio. The calculated Chi-square values gave a good fit for the ratio of 3 resistant to 1 susceptible. This type of segregation suggested that the resistance in Mexico 54 to isolate 63-55 of *Phaseoli griseola* is governed by a single dominant gene. The BC_1P_1 showed 1:1 segregation ratio. The BC_1P_2 had all the progenies resistant. Similar results were obtained by Namayanja (2003) for resistance to isolate 63-55. However, Mahuku *et al.* (2002) reported that the resistance in Mexico 54 was due to a single recessive gene. Caxieta (2002) also reported three dominant genes in Mexico 54. It is interesting to note that various authors (Mahuku *et al.* (2002), Caxieta (2002)) have identified different nature of resistance genes in Mexico 54. These differences in the gene action could be possibly due to the different susceptible backgrounds and pathotypes used, Pastor – Corrales *et al.*, (1994). It could also be that there are many genes for resistance in the line. Each pathotype can identify some, but not all.

5.2 Inheritance of common bacterial blight in yellow bean crosses

All Wilk 2 and VAX 6 plants were rated resistant to common bacterial blight while all of Lusaka Yellow and Pembela were susceptible. In the F_1 all the plants were resistant while they segregated in the 3:1 ratio for resistant to susceptible in the F_2. The BC_1P_1 segregated in the ratio of 1:1. All BC_1P_2 progenies were resistant. This is an indication of a possible dominant single gene for resistance to *Xanthomonas campestris* pv *phaseoli* in Wilk 2 and VAX 6. This is in line with the results of Singh and Muñoz, (1999) which showed that Wilk 2 and VAX 6 have high levels of resistance to common bacterial

blight. Furthermore, Deidré (2002) reported high levels of resistance in Wilk 2 and VAX 6 when they were used in a bean breeding program in South Africa.

(Pastor – Corrales *et al.*, 1994) reported that results of studies on the nature of inheritance greatly depend on the tester genotype used as the susceptible parent among other factors.

5.3 Inheritance of some quantitative traits in yellow bean crosses

Several genes govern quantitative traits; each gene has a small effect, which is usually cumulative. The environment considerably affects these traits. The large degree of variation seen among the parents used provided an excellent opportunity to observe the genetic basis for such differences. Heritability, which is the proportion of the total variation in a progeny that has genetic basis, was also calculated.

Days to Flowering: In most cases the parents and their progenies flowered fairly uniformly over a period of 5 days and showed uniform maturity. These conditions allowed the assessment of yield and its components without the confounding effects of crop duration.

Plant Height. This trait had a heritability of 74% leaving 26% being contributed by the environment. The effects seen in the progenies were contributed more by the gene effects making selection more effective.

Significant differences were observed in days to flowering between Lusaka Yellow and Mexico 54. The non-significance of the 100 seed weight among the progenies of this cross indicated the closeness of the parents in terms of seed size. The dominance of genes from Mexico 54 governing days to flowering, maturity and plant height was observed in the progenies. Moreover backcrosses to the donor parent had mean values above the mid parent, indicating the influence of the donor gene. The recombination of these two genotypes showed that it is possible to transfer resistance from the

Mesoamerican to the Andean pool. In the Lusaka Yellow and Wilk 2 cross, there was an improvement in the F_1 for plant height over the short parent (Wilk 2).

Four of the six crosses showed a higher heritability for grain yield as compared to number of pods per plant (Appendix 8). This could indicate that selection for yield based on the number of pods per plant would be less effective than that based on the grain yield. Bapna *et al* (1972) and Fernandez and Miller (1985), working with grain type cowpeas, also observed that pod number is the yield component most affected by the environment. The higher heritability observed in days to flowering, days to maturity, plant height, 100 seed weight and grain yield may indicate that the ambiguity of environmental influence may have been low under the experimental conditions. A striking heterotic effect for yield was noted in almost all the crosses in the study. Also the study showed some positive heterosis for almost all the crosses for 100 seed weight, number of pods per plant and plant height. The study did show some decrease in number of days to flowering as shown by negative heterosis values for this trait. This is a good characteristic in this case as earliness is preferred to lateness. Percent heterosis for pod length was low or negative which consequently resulted in negative percent heterosis for number of seeds per pod, which could mean that this trait is easily affected by the environment. The larger degree of transgressive segregation observed for most traits indicated ample opportunity for improvement of these traits. The study indicated both the presence of dominance gene action and additive gene effects.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

The objectives of this study were met by transferring resistance through crossing of a genotype from the Mesoamerican gene pool to those belonging to the Andean gene pool.

Resistance for angular leaf spot and common bacterial blight was transferred from the Mesoamerican gene pool to the Andean gene pool. This was first thought not possible. This has demonstrated that it is possible to transfer genes between the two gene pools.

The segregation of the F_2 generation for resistance to susceptible to *Phaeoisariopsis griseola* pathotype 63-35 of the crosses Lusaka Yellow x Mexico 54 and Pembela x Mexico 54 did not differ from the expected 3:1 ratio indicating that the resistance to angular leaf spot in Mexico 54 is governed by a single dominant gene. The segregation of the F_2 generation for resistance to susceptible to *Xanthomonas campestris* pv *phaseoli* in the crosses Lusaka Yellow x Wilk 2, Lusaka Yellow x VAX 6, Pembela x Wilk 2 and Pembela x VAX 6 did not differ from the expected 3:1 ratio indicating that the resistance to common bacterial blight in Wilk 2 and VAX 6 could be governed by a single dominant gene. The level of resistance to common bacterial blight in Wilk 2 and VAX 6 is high enough to protect those genotypes that carry the gene of resistance. These lines have been used in transferring common bacterial blight resistance to commercial varieties in South Africa through back crossing programmes. Mexico 54 has been used widely as a source of resistance to angular leaf spot and has been proved so.

It could be suggested here that the dominant nature of inheritance in the donor varieties used in this study could make transferring angular leaf spot and common bacterial blight resistance from the cultivars Mexico 54, Wilk 2 and VAX 6 relatively easy.

The existence of significant positive heterosis in the yield and yield components in the crosses made is encouraging as it indicates that gene combinations do exist which can

result in enhanced yield performance. Results showed that the gene for indeterminate (climbing ability) was dominant to the determinate as most of the crosses that were made between the two types resulted in progenies way above the mid parent value. The performance of the parents could be used to predict the performance of the progenies. The top yielders among the F_1 had at least one of the parent with high grain yield/ plot. This shows that parents to enter a breeding program should be high yielding. High heritability values are of importance as they indicate that the selection of parents bearing particular measurements will produce progenies of the same phenotype.

From this work it can be recommended that efforts should be aimed at improving local landraces. This helps in adoption and market. In this case Pembela and Lusaka Yellow are such two of the widely grown cultivars in the Zambia; hence their improvement would fill the gap of a high yielding yellow type for the country. Work is under way to combine resistance to both angular leaf spot and common bacterial blight in the same background of yellow beans.

The following recommendation can be made from this study

1 Mexico 54 can be used as a useful donor for angular leaf spot resistance
2 Wilk 2 and VAX 6 are quite good sources for resistance to common bacterial blight
3 Where determinate type are needed, sources other than Mexico 54 could be used as Mexico 54 is a semi-climber (type III).
4 When seed colour is critical it is recommended to cross lines that are in the same seed colour class to reduce the possibility of variability in colours of the progenies
5 It is possible to transfer genes between the two gene pools of Mesoamerican and Andean
6 More work involving Mexico 54, Wilk 2 and VAX 6 to confirm the genes governing resistance in these lines
7 More work involving different backgrounds is recommended to verify the influence of background on the resistance of these lines.
8 It is possible to develop angular leaf spot determinate lines using Mexico 54.

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APPENDICES

Appendix 1: Estimated area (in thousands of ha) and percentage production of the nine categories of bean seed types in Africa

Seed category	Eastern Africa	Southern Africa	As % of production
Calimas	650	90	22
Reds, small and medium-sized	510	160	20
Reds, large and kidney	230	120	10
Yellows, and tans	290	90	11
Creams	240	120	10
Navy	190	120	9
White, large and medium-sized	130	90	6
Purples	150	120	8
Blacks	100	30	3.5

(Source: Wortmann, *et al* (1998).

Appendix 2: Mean squares for Lusaka Yellow x Mexico 54 and progenies

Source of variation	df	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 seed weight (g)	Grain yield (kg ha^{-1})
Rep	2	0.2167	16.381	1308.6	177.94	0.1636	0.0966	102.71	235467
Progenies	5	1.5938*	6.200**	2227.1**	134.87	2.9052*	0.9727*	21.72	245789**
Residual	18(1)	0.8754	2.099	307.9	51.80	0.4218	0.1506	41.36	155136

* , ** - Significant at 5% and 1% level respectively

Appendix 3 Mean squares for Lusaka Yellow x Wilk 2 and progenies

Source of variation	df	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 seed weight (g)	Grain yield (kg ha^{-1})
Rep	2	1.4444	1.444	389.1	97.29	1.964	0.077	2.71	302732
Progenies	5	2.6167	1.150*	2337.7**	79.7	1.851	0.3278*	31.87**	120360
Residual	19	0.7737	2.282	168.1	58.08	2.815	0.1003	24.09	278422

*,** - Significant at 5% and 1% level respectively

Appendix 4: Mean squares for Lusaka Yellow x VAX 6 and progenies

Source of variation	df	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 seed weight (g)	Grain yield (kg ha^{-1})
Rep	2	0.1056	0.492	78.4	348.3	0.1548	0.2083	25.19	746350
Progenies	5	4.7101*	50.39**	567.**	936.1*	4.0387*	0.466	131.57**	987977*
Residual	18(1)	0.3322	4.219	280.7	226.4	0.3776	0.2478	16.77	165395

*,** - Significant at 5% and 1% level respectively.

Appendix 5: Mean squares for Pembela x Mexico 54 and progenies

Source of variation	df	Days to Flowering	Days to Maturity	Plant Height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 seed weight (g)	Grain yield (kg ha^{-1})
Rep	2	0.0370	2.704	420.8	387.2	0.8003	11.78	15.39	172077
Progenies	5	0.6315**	8.143**	5087.1**	262.5	2.720*	35.84*	36.52	532565**
Residual	19	0.5356	3.132	236.1	109.8	0.5788	13.22	20.36	153973

*,**- Significant at 5% and 1% level respectively

Appendix 6: Mean squares for Pembela x Wilk 2 and progenies

Source of variation	df	Days to flowering	Days to maturity	Plant height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 seed weight (g)	Grain yield (kg ha^{-1})
Rep	2	1.0	6.37	373.1	28.35	3.004	0.0424	5.68	54744
Progenies	5	1.6833*	1.793*	1286.2**	81.33	0.472*	0.4608	71.70**	361906*
Residual	19	0.9605	2.382	124.4	86.09	1.488	0.2518	32.22	142984

*, ** - Significant at 5% and 1% level respectively

Appendix 7: Mean squares for Pembela x VAX 6 and progenies

Source of variation	df	Days to flowering	Days to maturity	Plant height (cm)	Pods/ plant	Pod length (cm)	Seeds/ pod	100 seed weight (g)	Grain yield (kg ha^{-1})
Rep	2	0.4444	0.481	285.96	226.45	0.0578	0.0163	0.76	746350
Progenies	5	8.4667*	1.993*	283.58**	341.55*	1.0433	1.8922*	114.21*	987977*
Residual	19	0.3567	1.581	86.89	61.01	0.3782	0.1346	14.93	165395

*,**- Significant at 5% and 1% level respectively

Appendix 8: Percent F_1 heterosis above mid parent for selected traits of six bean crosses at Kabete, Nairobi, Kenya in 2004

Cross	Days to flowering	Days to maturity	Pods/ plant	Seeds/ pod	Pod length (cm)	Plant height (cm)	100 Seed weight (g)	Grain yield (kg ha-1)
Lsk x Mexico 54	-2	-2	52	-23	-15	15	6	10
Lsk x Wilk 2	2	-1	18	8	3	99	17	-5
Lsk x VAX 6	-3	4	112	-16	-8	47	12	5
Pem x Mex54	-2	3	68	-15	-8	52	21	11
Pem x Wilk 2	1	-1	26	2	7	52	14	27
[illegible] x VAX 6	-3	1	95	-6	-3	30	3	8

Appendix 9: Heritability (h^2) (%) for different traits in yellow bean crosses, at Kabete, Nairobi

Cross	Days to Flowering	Days to Maturity	Plant Height	Pods/ plant (cm)	Pod length (cm)	Seeds /pod	100 Seed weight (g)	Grain yield kg ha^{-1}
Lsk x Mex 54	22	52	42	61	39	41	65	57
Lsk x Wilk 2	56	71	62	40	23	13	52	55
Lsk x VAX 6	72	52	75	54	39	72	78	73
Pem x Mex 54	56	64	48	64	24	17	48	35
Pem x Wilk 2	86	54	58	14	15	43	42	58
Pem x VAX 6	73	46	59	57	55	39	81	78

Key: Lsk - Lusaka Yellow; Pem - Pembela.

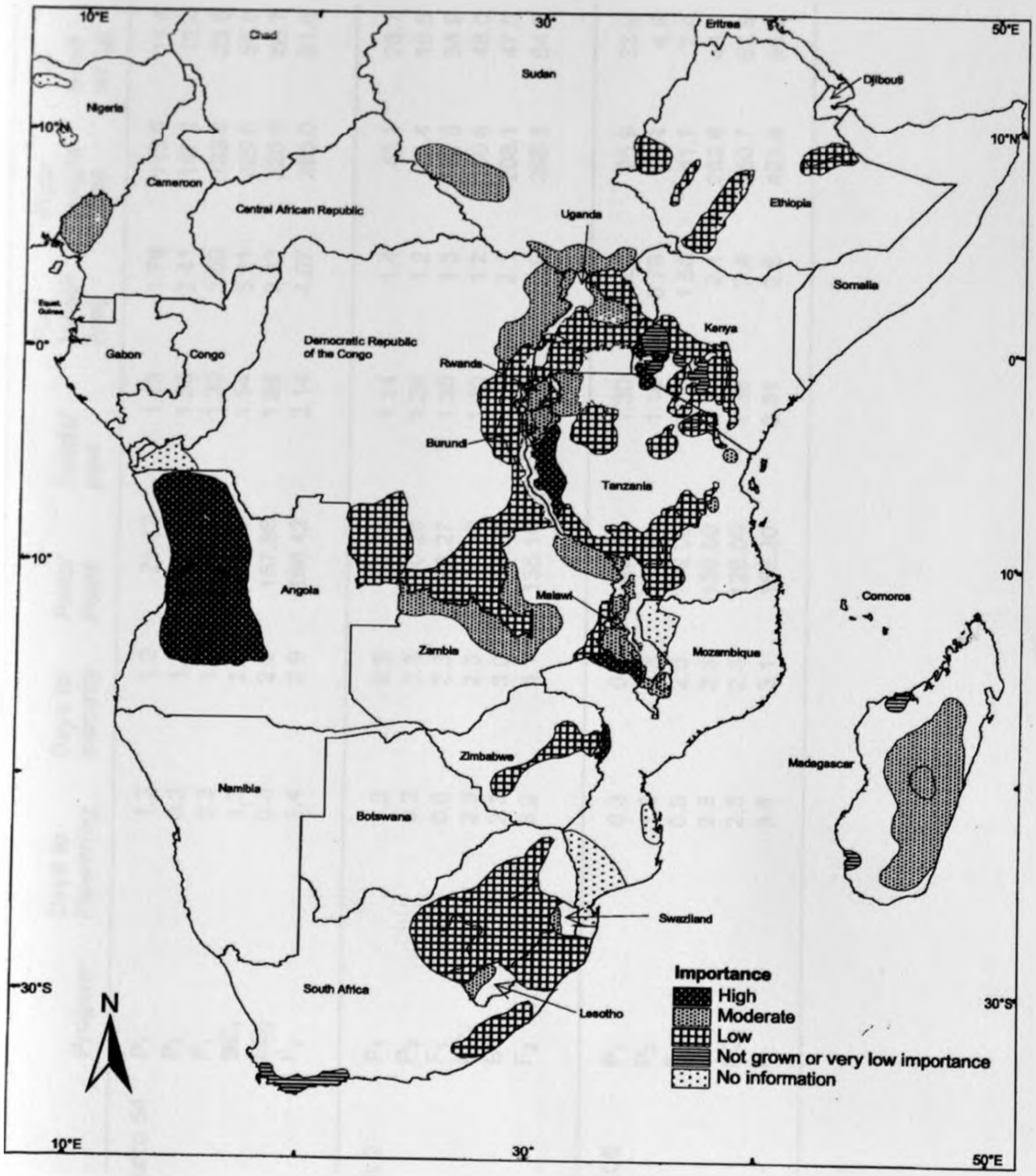
Appendix 10: Production losses in thousands Mt/year due diseases in sub-Saharan Africa

Constraint	Sub-Saharan Africa	Eastern Africa	Southern Africa
Angular leaf spot	384.2	281.3	93.5
Anthracnose	328.0	247.4	69.8
Common bacterial blight	220.4	145.9	69.8
Rust	191.4	118.7	72.4
Bean common mosaic	184.2	144.6	29.9
Root rot	221.1	179.8	31.0

Source: Wortmann, *et al* (1998).

Appendix 11: Importance of yellow and brown bean seed types in sub Saharan Africa

(Source: Wortmann, *et al*, 1998



Appendix 12: Variances for the selected traits of bean crosses at Kabete, Kenya in 2004

		Trait							
Cross	*Progeny*	*Days to Flowering*	*Days to maturity*	*Pods/ Plant*	*Seeds/ pod*	*Pod length (cm)*	*Plant Height (cm)*	*100 Seed wt (g)*	*Grain Yield Kg ha^{-1}*
Lusaka yellow x Mexico 54	P_1	1.3	1.0	21.43	1.13	1.79	113.9	11.4	72487.7
	P_2	0.3	1.3	49.03	1.20	2.41	147.8	13.4	60594.3
	F_1	0.3	1.3	68.88	1.30	3.00	133.9	23.8	80951.7
	BC_1	1.3	2.0	114.67	1.54	3.21	220.0	56.8	169660.0
	BC_2	0.3	2.3	157.86	1.85	3.33	230.0	66.7	105189.4
	F_2	1.4	2.9	196.42	2.14	4.07	285.0	91.6	192450.1
Lusaka yellow x Wilk 2	P_1	1.3	0.3	52.64	1.14	1.4	42.1	28.7	15847.8
	P_2	0.3	0.3	50.26	1.24	1.2	31.4	19.5	25498.5
	F_1	0.6	2.3	93.27	1.30	1.3	62.6	34.9	61077.5
	BC_1	2.3	2.3	102.10	1.40	1.2	160.6	48.0	55799.3
	BC_2	2.3	3.0	114.20	1.36	2.1	208.1	47.0	63834.6
	F_2	3.2	4.1	135.10	1.48	1.9	268.2	64.2	82510.3
Lusaka yellow x VAX 6	P_1	0.3	0.3	49.98	1.30	1.87	95.9	33.2	80832.1
	P_2	1.3	1.3	58.92	1.14	0.79	56.4	4.9	98009.4
	F_1	0.5	2.0	62.00	0.78	1.50	41.7	2.4	32121.0
	BC_1	2.3	2.3	139.50	1.59	2.1	252.8	48.3	337780.1
	BC_2	2.3	2.3	126.00	1.36	2.4	250.7	67.8	110457.7
	F_2	3.6	3.1	182.30	2.31	2.8	401.3	95.2	353615.7

Appendix 13: Variances for the selected traits of bean crosses at Kabete, Nairobi, Kenya in 2004

		Trait							
Cross	*Progeny*	*Days to Flowering*	*Days to maturity*	*Pods/ Plant*	*Seeds/ pod*	*Pod length (cm)*	*Plant Height (cm)*	*100 Seed wt (g)*	*Grain Yield Kg ha^{-1}*
Pembela x Mex 54	P_2	0.3	2.3	79.03	1.42	1.2	155.9	13.1	61320
	F_1	0.3	1.0	74.63	0.95	1.5	139.1	21.2	53933.1
	BC_1	1.0	2.3	188.69	1.23	1.8	227.9	34.7	76450.1
	BC_2	1.3	2.3	157.43	1.11	1.7	220.9	34.2	85250.2
	F_2	1.6	3.2	254.69	1.28	1.9	295.2	45.2	98250.1
Pembela x Wilk 2	P_1	0.3	0.3	124.10	0.66	1.5	77.4	25.1	23460.5
	P_2	0.6	2.3	148.44	0.71	1.6	33.9	22.2	24750.7
	F_1	0.3	1.3	145.17	0.98	1.4	61.2	32.8	18828.3
	BC_1	2.3	4.3	153.43	1.20	1.6	131.1	52.9	38438.0
	BC_2	1.3	2.3	153.86	1.02	1.7	198.1	42.9	48647.9
	F_2	3.2	4.6	165.24	1.41	1.8	232.1	60.8	61252.5
Pembela x VAX 6	P_1	0.3	2.3	46.50	0.72	1.3	42.4	8.9	8299.8
	P_2	0.3	2.3	37.98	1.054	0.5	51.7	2.8	26501.3
	F_1	1.3	2.3	66.24	1.04	1.1	63.8	4.2	16529.6
	BC_1	2.3	4.3	132.86	1.244	2.1	102.9	29.4	109938.7
	BC_2	1.0	4.3	112.64	1.539	1.2	159.5	17.56	133673.2
	F_2	2.6	4.9	171.24	1.721	2.3	186.2	39.441	201130.1