Genetic Studies on Host-Plant Resistance to Bean Fly (*Ophiomyia* spp.) and Seed Yield in Common Bean (*Phaseolus vulgaris*) under Semi-Arid Conditions

By

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Thesis abstract

Bean fly (*Ophiomyia* spp.) is a major pest of common bean (*Phaseolus vulgaris* L.) throughout eastern and southern Africa. In the semi-arid areas, apart from drought, the insect pest is reported to cause high crop losses up to 100%, particularly when drought occurs and under low soil fertility. Host-plant resistance is part of the integrated pest management strategies that have been widely employed against major insect pests of tropical legumes. However, information regarding its use in control of bean fly in common bean is limited. Therefore, the objectives of this study were to: (1) validate farmers' perceptions of major constraints responsible for yield losses, particularly the major insect pests of beans; (2) asses the level of adoption of improved bean varieties and determine factors that influence farmers' preferences of the varieties and criteria for selection; (3) identify sources of resistance to bean fly available in landraces; (4) determine the nature of gene action controlling bean fly resistance and seed yield in common bean; (5) describe a procedure for generating optimal bean fly populations for artificial cage screening for study of the mechanisms of resistance available in common bean against bean fly.

Farmers considered drought and insect pest problems as main causes for low yields. The adoption rate for improved varieties was high but self-sufficiency in beans stood at 23% in the dry transitional (DT) agro-ecology and at 18% in the dry mid-altitude (DM) agro-ecology, respectively. Drought, earliness, yield stability, and insect pest resistance were the factors determining the choice of varieties by farmers. Bean fly (*Ophiomyia* spp.), African bollworm (*Helicoverpa armigera*) and bean aphid (*Aphis fabae*) were identified as key crop pests of beans limiting yield.

The study to identify new sources of resistance included 64 genotypes consisting of landraces, bean fly resistant lines and local checks. The experiment was done under drought stressed (DS) and non-stressed (NS) environments and two bean fly treatments (insecticide sprayed and natural infestation) for three cropping seasons between 2008 and 2009. Genotypes differed in their reaction to natural bean fly attack under drought stressed (DS) and non-stressed environments (NS) over different cropping seasons. However, the effect of bean fly appeared to vary between the long rains (LR) and short rains (SR). It was observed that an increase in the number of pupae per stem resulted in a higher plant mortality. The range of seed yield was from 345 to1704 kg ha^{-1} under

natural infestation and from 591 to 2659 kg ha^{-1} under insecticide protection. Seed yield loss ranged from 3 to 69 %. The resistance of most of the bean fly resistant lines seemed to be ineffective in presence of DS.

To determine the nature of gene action controlling the inheritance of resistance to bean fly, four parents with known reaction to bean fly were crossed with four locally adapted genotypes in an 8 x 8 half-diallel mating design. Similarly, two resistant and two susceptible parents were selected and crossed to produce populations for generations means and variance components analysis. Results revealed that both general combining ability (GCA) and specific combining ability (SCA) mean squares were significant (p ≤ 0.05) for all four traits studied, except SCA for stem damage during one cropping season. Among the parents, GBK 047858 was the best general combiner for all the traits studied across seasons except for stem damage during LR 2009. Genotypes GBK 047821 and Kat x 69 (a locally adapted variety) were generally good general combiners for resistance traits as well as seed yield. General predictability ratio values ranging from 0.63 to 0.90 were obtained for plant mortality, stem damage, pupae in stem and seed yield across cropping seasons. These results established the predominance of additive gene effects (fixable variation) over the non-additive effects in controlling the traits. Low to moderate narrow sense heritability values ranging from 0.22 to 0.45 were obtained for pupae in stem. Such heritability estimates indicate that although additive gene components were critical in the inheritance of resistance for the trait, non-additive gene action was also important in addition to the environmental effects.

A major disadvantage in screening for resistance to bean fly in common bean by controlled means in net cages has been the lack of a method to use for raising adequate fly populations for screening. Due to this problem, a simple procedure for raising sufficient numbers of adult bean flies required for screening was described. Through this method, up to 62 % emergence of the adult flies was achieved. Moreover, the flies retained their ability to infest bean plants. To determine the presence of antibiosis and antixenosis mechanisms of resistance in common bean, five genotypes [CC 888 (G15430), GBK 047821, GBK 047858, Ikinimba and Macho (G22501)] and two local check varieties (Kat B1 and Kat B9) were screened under free-choice in outdoor net cages and no-choice conditions in net cages placed in a shadehouse. All the five resistant genotypes tested had relatively long internodes. It was established that long internode was a morphological trait associated with reduced pupation rate in bean stems, hence an antixenosis component of resistance. Both ovipositional non-preference and antibiosis mechanisms

were found to exist in three genotypes namely CC 888 (G45430), GBK 047858 and Macho (G22501). These genotypes were resistant when they were subjected to bean fly under both free-choice and no-choice conditions. They had fewer feeding/oviposition punctures, low number of pupae in the stem, reduced damage to the stems and low percent plant mortality. The remaining genotypes, Ikinimba and GBK 047821 only expressed antixenosis. To maximize the effectiveness of host-plant resistance against bean fly, multiple insect resistances should be incorporated into a single bean genotype in order to ensure durability. However, this should be within the background of integrated pest management strategy.

Declaration

I, **<u>Pascal Peter Okwiri Ojwang'</u>**, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. The thesis has not been submitted for any degree or examination at any other University
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. When other written sources have been quoted, then:
   a. Their words have been re-written but the general information attributed to them has been referenced;
   b. Where their exact words have been used, then their words have been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics and tables copied and pasted from internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference section.

Signed.. Date..

Pascal Peter Okwiri Ojwang' (Student)

As the candidate's supervisors, we agree to the submission of this dissertation for examination:

Signed.. Date ....

Prof. Rob Melis (Principal supervisor)

Signed .... Date ....

Dr. Mwangi Githiri (Co-supervisor)

Acknowledgements

Dedication

To my wife Jenipher and sons, Fortune and Job

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Publications pertaining to this thesis

Chapter 1

Ojwang', P.P.O., Melis, R., Githiri, M., Songa, J.M., 2011. Breeding options for improving common bean for resistance against bean fly (*Ophiomyia* spp.): A review of research in eastern and southern Africa. Euphytica (in press).

Chapter 2

Ojwang', P.P.O., Melis, R., Songa, J.M., Githiri, M., 2009. Participatory plant breeding approach for host plant resistance to bean fly in common bean under semi-arid Kenya conditions. Euphytica 170, 383-393.

Chapter 3

Ojwang', P.P.O., Melis, R., Songa, J.M., Githiri, M., 2010. Genotypic response of common bean genotypes to natural field populations of bean fly (*Ophiomyia phaseoli*) under diverse environmental conditions. Field Crops Res. 117, 139-145.

Chapter 4

Ojwang', P.P.O., Melis, R., Githiri, M., Songa, J.M., 2010. Genetic analysis for resistance to bean fly (*Ophiomyia phaseoli*) and seed yield among common bean genotypes in a semi-arid environment. Field Crops Res. 120, 223-229.

Introduction to thesis

1. Importance of common bean

Common bean (*Phaseolus vulgaris* L.) is one of the main food legumes grown in the tropics and the bulk of the production is concentrated in the developing countries under low input agriculture (Miklas et al., 2006). According to Broughton et al. (2003), beans are a key source of protein (~22%), macronutrients (Ca), micronutrients (Fe, Cu, Mn, Zn) and vitamins for the human diet, particularly in the developing countries. In Kenya beans rank second after maize as a source of food, predominantly among the rural communities (Korir et al., 2006). The total annual bean production figure is approximately 388, 796 metric tons (FAOSTAT, 2007), which makes Kenya one of the largest producers in sub-Saharan Africa. According to the Food and Agricultural Organisation, common bean accounts for about 10% of the total protein consumed in Kenya (FAOSTAT, 2002, 2003).

Bean production in semi-arid eastern Kenya region is carried out in marginal environments. The production is largely concentrated among the smallholder farmers whose cropping systems are diverse, ranging from monoculture to intercropping, mainly with cereals. A majority of these farmers are resource-poor and faced by numerous challenges. These include low soil fertility, disease and insect pest infestation, and drought stress (Letourneau, 1994; Ampofo and Massomo, 1998). Due to socio-economic factors, farmers are unable to combat these constraints effectively. Consequently, on-farm bean yields average less than 500 kg ha^{-1} (Graham and Ranalli, 1997; MoA, 2006) compared to about 1200 kg ha^{-1} under experimental conditions in the semi-arid areas (MoA, 2006).

2. Importance and distribution of bean fly

Bean fly (*Ophiomyia* spp.) is one of the most important insect pest of beans in major bean growing areas of eastern Africa (Ampofo and Massomo, 1998). Since farmers have limited land, they continue to cultivate the same areas over the years, using limited or no application of pesticides or fertilizer, which leads to a build-up of pests and reduction in soil fertility (Letourneau, 1994). Under such farming conditions, bean fly becomes the most important insect pest, causing significant yield losses (Greathead, 1968; Letourneau, 1995). The damage is increased under drought and on poor soils, leading to yield losses in the range of 30 to 100 % (Greathead, 1968; Ampofo and Massomo, 1998).

Despite the high yield losses reported, the significance of this pest is probably not well understood under farm conditions (Abate et al., 2000). Stem mining insects such as bean fly are economically important even at low densities, especially at seedlings (Edwards and Singh, 2006).

There are three main species of bean fly that attack beans in eastern Africa namely, *Ophiomyia phaseoli* Tyron*, O. spencerella* Greathead*,* and *O. centrosematis* de Meijere (Greathead, 1968; Letourneau, 1994; Songa and Ampofo, 1999). However, *O. phaseoli* and *O. spencerella* are the most important of the three species. This is because *O. centrosematis* only rarely occurs and in small numbers (Abate and Ampofo, 1996; Abate et al., 2000). The distribution of *O. phaseoli* and *O. centrosematis* extends all over tropical and subtropical Africa, Asia, and Australia, while *O. spencerella* has not been recorded outside Africa. In addition, location and season determine the species composition and pattern, where *O. phaseoli* and *O. centrosematis* are commonly found in warmer mid-altitude areas, while *O. spencerella* is more prevalent in cooler and wetter high-altitudes.

3. Genetics, mechanisms and sources of resistance

The genetics of insect resistance or tolerance in common bean is generally quantitative and polygenic (Miklas et al., 2006). Little information exists on the inheritance of resistance to bean fly. A report from a genetic study indicated the importance of additive gene effects over the non-additive gene effects for percent plant survival of beans under natural infestation of bean fly (Mushi and Slumpa, 1998). Nonetheless, a more detailed study that would consider more resistance parameters would be necessary in order to provide more comprehensive results.

Similar investigations in soybean revealed that the inheritance of resistance to agromyzid bean fly (*Melanagromyza sojae* Zehntner) was controlled by one major gene along with minor genes (Wang and Gai, 2001). Additive and dominant gene effects of the minor genes were less than for the major gene and varied from cross to cross. Heritability for the major gene was also higher compared to minor genes.

Like most plants, legumes rely on a set of defences for protection against insect pests (Edwards and Singh, 2006). Plant structural and chemical defences can discourage feeding by the herbivorous pests (antixenosis), by suppressing their growth and

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development (antibiosis), or by reducing the damage symptoms (tolerance) (Clement et al., 1994). Studies on the mechanisms of resistance to bean fly have mainly been conducted in soybean (Talekar and Hu, 1993; Taleker and Tengkano, 1993) and in mungbean (Talekar et al., 1988), where it has been reported that both morphological and chemical components present in certain soybean plants reduce the fecundity of bean fly. Limited information from studies of that nature exists in common bean (Edwards and Singh, 2006). However, Cardona and Kornegay (1999) suggested that both antibiosis and antixenosis mechanisms of resistance to insects could be found in bean plants.

Various sources of resistance to the bean fly in common bean germplasm have been reported (Greathead, 1968; Mushi and Slumpa, 1998; Ogecha et al., 2000). Abate et al. (1995) identified sources of resistance to bean fly among accessions obtained from the Centro Internacional de Agricultura Tropical (CIAT). A number of local landraces from Uganda and Tanzania were regarded as resistant due to their ability to produce adventitious roots and thickened hypocotyls (Greathead, 1968). Apart from common beans, host plant resistance against bean fly and related agromyzids has been reported in other leguminous crops such as mungbean, cowpea (Talekar et al., 1988) and soybean (Talekar et al., 1988; Wang and Gai, 2001).

4. Seed yield improvement and stability

Progress in breeding for high yield in common bean has been slow (Singh, 1991). Breeding for seed yield improvement requires an understanding of the factors that are important in yield increase (Yan and Wallace, 1995). Kelly et al. (1998) suggested that seed yield in common bean can be improved if the developed cultivars are bred to fit within the cropping season in the target environment. Specifically, efficient genotypes that can rapidly change from vegetative to reproductive growth phase for specific adaptation to definite local environments, are suitable. For the semi-arid areas, farmer fields represent multiple environments and are often very dissimilar to the experimental stations. In order to account for genotype (G) x environment (E) (GE) interactions, testing on farmer fields is a prerequisite (Ceccarelli and Grando, 2007).

5. Farmer perceptions of bean varieties and pests

In an effort to mitigate some of the crop production constraints experienced by the farmers, a number of improved bean varieties and agronomic packages for management of soil, pests and diseases have been recommended for the semi-aid areas. Apparently,

adoption of these technologies has been modest. Despite the adoption of some of the new varieties, self-sufficiency in beans has remained unachievable. Knowledge of farmers and their practices for managing pests is necessary for the development of management strategies that will better serve the farmers, and are thus likely to be adopted (Chitere and Omolo, 1993; Rubia et al., 1996: Tanzubil and Yakubu, 1997). Farmers perceive bean fly as a key pest of beans (Ngulu et al., 2004) and probably incorporate bean fly resistant cultivars into their cropping systems (Letourneau, 1994) by directly or indirectly selecting for resistance (Abate et al., 2000).

6. Research focus

Host plant resistance is one of the sustainable strategies that can be used to contain field pest populations below economic threshold levels. Success in incorporating insect resistance into commercial varieties through breeding has been difficult in many legume crops (Edwards and Singh, 2006). The lack of progress has been attributed to breeders not having access to a full range of available germplasm resources. Another problem has been the difficulty in achieving pest resistance without reducing agronomic quality (Edwards and Singh, 2006). The development of bean varieties with improved resistance to insect pests can help reduce the dependence on pesticides in high input systems, minimize yield loss from pests in low- and high-input systems, and enable stable bean production across diverse environments (Miklas et al., 2006). A combination of multiple qualities such as yield improvement along with pest resistance or tolerance to drought and low soil fertility is required for the development of bean cultivars that are adapted to a range of bean production agro-ecologies (Hillocks et al., 2006). In addition, farmers would be better served if such varieties are further improved for farmer preferred traits such as culinary qualities and market values (seed colour and seed size), which would improve adoption rates by small-scale farmers (Abate et al., 2000; Hillocks et al., 2006).

A key challenge in breeding common bean for resistance to bean fly is to develop a systematic screening procedure that would provide a constant bean fly populations to exert uniform pressure on the screening material (Hillocks, et al., 2006). Most of the screening has been based on open-field tests which has its own disadvantages. For example, low bean fly pressure could arise from high prevalence of natural enemies during certain periods that consequently reduce bean fly populations (Talekar and Tengkano, 1993). Therefore, there is need to develop a reliable technique that would help to positively identify resistant lines.

7. Research objectives

The main objective of the study was to develop insect resistant varieties with important farmer- preferred characteristics for the semi-arid bean growing agro-ecologies of eastern Africa.

The specific objectives were to:
1. Validate farmers' perceptions of major constraints responsible for yield losses, in particular the major insect pests of beans in semi-arid eastern Kenya.
2. Assess the level of adoption of improved bean varieties and determine factors that influence farmers' preferences of the varieties and criteria for selection.
3. Identify sources of resistance to bean fly available in landraces.
4. Determine the nature of gene action controlling bean fly resistance and seed yield in common bean.
5. Describe a procedure for generating optimal bean fly populations for artificial cage screening for the study of the mechanisms of resistance available in common bean against bean fly.

The thesis is structured in such a way that the chapters are in the form of research articles. Therefore, there could be a certain amount of overlap among the chapters.

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Chapter 1

Breeding options for improving common bean for resistance against bean fly (*Ophiomyia* spp.): A review of research in eastern and southern Africa

Abstract

Bean fly (*Ophiomyia* spp.) is a key pest of common bean (*Phaseolus vulgaris* L.) throughout eastern and southern Africa. It is known to cause total crop loss especially under drought stress and low soil fertility. This review underscores the importance of bean fly to bean production. It discusses the research achievements on genetic improvement of common bean for resistance against bean fly attack and highlights further opportunities available for rapid advance. The paper dwells on conventional breeding approaches and possibilities for utilization of marker-assisted selection. Mechanisms of common bean resistance to bean fly have been considered with a view to understand the genetic control. To maximize the effectiveness of host-plant resistance against bean fly, multiple insect resistances should be incorporated into a single bean genotype in order to ensure stability. However, this should be within the background of integrated pest management strategy.

Introduction

Common bean is (*Phaseolus vulgaris* L.) is a major food legume grown throughout the tropics but most widely in Latin America and eastern and southern Africa, where it is a key source of dietary protein (Hillocks et al., 2006). The bulk of bean production in the developing world, particularly in Africa, takes place under low input agriculture on small-scale farms and mainly by women farmers (Wortmann et al., 1998). Such farming conditions are extremely variable and the beans grown here are exposed to several biotic and abiotic stresses (Singh, 1992; Wortmann et al., 1998) which lead to low seed yield. Insect pests are reported to be a major component of biotic stress in subsistence production systems resulting from limited or no use of chemical pesticides and fertilizers (Letourneau, 1994). Of the major insect pests of common bean in eastern and southern Africa, bean fly (also known as bean stem maggot) (*Ophiomyia* spp.) is by far the most important pest of economic importance (Abate and Ampofo, 1996; Abate et al., 2000; Hillocks et al., 2006). Reports on yield losses arising from damage caused by this pest are varied but a range of 8% to 100% has been recorded (Greathead, 1968; Abate and Ampofo, 1996; Ojwang' et al., 2010). Despite the high yield losses reported, the significance of this pest is probably not well understood especially under farm conditions (Abate et al., 2000). Insects that destroy seedlings such as bean fly are economically important even at low densities (Edwards and Singh, 2006).

The majority of bean farmers in Africa rarely use chemical pesticides on their crop and instead rely upon traditional pest management practices (Abate et al., 2000). Besides, there is limited access to farm inputs including chemical pesticides, quality seed and chemical fertilizers among the resource-disadvantaged farmers arising from prohibitive costs (Ojwang' et al., 2009). Consequently, heavy yield losses resulting from insect pests such as bean fly are incurred. The damage caused by bean fly is more pronounced in the marginal areas particularly in dry than wet conditions and under low soil fertility (Greathead, 1968; Karel, 1985). Attempts by farmers to optimize production with limited resources available may lead to build up of crop pests (Letourneau, 1994; Letourneau, 1995). When effective pesticides are not used because of hazardous effects on the environment or due to lack of affordability, in that case management through cultural practices e.g. intercropping, earthing-up, early planting, timely weeding and/or by biological means such as the use of natural enemies, bio-pesticides or also by genetic means (host-plant resistance) can be critical. However, the short growing season of beans and the frequent fallow periods that follow crop harvest, lessen the efficacy of biological control (Kornegay and Cardona, 1991a).

Research on host-plant resistance against bean fly has been given due consideration by the main international bean improvement programmes run by the Centro Internacional de Agricultura Tropical (CIAT) under the regional establishments, East and Central Africa Bean Research Network (ECABREN) as well as Southern Africa Bean Research Network (SABRN) (Chirwa et al., 2003). This research has been done in collaboration with the National Agricultural Research Institutes (NARIs) of respective member countries. In spite of some resistant or tolerant varieties having been identified and availed to the farmers, these varieties have failed to achieve a major impact on subsistence food production. This could be attributed to the farmer varieties being well adapted and therefore more resistant/tolerant to local insect pests as a result of co-evolution and selection by farmers either knowingly or unknowingly over many years (Abate et al., 2000). However, local landraces are inherently low-yielding. Farmers in unstable and unpredictable environments plant mixtures of varieties that are more able to respond to extremely variable conditions such as unpredictable rainfall, variation in soil condition, and pest and disease problem. Besides, breeding physical qualities in varieties may have negative effects on taste, or cooking time, and therefore may be undesirable to farmers. Therefore, farmers are perhaps better served when offered a range of genotypes to chose from in order to exploit a highly erratic environment (Mekbib, 2006; Ceccarelli and Grando, 2007; Ojwang' et al., 2009).

The present review therefore considers bean fly as a major insect pest limiting bean production in sub-Saharan Africa. Progress made so far and possible challenges in breeding for resistance to bean fly as a component of host-plant resistance management strategy is highlighted. In the final section, the potential available for future research is suggested.

1.2 Distribution and biology of bean fly

1.2.1 Distribution of bean fly

There are three main bean fly species that have been reported to attack beans in various parts of the world namely, *Ophiomyia phaseoli* Tyron*, O. spencerella* Greathead*,* and *O. centrosematis* de Meijere (Greathead, 1968; Letourneau, 1994; Songa and Ampofo, 1999). The distribution of *O. phaseoli* and *O. centrosematis* stretches throughout tropical and subtropical Africa, Asia, and Australia, but *O. spencerella* has not been recorded beyond Africa (Abate et al., 2000). In eastern and southern Africa, bean fly infestation is

widespread, and has been confirmed in nearly all the countries in the region (Table 1.1). The population dynamics of bean fly species, composition and patterns of infestation vary with location and season. In warmer mid-altitude areas, *O. phaseoli* and *O. centrosematis* are common while *O. spencerella* is more prevalent in cooler and wetter high altitudes. However, of the three species, *O. phaseoli* and *O. spencerella* are the most important. This is because *O. centrosematis* only occurs rarely and in small numbers (Abate and Ampofo, 1996; Abate et al., 2000). Within a growing season, *O. phaseoli* is known to attack the earlier planted crops compared to *O. spencerella* which destroys the late planted crops. A study on the relative abundance of bean fly species and their population dynamics in semi-arid eastern Kenya revealed that the dominant species in this region are *O. phaseoli* and *O. spencerella* (Songa and Ampofo, 1999).

Table 1.1 Distribution of bean fly (*Ophiomyia* spp.) in eastern and southern Africa.

Country	Reference
Botswana	Greathead (1968)
Burundi	Karel (1985);Atrique (1989)
Ethiopia	Karel (1985); Abate (1990)
Kenya	Greathead (1968);
Malawi	Karel (1985); Letourneau (1994)
Mozambique	Davies (1998)
Tanzania	Greathead (1968); Karel and Maerere (1985)
Uganda	Greathead (1968); Spencer (1973)
Zambia	Karel (1985)
Zimbabwe	Karel (1985)

1.2.2 Bean fly biology

Karel (1985) provided a detailed description of the biology of *Ophiomyia* spp. The adult fly in all the species, is a tiny insect measuring 1.9 to 2.2 mm in length (Fig. 1.1a). The life cycles of *Ophiomyia* spp. are similar except that eggs of *O. phaseoli* are deposited in the leaf tissue (Fig. 1.1a), whereas eggs of *O. spencerella* and *O. centrosematis* are inserted in the hypocotyl or stem (Greathead, 1968). About 70 eggs are laid per female (Karel, 1985). The larvae hatch from eggs in two to four days and begin feeding on the stem tissue soon after emergence, tunnelling down the stem towards the soil surface. In their

feeding activities they damage vascular tissues thereby interfering with translocation activities of the plant. The total larval period lasts for eight to ten days in warm climates. The fully grown larvae pupate below the stem epidermis at the junction between the root and the stem. The larvae make a thin transparent window in the epidermis for emergence of the adult. The pupae of *O. phaseoli* are translucent yellow-brown (Fig. 1.1b) while those of *O. spencerella* are shiny black (Greathead, 1968; Karel, 1985). Species identification in adults can be done using the male genitalia (Greathead, 1968). The total life cycle from egg to adult emergence varies under different environmental conditions from an average of 20 days in warm weather to 42 days in cool weather. Heavy infestation on younger plants may cause severe damage to the vascular tissue causing the plant to wither and die just before flowering stage. In older plants, calloused growth develops on the stem around the injured areas mainly where the larvae pupate which result in stunted growth, yellowing of leaves and occasionally lodging of the plants.

Fig. 1.1 (a) Adult bean fly feeding on young trifoliate leaves and oviposition/feeding punctures visible on older leaves; (b) damage symptoms and *Ophiomyia phaseoli* pupa in the stem at the junction between the roots and stems.

1.3 Conventional breeding approaches for resistance to bean fly

1.3.1 Sources of resistance and gene introgression

Plant breeding has contributed to remarkable improvements in food supplies and crop productivity in many parts of the world. However, conventional plant breeding has had great impact in high potential production environments but falls far below expectation in marginal environments in developing countries where poverty levels are high (Ceccarelli

and Grando, 2007). Research work to improve common bean for resistance to bean fly in eastern and southern Africa has dwelt much on screening for genetic sources of resistance mainly from local landraces (farmer varieties), germpasm accessions and local varieties (Greathead, 1968; Kornegay and Cardona, 1991a; Abate et al., 1995; Ojwang' et al., 2010). Various sources of resistance to the bean fly in common bean germplasm have therefore been reported (Table 1.2). Bean fly resistance has also been reported from scarlet runner bean (*Phaseolus coccineus*) (Kornegay and Cardona, 1991a). Karel and Maerere (1985) found only low resistance among common bean genotypes to bean fly. Apart from common beans, resistance against bean fly and related agromyzids has been reported in other leguminous crops such as mungbean, cowpea and soybean (Talekar et al., 1988; Wang and Gai, 2001).

Table 1.2 Examples of sources of resistance in common bean genotypes in primary and secondary gene pools with high levels of resistance to bean fly.

Landrace/variety	Source[a]	Crop species	Reference
G5773, G2072	CIAT	*Phaseolus vulgaris*	Abate (1990); Abate et al. (1995); Mushi and Slumpa, (1996)
ZPV 292, G5773, A55, G2005	CIAT	*Phaseolus vulgaris*	Abate et al. (1995); Mushi and Slumpa (1996)
G35023, G35075	CIAT	*Phaseolus coccineus*	Kornegay and Cardona, (1991a)
A429, TMO	CIAT	*Phaseolus vulgaris*	Abate et al. (1995)
Mlama 49, Mlama 127, G22501	CIAT	*Phaseolus vulgaris*	Hillocks et al. (2006)
GBK 047810, GBK 047866, GBK 047821, GBK 036488,	NGBK	*Phaseolus vulgaris*	Ojwang' et al. (2010a)
G21212, CIM 9314-36, Ikinimba	CIAT	*Phaseolus vulgaris*	Mushi and Slumpa (1996); Ojwang' et al. (2010a)
G2472, EMP 81, G3844, BAT 16	CIAT	*Phaseolus vulgaris*	Mushi and Slumpa (1996); Mushi and Slumpa (1998)

[a] NGBK National Gene Bank of Kenya.

The use of crop diversity (intercropped systems) is one of the primary methods for bean fly control by small-scale farmers in sub-Saharan Africa (Abate and Ampofo, 1996). Breeding for resistance to bean fly should take into account not only increased levels of resistance through recombination of lines with different resistance mechanisms and genepools, but also through the development of bean varieties that are adapted to maize/bean and other mixed cropping systems. Although the overall level of resistance is low to moderate, it may provide sufficient protection in traditional farming systems.

Attempts to introduce resistance genes into locally adapted and widely adopted commercial varieties have been done on a limited scale and with mixed success. Abate et al. (1995) reported the presence of genetic variation in F_2 populations for crosses made between resistant lines with locally adapted varieties for both *O. phaseoli* and *O. spencerella*). Despite the reports, little evidence exists regarding the successful release and adoption of bean fly resistant varieties by farmers.

Sources of resistance presented in Table 1.2 could be exploited in breeding programmes in the Africa region to introduce resistance genes into improved varieties already cultivated by the farmers. However, initial testing of these materials may be required to confirm their resistance before inclusion into a breeding programme. In addition to the use of resistance sources from primary common bean gene pool, gene introgression through interspecific hybridization from secondary gene pool such as scarlet runner bean *phaseolus coccineus*, in which resistance to bean fly has been found could be exploited. For example, several progenies of interspecific crosses between *P. coccineus* and *P. vulgaris* showed no signs of infestation to *O. phaseoli* (Kornegay and Cardona, 1991a). However, success in incorporating insect resistance into commercial varieties through breeding has been difficult in many legume crops (Edwards and Singh, 2006). The lack of progress has been attributed to breeders not having access to the full range of available germplasm resources (Kornegay and Cardona, 1991a). More so, if traits are under polygenic control or have additivity, then achieving pest resistance without reducing the agronomic quality would be a challenge. This could be probably due to the polygenic nature of resistance bringing along undesirable traits due to linkage drag.

In the past, it has been suggested that failures in breeding for insect resistance arose from lack of establishment of proper links between researchers (entomologists) identifying the resistance and breeders who would introduce the resistance into commercial lines (Edwards and Singh, 2006). In the contrary, lack of directing attention to breeding for

bean fly resistance in common bean may not be fully due to the breeders not having had the support of entomologists to perform artificial infestation under controlled conditions. In fact, successful work has been done in the field under natural infestation at 'hot spot' using appropriate field design and good nursery management (Karel and Maerere, 1985; Abate et al., 1995; Ojwang' et al., 2010). A good field evaluation has the potential to identify resistant genotypes.

1.3.2 Gene pyramiding

Due to large differences found between common bean races and among gene pools, problems occasionally occur that affect recombination and gene exchange (Koinange and Gepts, 1992). However, introgressing and pyramiding of useful alleles from within and across cultivated races and gene pools, wild populations of common bean, and its secondary and tertiary gene pools would broaden genetic base, apart from taking advantage of gains from selection and increasing the durability of resistance to insects (Singh, 2001). Although some success in introducing a single insect resistance gene into commercial bean cultivars from wild common bean has been achieved (Kornegay and Cardona, 1991b), multiple insect and /or disease resistant varieties are greatly required (Clement et al. 1994) for increased commercial value. Pyramiding of multiple insect resistance traits and disease resistance simultaneously has not been common, but attempt so far made by Singh et al. (1998) show that this approach may be promising. Pyramiding favourable alleles has been used for leafhopper resistance from a cultivated race of common bean (Singh, 2001) but not for bean fly resistance. Due to the polygenic nature of resistance to many insect pests, pyramiding of resistance of many insect pests in a single genotype will remain a challenge (Miklas et al., 2006). Besides, it is difficult to breed for pest resistance when the resistance in itself reduces crop quality. Despite difficulties in developing true breeding lines from interspercific crosses, researchers have successfully introgressed disease resistance from *P. coccineus* (Miklas et al., 1998; Park and Dhanvantari, 1987).

1.4 Host-plant resistance: Mechanisms and genetic control

1.4.1 Application of host-plant resistance

Host-plant resistance is a part of integrated pest management approach that can be used to contain field pest populations below economic threshold levels. When a given pest is continuously present and happens to be the single most limiting factor in successful

cultivation of a crop in a wide crop area, then host-plant resistance has comparative advantage over other control strategies (Shanower et al., 1998). An example of such an insect pest is the bean fly. That is why, the development of bean varieties with reasonable levels of resistance to bean fly can help reduce direct cost to the small-scale farmers. Miklas et al., (2006) suggested that improving bean varieties for resistance to insect pests can help reduce the dependence on pesticides to enable stable bean production across varied and unfavourable environments. Moderate to high levels of resistance have been reported in soybean (Chiang and Norris, 1983; Taleker and Tengkano, 1993; Wang and Gai, 2001) and mungbean (Taleker and Hu, 1993). The identification of such sources of resistance has lead to wide use of host-plant resistance against bean fly and related agromyzids in mungbean and soybean (Chiang and Norris, 1983; Taleker et al., 1988; Taleker and Tengkano, 1993; Wang and Gai, 2001). The breeding methods applied in soybean were mainly conventional approaches (Taleker and Tengkano, 1993; Wang and Gai, 2001). Some effort to address the gap existing in breeding beans for host-plant resistance to bean fly has been made by CIAT through ECABREN and SABRN (Chirwa et al., 2003; Hillocks et al., 2006) and national breeding programmes of some countries (Abate et al., 1995; Ojwang' et al., 2011).

1.4.2 Mechanisms of resistance to bean fly

Due to evolution, pest populations are able to overcome vertical plant resistances. This suggests that resistance breakdown leads to susceptibility of such pest-resistant crops. In order to lengthen the usefulness of resistant cultivars, it has been suggested that breeding strategies should aim at developing cultivars with more than one resistance gene (Clement et al., 1994). Like most plants, legumes rely on a set of defences for protection against insect pests (Edwards and Singh, 2006). Plant structural and chemical defences can act directly on the herbivorous pests by discouraging the herbivore feeding (antixenosis), by suppressing herbivore growth and development (antibiosis), or by decreasing the damage symptoms (tolerance) (Clement et al., 1994). Cardona and Kornegay (1999) stated that the mechanisms of resistance to insects in common bean can be divided into antibiosis and antixenosis traits except for a few that chiefly have biochemical traits such as seed protein, or morphological traits for instance leaf hair (trichome) density. Other plant characters implicated in bean fly ovipositional non-preference include concentration of tannin-like substances beneath the outer epidermis and the thickness of the fibrous cell layer above the inner epidermis. Tolerance to bean fly was attributed to thickened hypocotyls (Greathead, 1968). Wei et al. (2006) demonstrated that bean plants emit volatile compounds in reaction to damage caused by

agromyzid flies. Many volatiles are produced when bean plants are wounded by insects or by artificial means, although some of these volatile compounds may not be associated with resistance.

Studies on mechanisms of resistance in soybean have also shown that the defence against herbivorous insects may involve morphological (Talekar and Tengkano, 1993; Talekar et al., 1988) or chemical mechanisms (Hartmann, 2004; Mattiacci et al., 2001; Wei et al., 2006). Resistant accessions had significantly smaller unifoliate leaves (Talekar and Tengkano, 1993). The unifoliate leaves of the resistant lines were pubescent and their hypocotyls had low dry matter. Besides, certain unconfirmed antibiotic factors appeared to be involved in conferring the resistance. Tolerance to stem damage in beans is a mechanism of resistance to *O. phaseoli*. Low egg counts were associated with high leaf pubescence, thin stems, and long internodes (Maerere and Karel, 1984). Stem characteristics such as pigmentation and degree of lignification may be vital resistance factors for *O. spencerella*. Plants with purple hypocotyls were viewed to have certain phenolic compounds associated with bean fly resistance (Talekar and Hu, 1993).

1.4.3 Genetics of resistance

The genetics of insect resistance or tolerance in common bean is generally quantitative and polygenic (Kornegay and Cardona, 1991a; Miklas et al., 2006). Only limited studies have been conducted on inheritance of resistance to bean fly in common bean, but no studies on chromosomal localization of genes (Miklas et al., 2006). Preliminary evidence from diallel experiments indicate the predominance of additive gene effects over the non-additive gene effects in determining the expression of resistance to bean fly (Mushi and Slumpa, 1996; Ojwang' et al., 2011). These studies were based on fixed effects models of diallel mating designs meaning that the inferences made only applied to the selected lines studied but not to the general population. Nonetheless, due to the importance of additive gene action, good progress could be made in selecting resistant lines among breeding populations obtained from such crosses.

Similar investigation into the genetic inheritance of resistance of soybean to the agromyzid bean fly (*Melanagromyza sojae* Zehntner), revealed that the resistance was controlled by one major gene along with polygenes (Wang and Gai, 2001). Additive and dominant gene effects of the polygenes were less than the major gene and varied from cross to cross. Heritability was higher for the major gene as opposed to polygenes.

1.5 Genotype x environment interactions and stability of resistance

Genotype (G) x environment (E) (GE) interactions are of major importance to plant breeders (Kang, 1993) particularly when developing improved varieties targeting extremely variable farm conditions. When different genotypes of a given crop are adequately evaluated in a range of environments, changes in rankings are usually common (Ceccarelli and Grando, 2007). Such changes may pose difficulty to show the superiority of a given variety across environments (Mekbib, 2003). Significant GE interactions cannot be disregarded. The options are to avoid them by selecting genotypes that are broadly adapted to a whole range of target environments or basically carry out selection for an array of genotypes, each adapted to a specific environment (Ceccarelli et al., 1991). Selecting for specific adaptation is important predominantly for crops grown under unfavourable conditions. This is mainly for the reason that unfavourable environments can be very different from each other (Ceccarelli, 1994; Ceccarelli and Grando, 2007). Therefore, breeding strategy to identify materials suitable for unfavourable environmental and variable seasonal conditions should exploit analysis of GE components. This is because seasonal variations of bean fly populations (Davies, 1998; Songa and Ampofo, 1999), negative or low correlation between farmer field and research stations (Ceccarelli and Grando, 2007) and rainfall patterns/drought pressure may complicate the breeder's selection process. As a result, this may hamper positive identification of superior materials for the intended specific target environment or a wide range of environments.

In order to extend the usefulness of insect resistant cultivars and achieve stability, Kennedy et al. (1987) and Smith (1989) suggested that breeding programmes should emphasize on breeding of insect-resistant cultivars with more than one type of resistance, deploy polygenic (horizontal) resistance and use tolerant cultivars. Where various types of resistance namely, antibiosis (toxicity), antixenosis (insect repellence) and tolerance are presumed to be associated with bean resistance to insects (Edwards and Singh, 2006), breeders may be able to avoid the breakdown of plant resistance by releasing cultivars with multiple types of insect resistance. However, this strategy may not work in a diverse environment where variation across the environments possibly arising from low soil fertility could cause resistance breakdown. This is because soil fertility is an important potential cause of GE. Besides, low soil fertility exacerbates the effects of bean fly (Letourneau, 1994). In such instances, testing in a wide range of environments offered by the small-scale scale farms may help identify stable genotypes. For the case of bean fly, polygenic resistance could be durable. However, to ensure stability of resistance, apart

from employing polygenic resistance, farmers should also take advantage of traditional management systems such as good cultural practice and use of bio-pesticides (Abate et al., 2000).

1.6 Conclusions and future research prospects

Challenges arising from raising fly populations for screening under artificial cages, lack of uniform distribution of pest populations during open-field tests sometimes due to seasonal variation of bean fly may call for application of alternative strategies. For example, identification and mapping of insect resistance genes is expected to facilitate the development of molecular markers for marker-assisted selection (MAS) as has been achieved for disease resistance. Key to the deployment of insect resistance genes will be their further characterization and genetic tagging either as qualitative or quantitative characters. Therefore, the implementation and adoption of MAS in combination with conventional breeding for bean fly resistance would result in rapid advance. The potential for developing bean cultivars with high levels of resistance to bean fly appears to be plausible. Several resistant genotypes have already been identified within the common bean germplasm and they appear to be from the both Andean and Mesoamerican gene pools and a range of market classes. Generally, one method of pest control may not provide a long term control because of variations arising from seasons, locations and crop management systems. An integrated approach is more sustainable which requires an interdisciplinary approach involving plant breeders and entomologists. However, the resistant genotypes need to be combined with high yield and consumer-preferred agronomic traits before they will be accepted by farmers.

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Chapter 2

Participatory plant breeding approach for host plant resistance to bean fly in common bean under semi-arid Kenya conditions

Abstract

Common bean (*Phaseolus vulgaris* L.) is the most important legume crop in Kenya. It is cultivated across a wide range of agro-ecologies which include high potential and marginal areas. Eastern Kenya alone, largely semi-arid, accounts for 35 % of the country's total bean production. Bean farmers mainly small-scale have limited access to quality seed, chemical pesticides and fertilizer. Therefore, bean yield under on-farm conditions still remains below 500 kg ha^{-1} while the potential is about 1200 kg ha^{-1} under semi-arid conditions. To assess the farmers' views on bean varieties and a key insect pest and associated constraints contributing to yield loss, research was undertaken. The research included a survey to quantify the yield loss and a Participatory Rural Appraisal to determine the level of adoption and criteria for variety choice in semi-arid eastern Kenya (SAEK). The results show that farmers consider drought and insect pest problems as main causes for low yields. The adoption rate for improved varieties is high but self-sufficiency in beans stands at 23% in the dry transitional (DT) agro-ecology and at 18% in the dry mid-altitude (DM) agro-ecology, respectively. This could be attributed to low adaptability since most of the improved varieties grown were selected for high potential areas but now found in marginal areas. Drought, earliness, yield stability, and insect pest resistance are the main reasons for choice of varieties by farmers. Bean fly (*Ophiomyia* spp.) was identified as one of the key crop pests of beans limiting yield. Besides, African bollworm (*Helicoverpa armigera*) and bean aphid (*Aphis fabae*) were ranked higher. Due to limitations of the conventional breeding approach, a participatory plant breeding approach is suggested so as to provide an opportunity to develop insect pest resistant varieties adapted to the SAEK region.

2.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the principal food legume in Kenya. According to the Food and Agricultural Organization (FAOSTAT, 2002, 2003), of the total 67 g/capita/day proteins consumed in the country beans contribute 7 g/capita/day, which accounts for 10 %. In the semi-arid eastern Kenya (SAEK) region, beans are largely grown in marginal environments where the growing conditions are very unfavourable. Besides, the production is largely concentrated among the small-scale farmers whose farming conditions are diverse. A majority of these farmers are resource-poor, and faced with a myriad of challenges (Letourneau, 1994; Ampofo and Massomo, 1998). Due to socio-economic reasons, farmers are unable to effectively alleviate the effects of these constraints. Consequently, high yield losses are experienced. On-farm bean yields average less than 500 kg ha^{-1} compared to as much as 1800 kg ha^{-1} under experimental conditions.

Bean fly (*Ophiomyia* spp.) is regarded as the most important insect pest of beans in East Africa where the problem is acute (Ampofo and Massomo, 1998). Since farmers have limited land, they continue to cultivate the same areas over the years, using limited or no application of pesticides or fertilizer which leads to a build up of pests (Letourneau, 1994). Under such farming conditions, bean fly becomes the key insect pest, causing significant yield losses (Greathead, 1968; Letourneau, 1995). The damage is magnified under drought conditions and poor soils leading to yield losses in the range of 30 to 100 % (Greathead, 1968). Despite the high yield losses reported, the significance of this pest is probably not well understood under farm conditions (Abate et al., 2000). Stem mining insects such as bean fly are economically important even at low densities, especially when they destroy seedlings (Edwards and Singh, 2006). There are three main species of bean fly in East Africa namely, *Ophiomyia phaseoli* Tyron, *O. spencerella* Greathead, and *O. centrosematis* de Meijere (Greathead, 1968; Letourneau, 1994; Songa and Ampofo, 1999). However, *O. phaseoli* and *O. spencerella* are the most important of the three species. *Ophiomyia. centrosematis* only occurs rarely and in small numbers (Abate and Ampofo, 1996; Abate et al., 2000).

Kenyan farmers are knowledgeable about the symptoms of bean fly as a pest of beans (Ogecha et al., 2000), but may not recognize the flies as the causal agent of those symptoms. Letourneau (1994) reported that Malawian farmers were aware of the symptoms caused by bean fly attack and probably incorporate bean fly resistant cultivars

into their cropping systems despite the fact that the tiny flies themselves were not commonly known.

Knowledge of farmers is necessary for the development of appropriate pest control management strategies in line with farmers' needs hence a high likelihood of adoption (Chitere and Omolo, 1993; Rubia et al., 1996; Tanzubil and Yakubu, 1997). Technologies developed for small-scale farmers with minimal or lack of local participation, and without consideration of farmers' indigenous knowledge, practices and needs are seldom adopted (Trutmann et al., 1996) and if adopted often fail to meet the farmers' needs. Farmers are dynamic and adapt to changing situations affecting their environment. However, farmer knowledge is locality specific and needs to be validated (Nkunika, 2002; Trutmann et al., 1996). A better understanding by farmers may enhance their knowledge of management practices (Letourneau, 1994). It will also help them make informed decisions on the choice of appropriate pest management options, such as a combination of host plant resistance and cultural practices (Songa and Ampofo, 1999). To be successfully adopted, a new bean variety should satisfy the grower, seed producer and the consumer (Graham and Ranalli, 1997).

Host plant resistance is one of the sustainable strategies that can be used to suppress field pest populations below economic threshold levels. Therefore, the development of bean varieties with improved resistance to insect pests through participatory plant breeding (PPB) can help reduce the reliance on pesticides in high input systems, avert risk of yield loss from pests in low input systems, and enable stable bean production across diverse and adverse environments (Miklas et al., 2006). If bean varieties with reasonable levels of resistance are developed, they can form an integral part of an integrated pest management programme for the bean fly, and reduce direct cost to the small-scale farmers.

To enhance adoption of improved technology, a participatory plant breeding (PPB) system which allows farmers, research scientists and extension agents to conduct research together is essential. Farmer fields provide multiple environments which allow avoiding genotype by environment interaction effect between the farmer fields and research stations given that in most cases they are never similar, particularly under the semi-arid conditions (Ceccarelli and Grando, 2007). Besides, farmers base their selection on criteria which may differ from researchers' criteria.

The Kenyan bean breeding research programmes, using conventional breeding approach, have released a number of bean varieties for the semi-arid areas, mainly under the grain legumes programme (GLP) at Kenya Agricultural Research Institute (KARI) Thika and also the National Dry Land grain legumes programme at KARI Katumani. Most of these varieties were released in the 1970s and 80s. Despite the adoption of these varieties in SAEK, self-sufficiency in beans is yet to be met. This is partly attributed to lack of adaptability of the varieties to the environment where they are grown. Apparently, most of these varieties were developed for the high potential environment but are now being grown in the marginal areas. According to Ceccarelli and Grando (2007), conventional plant breeding has been successful to farmers in high potential environments because they can afford farm inputs but has achieved little in marginal environments since such environments are highly diverse and the farmers are generally poor hence cannot afford inputs including the certified seed which are costly.

Despite the limited success of formal breeding in mitigating the challenges of bean farmers in marginal areas, the bean programmes are yet to come up with technologies that are able to meet the diverse needs. It is therefore imperative to orientate the research strategy to come up with possible solutions and therefore develop sustainable bean production systems under the prevailing circumstances.

Therefore the objectives of this study were to:

1. Identify the major constraints to bean production in semi-arid eastern Kenya.
2. Understand farmers' perceptions on yield losses associated with bean fly and other important insect pests.
3. Asses the level of adoption of improved bean varieties in semi-arid eastern Kenya.
4. Determine factors that influence farmers' preferences of the varieties and criteria for selection.

2.2 Materials and methods

2.2.1 Study site

The semi-arid eastern Kenya (SAEK) was selected for its importance in bean production. Machakos and Kitui districts are representative because they are the two key areas where beans are produced in SAEK. Four sites were randomly selected representing the two major agro-ecologies where beans are produced (Table 2.1). The farmers were invited at specific sites for the Participatory Rural Appraisal (PRA). The farmers were

drawn from a total of 29 villages. The participants were mainly small-scale farmers with < 0.4 hectares of land. These farmers rarely use farm inputs such as fertilizer or pesticides to control insect pests on beans. Continuous growing of the same crops for years without rotation is a poor cultural practice which supports a rich array of insect pests resulting in a significant yield loss (Letourneau, 1994). The study area is therefore important for assessment of farmer knowledge and perceptions of bean fly and yield losses.

Table 2.1 Descriptions of sites used for farmer surveys and focused group discussions representing the two major bean agro-ecologies in semi-arid eastern Kenya

Sites	Altitude (M asl)	Agro-ecological zone
Iveti	1675	Dry transitional (DT)
Kaewa	1423	Dry transitional (DT)
Makutano	1270	Dry mid-altitude (DM)
Mulango	1025	Dry mid-altitude (DM)

2.2.2 Farmer surveys

A reconnaissance survey was first conducted with a total of 12 key informants across the sites. These were mainly elderly farmers with wide experience in bean growing, village elders as well as the frontline extension staff. A checklist was used as guide throughout the interview process. The interview covered perceptions on adoption of bean varieties, bean yield losses due to insect pests, local knowledge of pests and cultural practices as a management option.

2.2.3 Focused group discussion

Because of traditional custom, the male farmers were interviewed separately from their female counterparts so as to allow women to express themselves freely. Overall, 220 farmers participated (98 males and 122 females). The focused group discussions followed a loosely structured questionnaire. The discussions covered bean cropping systems, bean cropping calendar, bean varieties grown over the past 5-10 years, the criteria used by the farmers to select the varieties and ranking the varieties based on their criteria, major constraints to bean production and ranking according to importance, and major field insect pests of beans and how they rank these pests in order of importance. Farmers were asked to give an account of crop losses incurred in terms of percentage loss under mild, moderate, severe and very severe bean fly attack. The group discussion was backed up with individual interviews of each participating farmer. The exercise was

organized in collaboration with the village elders, local area administrative officers, farmer groups, and extension staff from the ministry of agriculture, as well as individual farmers.

2.2.4 Data analysis

Data collected was subjected for descriptive statistics analysis using SPSS version 10, statistical software.

2.3 Results

2.3.1 Cropping calendar and cropping systems

During the group discussions, farmers presented the bean crop calendar (Fig. 2.1). The rainfall pattern in the SAEK region is bimodal with the short rains (October-December) more reliable than the long rains (March to May). Common beans are usually planted in both cropping seasons. Planting is done at the onset of the rains as a normal practice by the farmers. However, some farmers still plant their beans late. The farmers who plant late mentioned that they incur high insect pest attack, mainly the bean fly, as opposed to when they plant early. Bean fly (*Ophiomyia* spp.) attacks the crop immediately after emergence. The pest population then increases rapidly finding the late planted beans at a vulnerable stage. Early planting is therefore essential as an escape mechanism and the majority of farmers in this region employ this strategy to avoid a high pest infestation during the crop growing season. Timing of weeding was also mentioned as an essential activity. Farmers emphasized the importance of weeding which is a vital activity in the cropping calendar as a responsive means to reduce the pest prevalence.

Intercropping is a common practice by small-scale bean growers in SAEK as in other regions of the sub-Saharan Africa. Farmers plant their beans alongside other crops, mainly cereals and sometimes other legumes in mixture or rotation (Fig. 2.2). The most commonly practiced intercropping system was bean/maize which approximately 90 % of the farmers indicated that they practice. This was followed closely by bean/pigeonpea cropping system, practiced by about 75 % of the farmers. The other common cropping systems were the three crop combinations which included bean/maize/pigeonpea, bean/maize/cowpea and bean/cowpea/pigeonpea practiced by 50 %, 25 % and 12 % of the farmers, respectively. Apart from food security intercropping is a built-in mechanism as a cultural practice to control crop insect pests such as the bean fly (Abate et al., 2000).

	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sep	Oct	Nov	Dec
Planting		·		███							███	
Weedin				≡≡≡	≡≡≡						≡≡≡	
Harvest	////	////			////	////						
Rainfall			‖‖‖	‖‖‖	‖‖‖					‖‖‖	‖‖‖	‖‖‖
Bean fly				▒▒▒							▒▒▒	

███ Planting time	≡≡≡ Weeding time	//// Harvest period

‖‖‖‖ Rainfall season	▒▒▒▒ Bean fly infestation
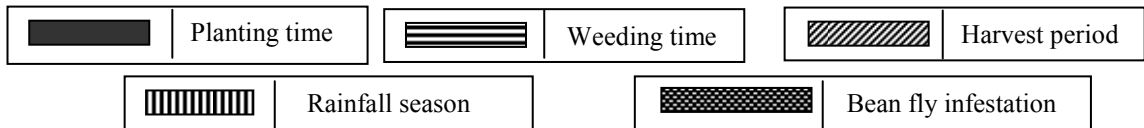
Fig. 2.1 Common bean cropping calendar in the semi-arid eastern Kenya.
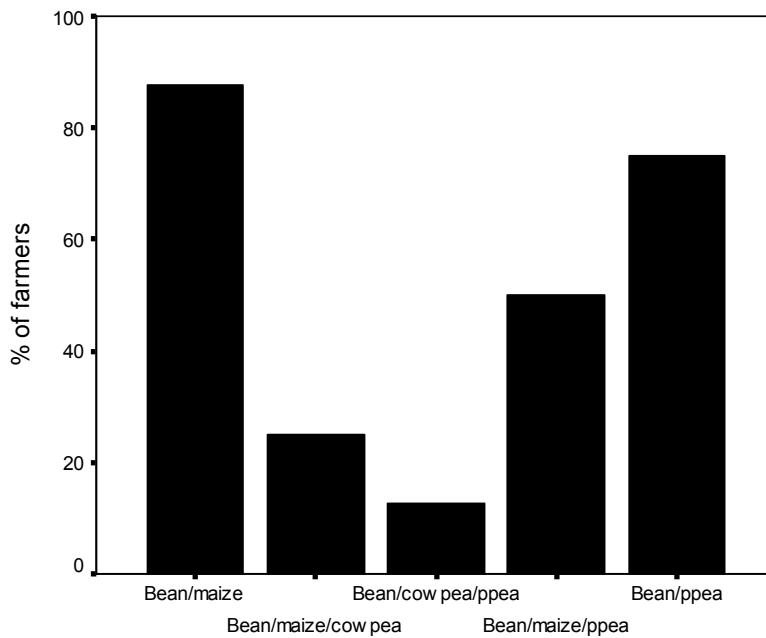
Fig. 2.2 Bean cropping systems commonly practiced in semi-arid eastern Kenya.

2.3.2 Common bean varieties preferred and reasons for adoption

Adoption of improved varieties developed through conventional breeding approach and formally released in the major bean growing agro-ecological zones was evident (Fig. 2.3). These varieties were developed under two separate national programmes with different objectives. The KARI Thika programme developed varieties for the medium and high potential areas, while the KARI Katumani programme developed bean varieties for the semi-arid regions. Results as represented in Fig. 2.3 show that farmers mainly grow improved varieties with only less than 10 % of the farmers growing the local varieties. The choice of variety to be planted as demonstrated is based on multiple criteria. According to farmers, drought tolerance, yield, early maturity and insect pest resistance are the most important for variety preference (Fig. 2.4). Other minor criteria include, marketability (market class), suitability for intercropping, determinate plant type as well as seed size. A bean variety that combines most of these traits is most preferred.

Even though similar varieties are grown across the semi-arid region, the choice of varieties varies from one locality to the next according to direct matrix ranking (Table 2.2). Different farmers have different specific needs suggesting that specific adaptation is important if these needs are to be met. This is shown by the change in ranking of varieties by farmers in different sites. Generally, the Katumani varieties are preferred for the drier areas (dry mid- altitude), whereas the Thika varieties seem to be more adapted to the dry transitional zone. Katumani varieties (Kat B1, Kat x 56 and Kat x 69) ranked higher in the DM ecology while the Thika varieties (GLP 2, GLP 24, GLP 1004, GLP x 92 and GLP 585) were ranked higher in the DT ecology. Farmers have abandoned most of the local varieties except a few such as Ngoso, Kakunzu, Ndumu and Ndamba. Reasons given by farmers who have retained the local varieties is that they are highly adapted and are good in culinary qualities even though their marketability is poor.
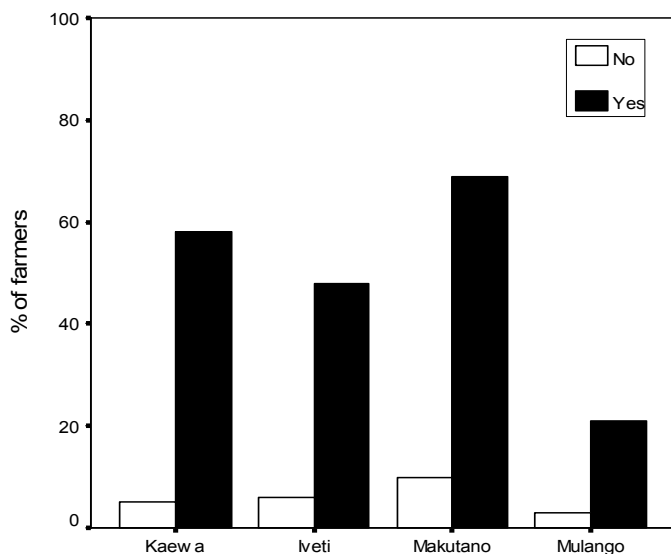
Fig. 2.3 Percent of adoption of improved bean varieties across the semi-arid eastern Kenya.
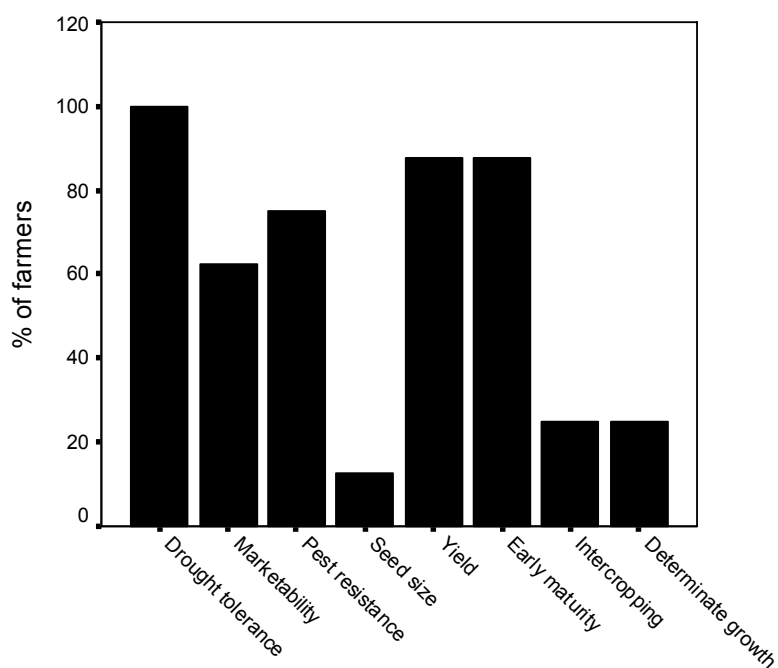
Fig. 2.4 Criteria used by farmers for variety choice.

In spite of the high adoption rate of improved varieties, an assessment in the villages at all sites demonstrated that indeed farmers are still far from self-sufficiency in beans for food (Fig. 2.5). In the DT zone, 23% of farmers said they were self-sufficient while only 18% in the DM zone said they were self-sufficient.

Table 2.2 Direct matrix ranking of bean varieties for yield, tolerance to biotic and abiotic stresses, earliness and marketing quality traits.

Kaewa in Machakos district, Dry transitional (DT) zone N = 63						Iveti in Machakos district, Dry transitional (DT) zone N = 54					
Varieties	Drought tolerance	Insect pest resistance	Early maturity	Yield stability	Multiple traits	Varieties	Drought tolerance	Insect pest resistance	Early maturity	Yield stability	Multiple traits
GLP 2 (Nyayo)	8	6	5	6	4	GLP 2 (Nyayo)	9	9	5	7	2
GLP x 92 (Katumbuka)	1	2	3	1	8	GLP x 92 (Katumbuka)	1	4	1	4	3
GLP 24 (Kitui)	7	7	7	4	3	GLP 24 (Kitui)	6	6	8	5	7
GLP 1004 (Mwezi moja)	3	5	4	5	2	GLP 1004 (Mwezi moja)	4	7	3	9	9
GLP 585 (Wairimu)	4	4	6	3	5	GLP 585 (Wairimu)	5	2	4	2	1
Kat B1 (Kathiika)	5	8	1	9	7	Kat B1 (Kathiika)	2	8	2	7	6
Zebra bean (Ngoso) [a]	2	1	2	2	1	Zebra bean (Ngoso) [a]	3	3	7	3	4
Kakunzu [a]	6	3	8	7	6	Kakunzu [a]	8	5	6	8	8
White haricot	9	9	9	8	9	Ndamba [a]	7	1	9	1	5
Makutano in Machakos district, Dry mid-altitude (DM) zone N = 79						Mulango in Kitui district, Dry mid-altitude (DM) zone N =24					
GLP 2 (Nyayo)	8	9	7	4	6	GLP 2 (Nyayo)	9	7	7	9	9
GLP x 92 (Katumbuka)	1	1	3	2	7	GLP x 92 (Katumbuka)	1	9	1	1	3
GLP 24 (Kitui)	7	2	8	7	2	GLP 1004 (Mwezi moja)	7	3	5	8	8
GLP 1004 (Mwezi moja)	4	6	2	9	9	Kat B1 (Kathiika)	6	6	2	7	1
GLP 585 (Wairimu)	3	4	9	1	3	Kavuti [a]	2	1	3	4	6
Kat B1 (Kathiika)	2	5	1	3	1	Kakunzu [a]	8	8	8	5	5
Kakunzu [a]	9	7	6	8	8	Kat x 56	3	4	6	3	2
Kat x 56	5	3	5	6	5	Kat x 69	4	5	4	6	4
Kat x 69	6	8	4	5	4	Ndumu [a]	5	2	9	2	7

Varieties abbreviated GLP (Grain Legumes Programme) were developed at KARI Thika, while Kat which is short form of Katumani were developed at KARI Katumani. The names in the parenthesis are local names given by the local communities. The ranking was done in groups by consensus. Multiple traits refer to how the variety is ranked overall on the basis of all traits considered in Fig. 2.4.

1 = Highest rank, 9 = Lowest rank.

[a] = Local variety.
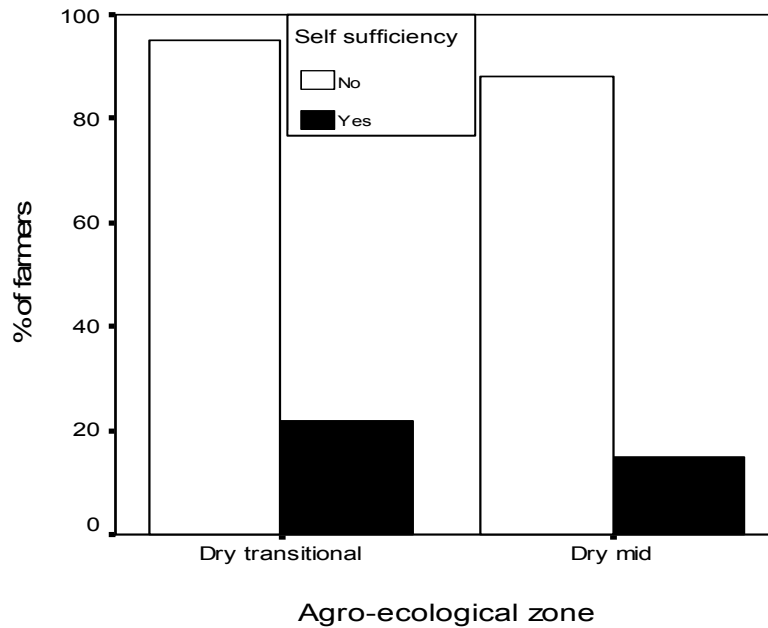
Fig. 2.5 Self sufficiency in bean production.

2.3.3 Limitations to bean production in the semi-arid Eastern Kenya

Small-scale bean farmers in the SAEK are faced by a wide range of challenges. Through a participatory process and by consensus by farmers, groups came up with a list of main production constraints which they ranked as shown in Table 2.3. Drought was consistently ranked top in nearly all sites except at Iveti which is a DT agro-ecology and thus receives slightly more rainfall compared to the DM ecologies. Insect pest problem also featured prominently as a main constraint. Farmers believe that insect pest problem is one of the main reasons why they are not able to realize high bean yield. Other significant constraints stated were crop diseases, lack of certified seed as well as low soil fertility. Farmers interviewed affirmed that they recycle their own seed. In situations where they have insufficient seed for planting, they reach out to their neighbours. Hence farmer to farmer system of seed acquisition is widespread.

The outcome of the farmer interviews indicates that crop losses incurred by the farmers due to insect pest attack vary according to the level of infestation. The loss ranges from 12 to 67 % in DM agro-ecology and between 10 and 50 % loss in DT agro-ecology (Fig. 2.6). In line with the direct matrix (Table 2.4), bean fly (*Ophiomyia* spp.) ranked among the key insects responsible for the bean crop losses in the different agro-ecologies. The African bollworm (*Helicoverpa armigera*), and bean aphid (*Aphis fabae*) were also mentioned.

Table 2.3 Direct matrix ranking of constraints experienced by common bean farmers.

Constraints	Kaewa		Iveti		Makutano		Mulango		Total score	Mean rank	Men rank	Women rank	Overall rank
	M	F	M	F	M	F	M	F					
Drought	1	1	6	6	1	2	1	1	19	2.38	9	10	1
Insect pests	2	4	3	4	4	3	2	3	25	3.13	11	14	2
Diseases	3	5	2	3	3	4	3	4	27	3.38	12	17	3
Lack of certified seed	6	2	5	1	2	5	4	6	31	3.88	17	14	4
High prices of inputs	4	6	4	2	5	6	6	2	35	4.38	19	16	5
Low soil fertility	5	3	1	5	6	1	5	5	31	3.88	17	14	4
No. of farmers	13	50	28	26	46	33	11	13					

1 = High, 6 = Low; M = Males, F = Females.

Table 2.4 Direct ranking of field pests by farmer groups according to their importance at randomly selected sites in semi-arid eastern Kenya.

Pests	Kaewa		Iveti		Makutano		Mulango		Total score	Mean rank	Men rank	Women rank	Overall rank
	M	F	M	F	M	F	M	F					
Bean fly	3	2	3	4	4	3	2	4	25	3.13	12	13	2
Bean aphid	2	4	1	1	6	4	4	3	25	3.13	13	12	2
African bollworm	1	3	2	3	1	1	3	5	19	2.38	7	12	1
Cutworm	4	1	4	5	2	5	5	1	27	3.38	15	12	3
White fly	6	5	5	6	5	6	1	6	40	5.00	17	23	5
Chaffer grab	5	6	6	2	3	2	6	2	32	4.50	20	12	4
No. of farmers	13	50	28	26	46	33	11	13					

1 = High, 6 = Low; M = Males, F = Females.
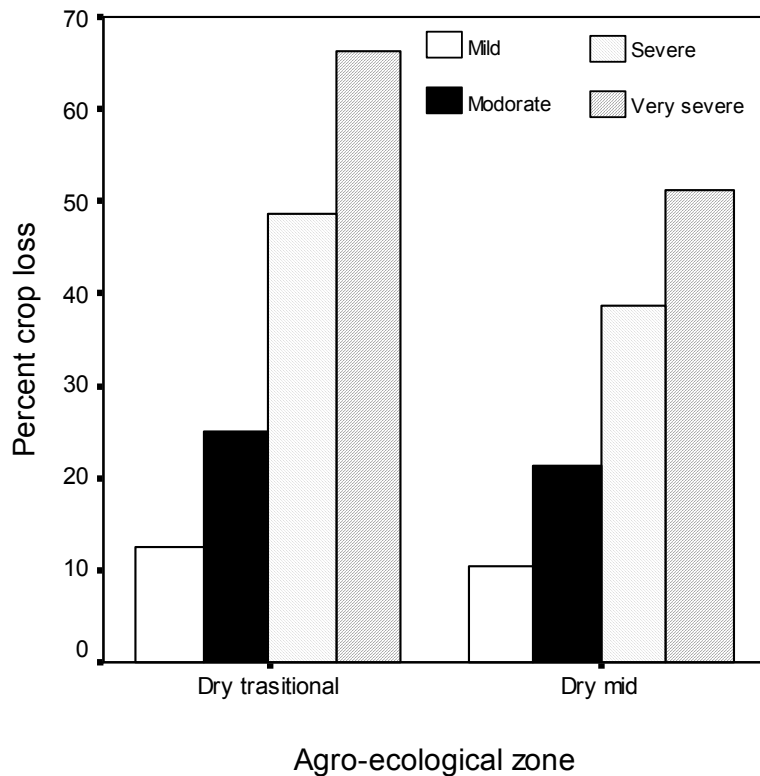
Fig. 2.6 Crop losse experienced by farmers due to bean fly attack in the bean growing agro-ecologies of the semi-arid eastern Kenya.

2.4 Discussion

Apart from drought, small-scale farmers in semi-arid eastern Kenya region (SAEK) are faced with insect pest problem which is another major challenge (Table 2.3). Coupled with a complex socio-economic environment, it makes it difficult for the farmers to attain bean yields close to what is reported from experimental stations. So far, the actual yield under small-scale on-farm conditions still remains below 500 kg ha^{-1}, while the potential is about 1200 kg ha^{-1} (MoA, 2006). This is aggravated by the fact that until recently bean breeding programmes in Kenya were purely on-station and based on conventional methods. When selection of lines is carried out in research stations they tend to adapt to farm conditions similar to the research station and not to those which are very different (Ceccarelli and Grando, 2007). Besides, such materials tend to have a high genetic load making them succumb easily to adverse environmental conditions (Mekbib, 2006). The selection process results in valuable genetic materials being discarded. In such a situation a participatory plant breeding approach becomes an essential tool. A

decentralized system results in development of materials that are broad genetic based. Such materials should have specific adaptability to given farmer conditions.

In this study, farmers' ranking of constraints placed insect pest problem second to drought (Table 2.3). Similar results were obtained by De Groote et al. (2004) on maize. Comparable results were also obtained in another study carried out in SAEK on maize, where insect pest damage was ranked third after water stress and low soil fertility (Songa and Songa, 1996). An array of insect pests attack beans in the region causing significant yield reduction (Abate and Ampofo, 1996). Bean fly (*Ophiomyia* spp.), African bollworm (*Helicoverpa armigera*) and bean aphid (*Aphis fabae*) were among the most important as per the farmers' ranking (Table 2.4). Of these major bean pests, bean fly is possibly the most important pest of common bean across the main bean growing areas of eastern and southern Africa. Reports on yield loss arising from these pests are varied, but a range of 8 to 100 % is documented (Greathead, 1968).

Farmers are dynamic and understand their situation well. Because of their rich knowledge and experience, they have over the years selected and tested varieties based on multiple criteria. Drought, yield stability, insect pests and early maturity were among the important criteria mentioned. Apparently, these criteria are not different from those used by the breeders for selection under on-station conditions. The obvious reason for low performance of the improved varieties is that the testing sites are different from the farmers' growing conditions. On the other hand, poor crop management and growing of varieties meant for high potential areas in the marginal areas due to lack of information by farmers in a way also contributes negatively. For example, the majority of the farmers obtain their seed informally from neighbours, local markets and own farm saved which compromise the quality of their seed. Therefore, close collaboration among the stakeholders provides an opportunity for the development of technologies that will be relevant (Ceccarelli and Grando, 2007).

According to Letourneau (1994) Malawian farmers were found to be aware of the symptoms caused by bean fly attack and probably inadvertently incorporated bean fly resistant cultivars in their cropping system schemes although the tiny flies that visit their farms mostly in the morning are not known. Such perceptions were revealed during the interviews with farmers in the SAEK region, where the farmers were aware of the symptoms and some of them identified the pupae in the bean stems, but the adult flies were not known. Farmers had little knowledge on the

ecology of the bean fly as they perceived the bean fly to be soil-borne. Some of the farmers confused the bean fly damage symptoms with disease and in some instances with drought.

Major emphasis has been given to host plant resistance as an insect pest control measure yet this has not made much impact due high variable and unstable local environment. It has been hard for resistant varieties developed by conventional breeding procedures to withstand the pest pressure under diverse farmer conditions. In contrast, the local varieties may have better adaptability due to co-evolution with local pests and disease, fluctuations in soil conditions and rainfall. However, during group discussions farmers argued that their local varieties lack good commercial values, thus fetch low market price hence their abandonment. Even though the approach of host plant resistance is sustainable to small-scale farmers, a change in strategy is necessary. Selection for specific adaptation is reasonable when dealing with a diverse environment. This is because the approaches employed in the past have mainly targeted modified environments, which are more favourable and have to some extent contributed to the failure. According to the farmers, there is potential demand for new varieties resistant to bean fly but adoption of the new technologies would be enhanced if they were allowed to participate in the selection and testing process.

At present, small-scale farmers in the SAEK region like other farmers in many parts of Africa rely upon traditional pest management practices. The control practices are based on cultural practices such as intercropping and crop rotation or specific responsive actions to reduce pest attack such as timing of weeding and adjusting planting time to escape damage (Abate et al., 2000; Karel, 1991). Diversity of crop species planted on the same piece of land reduces bean fly populations (Karel, 1991) and is a food security measure in that it not only averts food shortage but also helps to meet nutritional requirements especially when legumes are planted in association with cereals. Bean fly infestation was significantly lower in intercropped beans compared to pure stand (Karel, 1991). Farmers are already taking advantage of existing genetic diversity in different bean varieties by growing more than one bean variety on their farms to try and manage various pests which include the bean fly. Farmers confirmed that they suffer less losses to bean fly and other insect pest when they intercrop beans than when they plant sole crop.

In spite of the high adoption rate of improved varieties, a survey in the villages at all sites demonstrated that indeed farmers are still far from self-sufficient in beans (Fig. 2.5). In the DT zone, 23% of farmers said they were self-sufficient while only 18% in the DM zone indicating they were self- sufficient. Conventional plant breeding has offered more benefits in high potential environment where farmers are capable of improving the environment by supplementing inputs to maximize production from the new varieties. This is in contrast to the farmer in risk prone environment where farmers are not able to modify their environment so as to realize high yield and as a result crop failures are frequent. Therefore, a participatory plant breeding (PPB) approach could provide the opportunity to overcome some of these limitations by empowering the farmers to identify varieties that are tailor-made for their own environmental conditions and needs as opposed to the conventional approach.

2.5 Conclusions

Breeding for improved and stable yield requires an understanding of the factors that are important in yield accumulation. Yield is a constant capacity system and a component of interdependent traits (Yan and Wallace, 1995). Therefore, increasing one component may result in overall reduction of another. In the context of participatory plant breeding, apart from adjusting the various components to maximize the functioning of the system, the socio-economic environmental aspect should be considered as part of system so as to achieve yield stability. This is so considering that a wide biophysical and socio-economic environment exists.

Kelly et al. (1998) suggested that seed yield in common bean can be improved if the developed cultivars are bred to fit in the target environment. Good genetic control for important traits such as yield, quality and resistance to important biotic and abiotic constraints found within the bean gene pools of both Andean and Mesoamerican races should be explored. Specifically, efficient genotypes that are swift in changing from vegetative to reproductive growth phase for specific adaptation to given local environments, should be considered as candidate entries to a participatory selection process. This should be in a wide range of environmental conditions provided by small-scale farmer conditions to give adequate testing for assessing the importance of genotype x environment interaction mainly genotype x location, genotype x year and genotype x location x year.

Effective control strategy for bean fly and other important insect pest should take advantage of an integrated approach that is already inbuilt within the farming systems. These may include host plant resistance, cultural practice, biological pest control, and the use of bio-pesticides. Besides being environmentally friendly such strategies are sustainable and may require no money or expertise. The knowledge required is already with the farmers but since it is locality specific, a participatory approach can ensure that a wider population of farmers is reached.

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Chapter 3

Genotypic response of common bean to natural field populations of bean fly (*Ophiomyia phaseoli*) under diverse environmental conditions

Abstract

Bean fly (*Ophiomyia* spp.) is a significant pest of common bean (*Phaseolus vulgaris* L.) in semi-arid areas of eastern Africa. Apart from inadequate moisture in the dry land, bean fly simultaneously contributes negatively, thereby adversely affecting bean productivity. The objectives of this study were to (1) identify sources of resistance to bean fly available in landraces (2) confirm stability of host plant resistance in drought stress and (3) determine the effect of drought stress and seasonal variation on common bean genotypes in relation to bean fly attack for adaptability to the semi-arid areas of eastern Africa. Sixty four genotypes including landraces, bean fly resistant lines and local checks were evaluated for seed yield, 100-seed weight, days to maturity, plant mortality and number of pupae in stem in an alpha lattice design with two replications. This was under drought stressed (DS) and non-stressed (NS) environments and two treatments (insecticide sprayed and natural infestation) for three cropping seasons between 2008 and 2009. Genotypes differed in their reaction to natural bean fly attack under drought stressed (DS) and non-stressed environments (NS) over different cropping seasons. However, the effect of bean fly appeared to vary between the long rains (LR) and short rains (SR). It was observed that an increase in number of pupae per stem resulted in a higher plant mortality. The range of seed yield was from 345 to 1704 kg ha^{-1} under natural infestation and from 591 to 2659 kg ha^{-1} under insecticide protection. Seed yield loss ranged from 3 to 69 %. The resistance of most of the bean fly resistant lines seemed to break down in presence of DS. Screening of genetic resources in common bean to breed for host plant resistance to bean fly offers high potential of success if researchers take full advantage of the diversity available within the landraces.

3.1 Introduction

Bean fly (*Ophiomyia* spp.) is considered the most important field insect pest of beans in major bean-growing areas of eastern Africa (Abate and Ampofo, 1996; Ampofo and Massomo, 1998). *Ophiomyia phaseoli* Tyron and *Ophiomyia spencerella* Greathead are the two main species of economic importance reported to attack beans in east Africa (Greathead, 1968). Yield losses reported under field conditions resulting from bean fly can be up to 100% (Abate and Ampofo, 1996). Decline in rainfall resulted in high pupae numbers of bean fly in bean stems (Songa and Ampofo, 1999). The importance and distribution of bean fly varies with location and season. In the lower semi-arid areas of eastern Africa where beans are more prone to perennial drought, *O. phaseoli* is the predominant species. Although *O. spencerella* is the more abundant species at higher altitudes of semi-arid areas, *O. phaseoli* has been reported to be to more prevalent early in the season at such elevations (Songa and Ampofo, 1999) and this is when the bean plant is more vulnerable.

Small-scale farmers in the semi-arid regions of Africa like the other parts of sub-Saharan Africa are compelled to rely upon traditional pest management practices (Abate and Ampofo, 1996) mainly due to financial constraints. Poor crop management practices, in addition to the adverse biophysical environment, leads to build-up of field pests such as bean fly (Letourneau, 1994).

A range of bean fly management methods have been suggested for beans and these include: biological control, agronomic or cultural practices, use of genetic diversity (local landraces) and host plant resistance (Ampofo and Massomo, 1998; Byabagambi et al., 1999; Greathead, 1968; Letourneau, 1994; Letourneau, 1995). Farmers exploiting the diversity available in landraces and cultivars reduce the risk of bean fly infestation (Letourneau, 1994).

Conventional methods such as open-field tests have been successfully used in screening grain legumes to differentiate them for host plant resistance to common field pests (Clement et al., 1994). The identification of useful sources of resistance to the most important pests is valuable in that such sources could be used to confer resistance to locally adapted materials. However, breeding programmes should place more emphasis on development of crop cultivars with more than one mechanism of resistance. Structural and physiological defences can act directly on the agromyzid bean flies by preventing feeding and oviposition (antixenosis), by suppressing growth

and larval development (antibiosis), or by reducing yield loss from plant injury (tolerance) (Clement et al., 1994).

Resistance genes may be found within the landrace populations due to co-evolution between crops and pests, natural selection and or artificial selection by farmers for many years. A number of local landraces were regarded as resistant due to thickened hypocotyls (Greathead, 1968). Sources of resistance to the bean fly in common bean germplasm have been reported (Greathead, 1968; Mushi and Slumpa, 1998). Abate et al. (1995) identified sources of resistance to bean fly from accessions obtained from CIAT. Bean fly resistance has also been reported from scarlet runner bean (*Phaseolus coccineus*) (Miklas et al., 2006). Ogecha et al. (2000) conducted on-farm trials to evaluate bean genotypes for their resistance to bean fly in south-western Kenya and reported that some genotypes had significantly lower percent mortality. Apart from common beans, host plant resistance against bean fly and related agromyzids has been reported in other leguminous crops such as mungbean and cowpea (Talekar et al., 1988) and soybean (Talekar et al., 1988; Wang and Gai, 2001).

According to Edwards and Singh (2006), slow progress in incorporating insect resistance into commercial varieties through breeding has been largely attributed to breeders not having access to full range of available germplasm resources. Besides, the linkage of resistance genes to undesirable traits compromises the agronomic quality. However, in tropical bean cropping systems for example, there have been some successes in developing cultivars resistant to a single pest.

Apart from yield reduction caused by insect pest (bean fly) attack, drought is a perennial problem to the semi-arid areas of east Africa. Therefore, adaptation of bean genotypes to the drought endemic environment requires reasonable levels of drought resistance in addition to insect pest resistance. This is because the effect of drought stress on common bean has been well documented (Frahm et al., 2004; Rosales-Serna et al., 2000; Singh, 1995; Terán and Singh, 2002a). Drought resistance is described on the basis of comparative yield of a genotype in regard to other genotypes subjected to equal drought. Although identification of different crop genotypes for their adaptation to drought stress environments has been carried out using selection indices, geometric mean (GM) has been shown to be the useful selection index for resistance to drought in common bean (Abebe et al., 1998; Terán and Singh, 2002a).

According to Ceccarelli and Grando (2007) when different genotypes of a given crop are evaluated adequately in a range of environments, genotype (G) x environment (E) (GE) interactions of cross-over type appear to be quite common. Significant GE interactions cannot be disregarded. The options are to manage them by selecting genotypes that are broadly adapted to whole range of target environments or basically carry out selection for an array of genotypes each adapted to a specific environment. Such selection requires separate GE analyses namely genotype (G) x year (Y) (GY), that is highly unpredictable and genotype x location (L) (GL), that if repeated identifies distinct target environment. Selecting for specific adaptation is important predominantly for crops grown under unfavourable conditions, mainly because unfavourable environments can be very different from each other (Ceccarelli and Grando, 2007). Therefore, breeding strategy to identify materials suitable for unfavourable environmental and variable seasonal conditions should exploit analysis of GE components. This is because seasonal variation of bean fly populations, rainfall patterns, drought pressure, negative or low correlation between farmer field and research stations may complicate the breeder's selection process. Hence, it may hamper positive identification of superior materials for the intended specific target environment or a wide range of environments.

The objectives of this study were, therefore, to (1) identify sources of resistance to bean fly available in landraces, (2) confirm stability of host plant resistance to bean fly in drought stressed environment, and (3) determine the effect of drought stress and seasonal variation on common bean genotypes in relation to bean fly attack under semi-arid areas of eastern Africa.

3.2 Materials and methods

3.2.1 Experimental site

The field experiments were carried out during the 2008 and 2009 cropping seasons at Kenya Agricultural Research Institute (KARI) Katumani, Kiboko sub-centre, Kenya. Kiboko is located 2^0 3' S, 37^0 43' E in semi-arid eastern Kenya region, at an elevation of 938 m above sea level. The annual average temperatures range is 21.6 - 24.0^0 C. It receives an annual average rainfall of 650 - 750 mm distributed over two cropping seasons, but the rainfall is usually erratic and unreliable. During the experimental period, below average rainfall was received (Table 3.1), which was insufficient to raise the bean crop and thereby supplementary irrigation was used. The long rainy (LR) season begins in mid-March to early-June and the short rainy (SR) season

from mid-October to early-January. The optimal sowing time for beans is normally at the onset of both rainy seasons.

The Kiboko site was selected because previous bean fly screening work has shown that it has a high natural population of *O. phaseoli* hence a 'hot spot' for screening (Songa and Ampofo, 1999). The biggest challenge to bean breeders working on insect resistance is the attainment of optimal pest pressure (Hillocks et al., 2006) under natural field conditions to effectively differentiate the genotypes and to avoid escapes. However, taking advantage of the available irrigation facility, it was possible to delay planting by 2 weeks at the onset of every cropping season so as to enhance the bean fly populations. The population dynamics of bean fly is known to depend on the time of the season, and delayed planting results in *O. phaseoli* build-up. However, to quantify the amount of water used for two environments (drought stressed and non-stressed environments), three rain gauges were placed diagonally across the fields in each environment and the amount of water recorded after every irrigation cumulatively for the entire growth period (Table 3.1). The NS plots received optimal amount of water in addition to the seasonal rainfall for the entire growth period until pod maturity. On the other hand the genotypes in DS environment were exposed to drought stress conditions twice. First, by withholding water 7 days after emergence for a period of 10 days to expose genotypes to bean fly attack since this is the stage when the crop is most vulnerable. Water was again withheld at the commencement of the flowering and early pod development stages, respectively. Similarly, maximum and minimum growing temperatures and cumulative rainfall were recorded (Table 3.1). Besides, the drought intensity indices (DII) were estimated.

Table 3.1 Cumulative rainfall, amount of water applied, growing season temperature and drought intensity index for three cropping seasons between 2008 and 2009 used to evaluate 64 common bean genotypes at KARI Katumani, Kiboko sub-centre, Kenya.

Cropping season [a]	Cumulative rainfall (mm)	Amount of water applied [b] (mm)		Growing season temperature (^{0}C)		Drought intensity index [c]
		DS	NS	Max	Min	
2008 (LR)	114.7	318.3	408.2	30.6	16.6	0.46
2008/09 (SR)	35.8	253.1	390.8	32.4	18.1	0.41
2009 (LR)	36.8	298.8	379.5	31.4	17.9	0.85

[a] SR, short rains (mid-October to early-January); LR, long rains (mid-March to early-June).

[b] DS, drought-stressed; NS, non-stressed.

[c] Drought intensity index (DII) = 1 - X_{DS}/X_{NS}, where X_{DS} and X_{NS} are the mean yield of all genotypes (64 entries) in drought-stressed and non-stressed environments, respectively.

3.2.2 Plant material and trial design

The materials for the study were 64 common bean genotypes. These were mainly resistant lines acquired from the regional bean fly nursery mainly assembled by East and Central Africa Bean Research Network (ECABREN) which is Centro Internacional de Agricultura Tropical (CIAT) regional body, landraces from the National Gene Bank of Kenya (NGBK) specifically collected from semi-arid areas of Kenya, and improved varieties mainly released from Kenya Agricultural Research Institute (KARI).

In order to identify adapted genotypes to be used for the experiments from introductions, a preliminary screening trial was first conducted during SR 2007/08 before the main trials. This was due to heat problems at Kiboko and as expected some introductions could not survive the heat. A complete set of 64 genotypes was then assembled for the main trials.

3.2.2.1 Experiment 1

During the long rains (LR) 2008, short rains (SR) 2008/09 and LR 2009, 64 bean genotypes were tested in an alpha lattice (16 rows x 4 columns). Each set of the 64 genotypes were grown in two environments, drought stressed (DS) and non-stressed (NS) and replicated twice (Fig.

3.1). Although, the DS and the NS environments were adjacent to each other on the same field they were placed about 50 m apart and this was mainly to avoid water from the NS plots interfering with the DS plots. The observation was made under natural bean fly populations. Every experimental unit consisted of two 3 m long rows spaced 0.15 m apart.

At 1 week after emergence, the numbers of dead plants were recorded until 7 weeks for computation of plant mortality on plot basis. During the same 7-week period the pupae in the stems of the dead plants were removed and physically counted. Similarly, days to physiological maturity, 100-seed weight and seed yield were recorded. Days to maturity were estimated as the number of days from planting to when 75 % of plants in the plot had attained the light brown colour. The 100-weight was measured as weight of hundred seeds. Whole plots were harvested for estimation of seed yield. The GM was computed using yield measured under drought stress (Y_{DS}) and non-stress (Y_{NS}) conditions where GM = $(Y_{DS} \times Y_{NS})^{1/2}$.

All the quantitative data collected were subjected to residual (or restricted) maximum likelihood (REML) spatial model analysis to fit the variance-components using a computer software programme, GENSTAT version 9. Data were combined over environments and cropping seasons (years). Means were separated by LSD test using suitable error term. Genotypes, environments and cropping seasons were considered fixed terms while replications, rows and columns were considered random terms. In order to asses yield stability, data were subjected to genotype (G) x environment interactions (E) (GE) component analysis. Besides, a regression analysis was done to relate number of bean fly pupae in stem with percent plant mortality using the same statistical software programme.
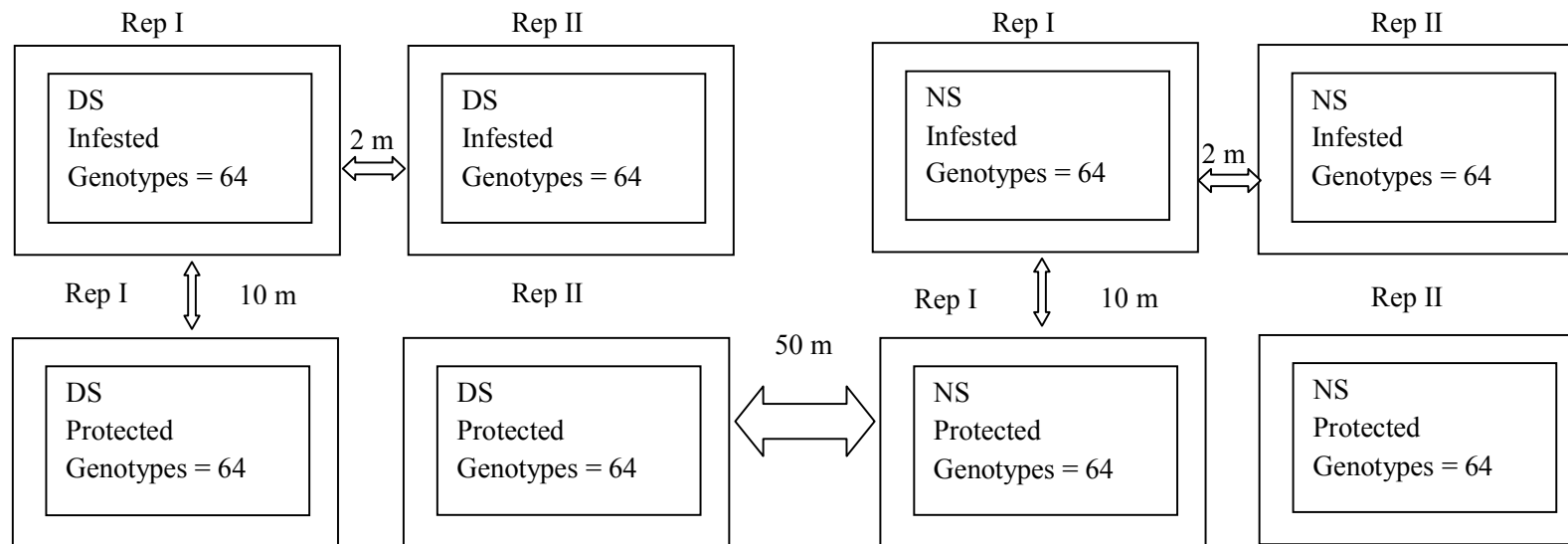
Fig. 3.1 Experimental design layout used to screen common bean genotypes for resistance to bean fly under drought stressed (DS) and non-stressed (NS) at KARI Katumani research centre (Kiboko sub-centre), Kenya.

3.2.2.2 Experiment 2

The second experiment was conducted during the SR 2008/2009 and LR 2009 with the 64 entries also planted in an alpha lattice (16 rows x 4 columns) as in the first experiment. There were two environments (DS and NS) and two treatments or insecticide levels (no spray and completely protected) each with a complete set of 64 entries (Fig. 3.1). In order to prevent the insecticide drifting from sprayed to unsprayed (naturally infested) plots and to avoid water from the NS environment interfering with the DS environment, separation was done (Fig. 3.1). In the DS environment, both sprayed and unsprayed plots were placed 10 m apart but adjacent to each other on the same field. A similar arrangement was done for the NS environment. However, the DS and NS experimental units were placed 50 m away from each other but on the same field. The protected plots were sprayed with dimethoate (insecticide) at the rate of 2 l in 1000 l of water ha^{-1}, while control (no spray) was exposed to the natural bean fly populations. This experiment was replicated twice and the experimental units likewise consisted of two 3 m long rows spaced 0.15 m apart.

The numbers of dead plants were recorded beginning at one week after emergence up to the 7-week for the plots subjected to natural bean fly infestation. This was mainly for computation of plant mortality on plot basis. During the same 7 week period the pupae in the stems of dead plants were removed and counted. Days to physiological maturity, 100-seed weight and seed yield were recorded on both naturally infested and control plots. Days to maturity was estimated as the number of days from planting to when 75 % of plants in the plot had attained the light brown colour. The 100-weight was measured as weight of hundred seeds. Whole plots were harvested for estimation of seed yield.

The data were combined over environments, treatments and cropping seasons (years) into spatial analysis using REML procedure of GENSTAT version 9 statistical software programme to fit the variance-components. Genotypes, environments, treatments and cropping seasons were considered fixed terms whereas replications, rows and columns were considered random terms. Multivariate data analysis was conducted to assess GE interactions. Means were separated by LSD test using suitable error term.

3.3 Results

3.3.1 Experiment 1

The level of drought stress is represented in the drought intensity index (DII) values for each cropping season (Table 3.1). A moderate to high level of stress was achieved, with cropping season SR 2008/2009 having the lowest DII value of 0.41 while LR 2009 had the highest value of 0.85. These levels of drought were comparable to those obtained by Terán and Singh (2002b) and Schneider et al. (1997)

Significant main effects ($p ≤ 0.01$) of genotypes, environment and cropping seasons were obtained for all the five traits measured (Appendix 1a). The two-way interaction between genotypes (G) and cropping season or years (Y) (GY) were significant ($p ≤ 0.01$) for all traits except pupae in stem (Appendix 1a). Conversely, interaction between genotypes and stress environments (S) (GS) was non-significant for all traits except for pupae in stem. The three-way interaction GYS was not significant for all the traits measured.

Results of GE interactions in particular GY interactions showed that the cropping seasons (years) were significantly different (Fig. 3.2). As illustrated, there was low or negative correlation between the long rains (LR) and short rains (SR). Furthermore, the LR 2008 and 2009 were rather similar as opposed to the SR 2008/09. On the other hand, the drought stressed (DS) and the non-stressed (NS) environments were similar for every cropping season and that probably explains the non-significant GS interaction. Looking at the genotypic performance in a bid to identify individuals with specific adaptation to each target environment, we found that; GBK 047826 (8), GBK 047818 (10), G 21212 (55), GBK 047880 (49), GBK 036488 (13) and IKINIMBA (34) were associated with LR season especially under DS. Genotypes, GBK 047821 (1), GBK 047815 (59) and GBK 047858 (2) were better adapted to the SR. These results indicate that a farmer growing beans during LR and SR in areas prone to bean fly will experience a cross-over type of interaction of his or her variety between the cropping seasons.
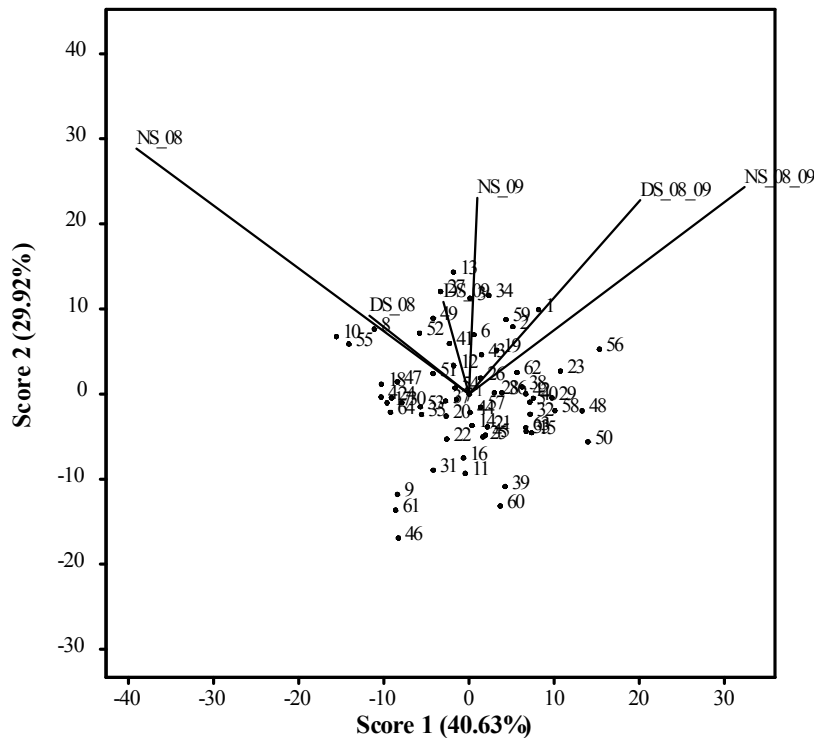
Fig. 3.2 Biplots of seed yield for 64 common bean genotypes for three cropping seasons in two environments, drought stressed (DS) and non-stressed (NS) subjected to natural bean fly infestation at Kiboko, Kenya. Genotypes are indicated by numbers and environments by vectors.

Significant GY interaction for seed yield indicates that possibly seasonal variation affected yield performance of common bean under natural infestation of bean fly. Severity of bean fly was found to depend on seasonal variation (Davies, 1998). Genotypic variation was observed among the 64 genotypes (Table 3.2). Only two lines GBK 047820 and GBK 036488 significantly yielded higher than the best check GLP x 92 in NS environment. On the other hand, IKINIMBA was the only genotype that significantly yielded higher than all the local checks in DS environment. Lack of GS interaction indicated that mean yield performance among the genotypes was relatively consistent under both NS and DS environments across seasons. Considering the genotypic performance based on the geometric mean (GM) which is associated with yield performance under drought, a number of genotypes, both landraces and bean fly resistant lines (introductions from CIAT) consistently outperformed the local checks, indicating broad adaptation under varied stress levels (bean fly and drought). IKINIMBA, a bean fly resistant line from CIAT and two local landraces (GBK 036488 and GKB 047821) were outstanding.

A range of seed sizes existed among the genotypes from small to large. The top two performing lines had relatively smaller seed sizes especially under drought stress conditions. For example, GBK 036488 and IKINIMBA had 28 and 21 g per 100 seeds in DS environment, respectively. However, GBK 036488 had small seed size in both DS and NS environments (Table 3.2). Apart from IKINIMBA and GBK 036488, no further clear pattern was observed, as it could be seen that among the top 20 performing lines some had small, medium as well as large seeds. Such observations were also made by Terán and Singh (2002b), where most of drought resistant common bean lines were small seeded but others had large seeds.

Although genotypes were significantly different for maturity, most genotypes were medium and early maturing (Table 3.2). Lack of GS interaction indicated that genotypes were consistent in maturity under both DS and NS environments when pooled over the years. Nonetheless, seasonal variation affected different genotypes as revealed by significant GY interaction. A range of lines had significant reduction of days to maturity when subjected to DS while others did not show any reduction.

Despite significant effects among the genotypes for pupae in stem, there seemed to be limited variation (Table 3.2). However, as indicated by significant GE interaction, the presence of pupae in stem depended on the drought condition. This implies that beans were more affected by pupae in stem in DS as opposed to NS conditions. In the NS environment, plants expressed tolerance possibly due to compensatory growth when they received more water thus lowering the effect of bean fly. This is supported by the fact that a high plant mortality percent was recorded in DS (44%) than in NS (20%) environment.

Seasonal factors such as planting time (early or delayed), month of sowing (Davies, 1998; Songa and Ampofo, 1999) as well as environmental factors like temperature, relative humidity and number of rain-free days have been considered important for severity of bean fly infestation (Talekar and Lee, 1989). A simple regression analysis of number of bean fly pupae in stem as a function of percent plant mortality under DS and NS environments revealed a significant positive relationship [slope (b) ± standard error = 5.41 ± 1.72, intercept (a) = 32.14, Student's t-value (t) = 3.15, p = 0.002 and coefficient of determination (r^2) = 0.35].

Table 3.2 Mean seed yield, 100-seed weight, days to maturity, plant mortality and pupae in stem for top 20 and bottom 1 out of 64 genotypes evaluated over three cropping seasons (LR 2008, SR 2008/09 and LR 2009) in drought stressed (DS) and non-stressed (NS) environments under natural bean fly infestation at Kiboko, Kenya.

Genotype	Source[a]	GM (kg ha^{-1})	Seed yield (kg ha^{-1})		100-seed weight (g)		Days to maturity (d)		Plant mortality (%)		Pupae in stem	
			NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
IKINIMBA	CIAT	1052	1241	891	31	28	74	68	26	41	1.6	2.8
GBK 036488	NGBK	1030	1529	694	24	21	75	71	10	35	1.9	1.4
GBK 047821	NGBK	998	1247	799	42	38	69	68	23	44	1.7	1.3
GBK 047880	NGBK	926	1183	724	31	28	77	74	20	36	1.1	1.5
GBK 047804	NGBK	912	1201	693	43	39	71	70	20	47	1.4	1.4
GBK 047858	NGBK	905	1298	631	38	31	76	72	19	50	1.4	1.8
GBK 047815	NGBK	904	1173	697	35	27	74	72	27	48	1.4	1.1
GBK 047826	NGBK	902	1263	644	36	34	74	72	17	43	2.6	1.5
GBK 047866	NGBK	890	1344	589	30	25	74	71	10	37	2.4	1.3
GBK 047849	NGBK	868	1142	659	23	20	75	74	9	50	1.2	2.5
MKOMBOZI(G11746)	CIAT	866	1087	690	39	34	71	69	20	43	2.1	1.5
GBK 047820	NGBK	864	1428	524	42	37	71	68	12	59	1.6	1.4
GBK 047829	NGBK	860	1159	638	19	18	73	69	28	34	1.4	1.5
IKISINONI	CIAT	854	1146	637	39	37	73	72	17	35	2.0	1.5
GBK 047790	NGBK	848	1105	651	31	29	75	74	23	37	1.3	1.5
CIM 9314-36	CIAT	833	1200	578	41	39	78	73	15	28	1.7	1.6
GBK 047810	NGBK	831	1402	493	31	25	78	75	26	55	1.6	1.5
G 21212	CIAT	821	1202	561	21	20	76	75	26	52	1.8	1.5
GBK 047813	NGBK	820	1178	571	25	21	75	72	17	54	1.7	1.3
GBK 047828	NGBK	819	1114	602	35	32	76	75	16	28	2.0	1.1
GBK 047861	NGBK	479	764	301	32	26	80	74	24	41	1.8	2.3
Checks												
KAT X 69	KARI	722	974	536	36	35	72	70	12	30	1.6	2.0
KAT B1	KARI	719	956	541	34	32	67	65	9	42	1.6	1.1
KAT X 56	KARI	695	1000	483	34	30	70	69	21	42	1.8	1.6
KAT B9	KARI	625	977	400	34	32	70	69	15	46	1.5	1.6
GLP X 92	KARI	620	1134	339	33	28	74	71	16	42	1.6	1.9
Mean		748	1068	528	33	30	74	71	20	44	1.7	1.6
LSD (0.05)			291	291	4.7	4.7	2.9	2.9	15.5	15.5	0.6	0.6

GM, geometric mean; NS, non-stressed, DS, drought stressed.

[a]NGBK, National Gene Bank of Kenya; KARI, Kenya Agricultural Research Institute.

3.3.2 Experiment 2

Significant main effects (p ≤ 0.01) for genotypes, treatments (insecticide or no insecticide), cropping seasons and environments were recorded for all the three traits measured namely, seed yield, 100-seed weight and days to maturity (Appendix 1b). The two-way interaction between genotype (G) and treatment (T) (GT) was significant (p ≤ 0.01) for seed yield and days to maturity but not for 100-seed weight (Appendix 1b). Genotype x stress environment (S) (GS) interaction was significant (p ≤ 0.05) for seed yield and (p ≤ 0.01) for 100-seed weight and days to maturity. The GY interactions was significant (p ≤ 0.01) for all the traits measured. The three-way interactions among genotype, treatment and cropping season GTY were significant (p ≤ 0.01) for all traits except 100-seed weight.

The presence of GT, GS and GY interactions for seed yield indicated that mean yield performance of common bean genotypes varied across seasons, and also between environments and treatments. Significant three-way interaction (GTY) signified that seed yield performance among the genotypes is controlled by both genetic and environmental factors including but not limited to drought, cropping season and bean fly attack. Significant genetic variation was observed for seed yield across environments as shown by the range under sprayed and infested conditions (Table 3.3). The seed yield loss from bean fly damage observed over the two cropping seasons was comparable. For instance the range of seed yield loss was 3 to 69 % in SR 2008/09 and 6 to 65 % during LR 2009. As expected, application of insecticide resulted in seed yield improvement. In spite of this, small-scale farmers rarely use insecticides but rely upon cultural practices to control insect pests (Abate and Ampofo, 1996).

Analysis of GE interactions (Fig. 3.3) revealed interactions existed among the genotypes between seasons and treatments. Treatments (infested and sprayed) were associated during LR 2009 as opposed to the dissimilarity observed in SR 2008/09. A cross-over type of interaction was observed among the genotypes. For example, genotypes GBK 047810 (27), GBK 047866 (52) and G21212 (55) performed well under bean fly attack during the LR 2009 period. For the SR 2008/09, the following genotypes, GBK 047821 (1), GBK 036488 (13), GBK 047812 (48), CIM 9314-36 (43), GBK 047803 (56) and IKINIMBA (34) were associated.

The non-significant GT interaction for 100-seed weight showed that the trait was not affected by bean fly infestation. However, seed weight was affected by both drought stress and

cropping season as revealed by significant GS and GY interactions. For instance, the mean 100-seed weight was significantly higher during the SR 2008/09 under both infested (34 g) and sprayed (36 g) compared to LR 2009 when only a mean of 27 g was recorded in both conditions.

Table 3.3 Range and mean for seed yield, 100-seed weight and days to maturity for 64 common bean genotypes grown in two environments (drought stressed and non-stressed) and two levels of insecticide application (no spray and completely protected) for two cropping seasons at KARI Katumani (Kiboko sub-centre), Kenya.

		Seed yield (kg ha^{-1})		100-seed weight (g)		Days to maturity (d)	
		Range	Mean	Range	Mean	Range	Mean
SR 2008/09	Infested	542-1704	1161	20-45	34	62-76	68
	Control (sprayed)	837-2659	1686	21-46	36	70-82	75
	Yield loss (%)	3-69	30				
LR 2009	Infested	345-999	619	15-36	27	58-72	62
	Control (sprayed)	591-1815	1041	15-36	27	58-74	63
	Yield loss (%)	6-65	40				

Yield loss (%) = Calculated as {[1-(yield of infested plot/yield of protected plot] x 100}.
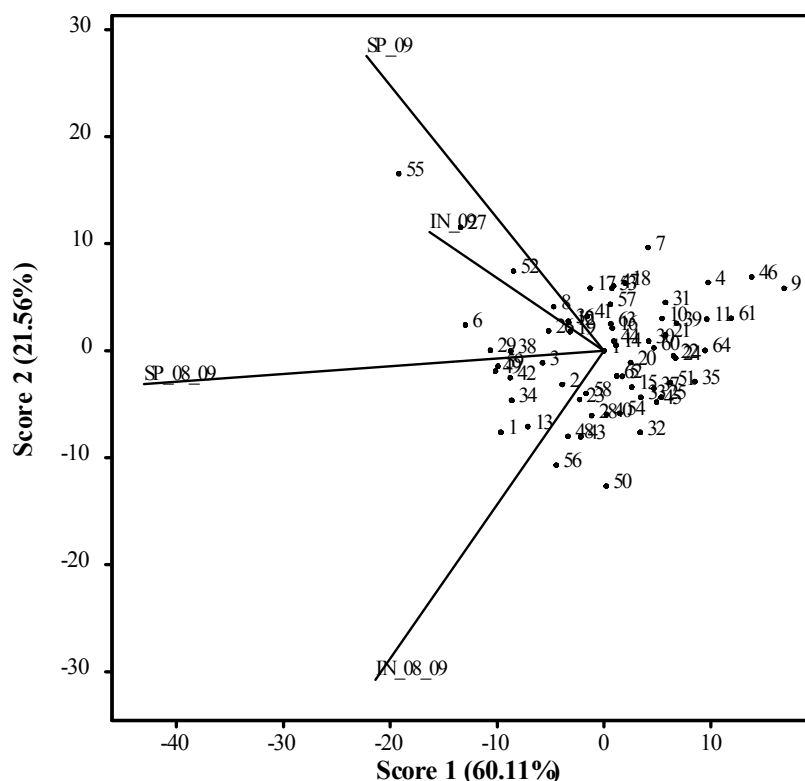
Fig. 3.3 Biplots of seed yield for 64 common bean genotypes grown for two cropping seasons (SR 2008/09 and LR 2009) under two treatments, natural infestation (IN) and insecticide protected or sprayed (SP) at Kiboko, Kenya. Genotypes are indicated by numbers and the treatments by vectors.

The significant GTY interaction for days to maturity (p≤ 0.01) indicated that the phenotypic expression of maturity was largely determined by both genetic as well as environmental factors. Significant GT interaction showed that bean fly infestation caused beans to mature a bit early (Table 3.3). A reduction in maturity period could compromise yield performance due to reduced physiological efficiency (Wallace et al., 1993).

3.4 Discussion

To effectively breed crops for resistance to both biotic and abiotic stresses, a control screening method is critical so as to identify superior genotypes. For drought screening, drought stressed (DS) and non-stressed (NS) environments have been traditionally used. Apart from drought, exposure to another source of stress such insect pest is required during screening to enable identification of crop genotypes combining the drought and insect pest resistance for adaptability to drought endemic environments. The use of a selection index such as GM alone is not enough to select superior genotypes in presence of both abiotic (drought) and biotic (insect pests especially bean fly) stresses. Therefore, where multiple

trait selection is involved, a combination of approaches becomes handy. Consequently, the use of both selection indices and genotype x environment (GE) components analysis as observed in this study would provide positive results. A well-adapted genotype should posses multiple trait resistance. This is because small-scale farmers in the semi-arid region rarely use chemical pesticides (Abate et al., 2000) and therefore, apart from drought, their crops are exposed to insect pests such as bean fly which is a major pest that contributes significant yield loss (Ojwang' et al., 2009).

Variation in temperature during the growing seasons was minimal (Table 3.1). However, a relatively low drought intensity index (DII) was obtained during SR 2008/09 cropping season which resulted in a higher mean performance for seed yield, 100-seed weight and days to maturity (Table 3.3). Such difference in the attainment of DII due to seasonal variation coupled with a lower bean fly infestation was probably the main cause for significant interactions between genotypes and cropping seasons. In contrast, no seasonal variation was observed for levels of bean fly infestation (Songa and Ampofo, 1999).

The results of regression analysis revealed that percent plant mortality increases as a linear function of pupae per stem. A similar trend was demonstrated by Davies (1998). Decrease in precipitation leads to higher pupae numbers of bean fly in bean stems (Songa and Ampofo, 1999). Therefore the severity of bean fly attack is dependent on environmental factors including but not limited to amount of rainfall (drought condition), temperature and relative humidity (Davies, 1998).

The range of yield reduction (loss) recorded in this study was similar to those reported by Abate and Ampofo (1996). During screening and evaluation, research scientist working on insect pest resistance of grain legumes should consider the pleiotropic effect of plant resistance mechanisms affecting the physiology of the crop that would result in more or less yield (Clement et al., 1994). Consequently, resistance in presence of the pest reduces the damage thereby resulting in relatively high yields. In general, a number of landraces and a few introductions (CIAT lines) had a lower percent yield reduction compared to the local checks indicating the presence of resistance genes within these gene pools. However, it is not clear whether the resistance operating within these gene pools is antibiosis or antixenosis. Tolerance could also play a part in reduced yield loss for some genotypes.

Based on both the geometric mean GM and GE component analysis, a number of genotypes among those evaluated, introductions from both CIAT and landraces appeared to somewhat to perform well under drought as well under bean fly infestation (Table 3.2 and Figs. 3.1 and 3.2). These genotypes were GBK 047810, GBK 047866, G21212, GBK 047821, GBK 036488, CIM 9314-36 and IKINIMBA. These genotypes were able to combine drought tolerance with bean fly resistance. Such genotypes could be useful to farmers since they would give comparative yield advantage under bean fly pest attack as well as drought, common to semi-arid environments. Similarly these genotypes would also do well in a good season occasionally experienced during the periods when above normal rainfall is received. Taking advantage of the wide genetic base available in the common bean landraces, there is a high potential for discovering more resistance that could be incorporated in adapted cultivars (Edwards and Singh, 2006). These sources of resistance could be important for a breeding programme aimed at developing insect resistant cultivars. Previous reports show that bean fly resistant lines had been identified (Abate et al., 1995). However, the resistance of quite a number of bean fly resistant lines obtained from a CIAT regional nursery appeared to break down as a result of drought stress and heat problems owing to their poor performance. Careful consideration is needed by breeders screening for insect pests exclusive to semi-arid areas so as to avoid confounding interaction with performance-based traits contributing to yield which can cause difficulties for the breeder in identifying superior lines (Frahm et al., 2004). For instance, the effect of bean fly may have confounding and antagonistic interactions with performance-based traits that can complicate identification of superior lines adapted to adverse environments.

According to Terán and Singh (2002b) breeding crops for adaptability to rain-fed dry land environment is a time-consuming and a complex process. The reason is that such environments are characterized by poorly distributed and unreliable rainfall which fluctuates from time to time and often results in interactions between seasonal factors and environments. In addition to drought, there exists biotic stress (insect pest or disease) that is seldom considered by the breeders working in drought endemic environment. Screening for bean fly resistance under both DS and NS environments over different cropping seasons (LR and SR) could be a useful step towards considering this goal. Moreover, small-scale farmers growing beans in semi-arid areas of East Africa rarely use chemical pesticides but rely upon natural pest control methods by combining cultural practices with host plant resistance (Abate et al., 2000). Therefore, the development of a successful variety may require a multiple trait approach combining drought with a major biotic stress resistance such as bean fly resistance commonly encountered by the farmers. If such factors are

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considered, breeding of common bean genotypes with specific adaptability that could yield well in presence of both stresses could be achieved. That is, by incorporating insect resistance genes in locally adapted materials. Hence, could result in avoidance of genotype x environment interactions due to the effects of insect pest confounding genotypic performance for seed yield under drought. According to Ceccarelli and Grando (2007) selection for specific adaptation is important for crops grown predominantly in unfavourable environments.

3.5 Conclusions

The results show that resistance sources exist in the landrace collections and this is probably due to co-evolution between the bean fly and the common bean genotypes. Genotypes, GBK 047810, GBK 047866, G21212, GBK 047821, GBK 036488, CIM 9314-36 and IKINIMBA were identified as useful sources of bean fly resistance with adaptation to the semi-arid bean-growing region of East Africa. These sources of resistance can be exploited and used in breeding programmes for the development of bean fly resistant lines, which can effectively help reduce the damage and yield reduction arising from bean fly attack under drought. For faster progress, elite by elite crosses should be attempted in order to aim at the apex of the breeding pyramid for common bean improvement (Kelly et al., 1998).

Screening of genetic resources in common bean, combined with conventional (Edwards and Singh, 2006) as well participatory breeding approaches (Ojwang' et al., 2009), offers high potential of success if researchers take full advantage of the diversity available within the landraces and obtained either locally or from regional (CIAT) nurseries. But initial testing should be done for specific adaptability since the resistances of some genotypes may break down especially under adverse environmental conditions.

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Chapter 4

Genetic analysis for resistance to bean fly (*Ophiomyia phaseoli*) and seed yield among common bean genotypes in a semi-arid environment

Abstract

Bean fly (*Ophiomyia* spp.) is a major field pest limiting common bean (*Phaseolus vulgaris*) production in eastern Africa. The genetic enhancement of beans for resistance to insect pests is essential for minimizing yield losses arising from crop damage. The objectives of this study were to (1) assess combining ability for bean fly resistance and seed yield in common bean and (2) estimate genetic parameters associated with resistance for formulating a further breeding strategy. Eight parents four of which with known reaction to bean fly and another four locally adapted genotypes were crossed in an 8 x 8 half-diallel mating design. Parents and F_2 progenies were grown in an alpha-lattice design replicated twice in an open-field and subjected to natural populations of bean fly for two cropping seasons under semi-arid conditions. Similarly, two resistant and two susceptible parents were selected and crossed to produce populations for generation means and variances components analysis. Results revealed that both general combining ability (GCA) and specific combining ability (SCA) mean squares were significant ($p \leq 0.05$) for all four traits studied except SCA for stem damage during one cropping season. Among the parents, GBK 047858 was the best general combiner for all the traits studied across seasons except for stem damage during long rains (LR) 2009. Besides, genotypes GBK 047821 and Kat x 69 (a locally adapted variety) were generally good general combiners for resistance traits as well as seed yield. General predictability ratio (GPR) values ranging from 0.63 to 0.90 were obtained for plant mortality, stem damage, pupae in stem and seed yield across cropping seasons. These results established the predominance of additive gene effects (fixable variation) over the non-additive effects in controlling the traits. Low to moderate narrow sense heritability values ranging from 0.22 to 0.45 were obtained for pupae in stem. Such heritability estimates indicate that although additive gene components were critical in the inheritance of resistance for the trait, non-additive gene action were also important in addition to the environmental effects.

4.1 Introduction

Bean fly (*Ophiomyia* spp.) is probably the most important insect pest of common bean (*Phaseolus vulgaris*) in eastern Africa (Abate et al., 2000). Several species of *Ophiomyia* attack beans; but *O. phaseoli* (Kornegay and Cardona, 1991) is the most prevalent. For semi-arid lowland areas, *O. phaseoli* is the main species. In the higher elevations, *O. phaseoli* is prevalent early and *O. spenceralla* later in the season (Songa and Ampofo, 1999). Bean fly damage is most critical at the seedling stage when it often causes plant death. Bean crop damage reports from on-station and on-farm are varied, but both generally conclude that early season infestations can result in considerable yield loss approaching 100% (Greathead 1968; Abate and Ampofo, 1996; Ojwang' et al., 2009).

Chemical control to combat bean fly damage can be effective under high insect pressure, but most beans in the semi-arid regions of eastern Africa are produced by small-scale farmers who lack the financial capacity to purchase chemical pesticides. Instead, subsistence farmers rely upon traditional pest control approaches that are less effective for control of bean fly (Abate et al., 2000). Host plant resistance is a promising approach for an integrated insect pest management system in common bean (Miklas et al., 2006). Development of varieties with some level of genetic resistance to bean fly would greatly benefit small- and large-scale farmers as a cost effective and sustainable measure. Such varieties could be deployed as an important component of an integrated pest management. In addition, a combination of multiple traits, for instance yield improvement and tolerance to drought or low soil fertility are requisite for adaptability to a range of bean production agro-ecologies (Hillocks et al., 2006). Furthermore, such attributes ought to be combined with others such as seed size, seed colour, suitable taste and good cooking qualities so as to make the variety outstanding to small-scale farmers.

Precise understanding of gene action involved in resistance and available resistance genes in the germplasm are pre-requisites for the achievement of the desirable resistance breeding goal. Genetic variability for resistance to bean fly exists in common bean (Ojwang' et al., 2010). A few reports showed some evidence of quantitative inheritance for resistance to bean fly, where significant general combining ability (GCA) was more important than specific combining ability (SCA) (Mushi and Slumpa, 1996; Mushi and Slumpa, 1998). According to Miklas et al. (2006), tolerance and/or resistance to insect pests are not well studied. More genetic information is needed to facilitate breeding for resistance to bean fly and insect pests in general.

Griffing (1956) postulated a diallel technique for estimating the combining ability of lines and characterizing the nature and extent of gene action (additive and dominance effects). Even though the diallel analysis largely involves the use of F_1 progeny means from a set of crosses, F_2 progeny means and in some cases a combination of F_1 and F_2 generations means have been used (Christie and Shattuk, 1992; Hill et al., 2001; Dhliwayo et al., 2005; Kandalkar, 2005). The use of F_2 rather than F_1 in the implementation of the diallel experiment could arise from cost implications involved resulting from difficulty in obtaining adequate F_1 seed. However, the genetic expectations for the diallel of F_2 is same as that for an F_1 generation, (Hill et al., 2001) but decreased heterozygosity occurs due to selfing and as a result, the dominance contribution to SCA is half (Falconer and Mackay, 1996; Hill et al., 2001). In order to maximize genetic information from a set of parents, means from other generations may be required. If an additive-dominance model fits the data adequately, then non-allelic interactions (epistasis) are absent and genetic components may be estimated based on a five parameter or a six parameter model (Mather and Jinks, 1971; Wang and Gai, 2001). Consequential model fitting using least-square estimates of the components and the goodness of fit of the resultant model can be tested (Kearsey and Pooni, 1996).

The objectives of this study were to (1) assess combining ability for bean fly resistance and seed yield in common bean and (2) estimate genetic parameters associated with resistance for formulating further breeding strategy.

4.2 Materials and methods

4.2.1 Experimental sites

The crosses were made in a shadehouse at Kenya Agricultural Research Institute (KARI) Katumani Research Centre, situated at 1^0 35' S, 37^0 15' E; 1611 m above sea level, in semi-arid eastern Kenya region. The field experiments were carried out during the 2009 and 2010 cropping seasons at Kenya Agricultural Research Institute (KARI) Katumani, Kiboko sub-centre, Kenya. Kiboko is located 2^0 3' S, 37^0 43' E also in semi-arid eastern Kenya region, at an elevation of 938 m above sea level. Both sites have a bimodal pattern of rainfall with the long rainy season from mid-March to July and the short rainy season from mid-October to January. Kiboko was chosen for field screening because it is considered a 'hot spot' for bean fly (*Ophiomyia phaseoli*) infestation, thus it has consistent natural levels of bean fly populations (Songa and Ampofo, 1999).

4.2.2 Diallel experiment

4.2.2.1 Parental lines and field procedures

Eight common bean lines (Table 4.1) were crossed using a diallel mating design. The lines were CC 888 (G15430) and Macho (G22501) selected from a bean fly resistant nursery tested and compiled by CIAT regional body, East and Central Africa Bean Research Network (ECABREN), pure lines selected from landraces (GBK 047821 and GBK 047858), and locally adapted varieties (Kat x 69, Kat B9, Kat x 56 and Kat B1). The eight lines were selected based on genetic diversity and reaction to bean fly infestation in an earlier field screening trial (Ojwang' et al., 2010). Growth habit were types I, II and III (I determinate, II semi-determinate, and III indeterminate). All the parents used in the crosses were medium and large seeded belonging to both Andean gene and Mesoamerican gene pools and a range of market classes represented by seed colour. The F_2 seed was obtained by selfing F_1 plants for every cross.

The eight parents together with the 28 F_2 progenies from a diallel mating design, excluding reciprocals, were evaluated during the long rains (March to June) 2009 and repeated during the short rains 2009/2010 (October to January) in an open-field test, relying on natural bean fly infestation at Kiboko. Planting was deliberately delayed by two weeks from the on-set of rainfall to ensure that optimal bean fly pressure was achieved (Songa and Ampofo, 1999). This is because delayed planting and drought condition results in increased bean fly pressure. Due to below average rainfall received at Kiboko during the experimental period, irrigation was applied whenever necessary to ensure optimal growth conditions.

The 36 entries (8 parents and 28 F_2s) were planted using an alpha-lattice design with two replications. A plot consisted of 5 rows, 4 m long, 0.50 m apart, with a spacing of 0.10 m between plants within the row. Dead plants were recorded from 2 to 7 weeks after emergence to establish percent plant mortality. To avoid inclusion of dead plants resulting from causes other than bean fly, stems of the dead plants were examined for damage and presence of pupae for confirmation. At 28 days after emergence, 50 plants were randomly sampled from each plot. This was done by uprooting the plants, splitting the stem longitudinally at the junction between the root and stem and then recording the number of pupae per plant. Similarly, rating of stem damage was done using a score of 1-9 (1 = immune and 9 = extremely susceptible) (appendix 2a). The outer rows in each plot were used for destructive sampling while leaving the middle three rows for yield estimation. At maturity, three middle rows were harvested for seed yield estimation.

Table 4.1 Response of parent lines to infestation by *Ophiomyia phaseoli*, source and agronomic traits.

Parent	Source[a]	Growth habit[b]	Seed size	Seed colour	Yield range (Kg ha^{-1})[c]	Bean fly reaction	Other merits
P$_1$ = GBK 047821	NGBK	I	Large	Red	800-1250	Moderately resistant	Drought tolerant
P$_2$ = GBK 047858	NGBK	III	Large	Navy blue	630-1300	Resistant	Drought tolerant
P$_3$ = CC 888 (G15430)	CIAT	II	Medium	Grey + cream speckles	450-1200	Resistant	Early maturing
P$_4$ = Macho (G22501)	CIAT	I	Medium	Light yellow	600-1050	Moderately resistant	Early maturing
P$_5$ = Kat x 69	KARI	I	Large	Red mottled	550-1000	Moderately resistant	Marketable class, early maturing
P$_6$ = Kat B9	KARI	I	Medium	Red	550-1000	Susceptible	Early maturing, marketable class
P$_7$ = Kat x 56	KARI	II	Medium	Red	500-1000	Moderately susceptible	Early maturing, marketable class
P$_8$ = Kat B1	KARI	I	Medium	Yellow	550-1000	Susceptible	Cooks fast, tasty, early maturing

[a]NGBK   National Gene Bank of Kenya, CIAT Centro Internacional de Agricultura Tropical, KARI   Kenya Agricultural Research Institute.

[b]I determinate, II semi-determinate, III indeterminate, [c]Yield range under semi-arid conditions.

4.2.3 Generation means analyses

Screening for resistance to bean fly was conducted during the 2009 and 2010 cropping seasons at Kiboko, Kenya. The evaluation was done under natural field infestation. Four crosses among resistant and susceptible parental lines [GBK 047858 x Kat B9 , GBK 047858 x Kat B1 , CC 888 (G15430) x Kat B9, and CC 888 (G15430) x Kat B1] were made at KARI, Katumani in a shadehouse during the long rains (March to June) of 2008. The F_1s were planted during the off-season in the shadehouse between July and October 2008. The F_2s were also grown in the shadehouse during the short rains November 2008 to January 2009. Testing of all the three generations P_1, F_1, P_2, F_2 and $F_{2:3}$ families for bean fly resistance was conducted in the field at Kiboko during the short rains between November and February (2009/2010). The field experimental design used was similar to a split-plot arrangement where the generations were considered main plots. Parents and F_1s (non-segregating generations) were grown on a given block, the F_2s were grown on another block while the $F_{2:3}$s were also grouped on their own on a separate block. Therefore, the parental lines and families formed the sub-plots. The parents and F_1s were grown in three 2 m long row plots spaced 0.50 m apart and 0.10 m between plants within the rows, each plot having a total of 60 plants. On the other hand, the F_2s and $F_{2:3}$ populations were grown in five 4 m long row plots also spaced at 0.10 m apart giving a total of 200 plants per plot. To ensure that optimal bean fly population was attained during the screening period delayed planting was done (Songa and Ampofo, 1999). However, supplementary irrigation had to be applied to sustain the crop due to insufficient rainfall received during the crop growing period. Similarly, irrigation water was withheld 7 days after planting to enhance bean fly pressure(Ojwang' et al., 2010).

Sampling for the number of pupae per plant was done 28 days after emergence. Mean pupae in stem were used as the resistance parameter. Approximately, 20 plants were randomly sampled for the non-segregating generations and 100 plants for the segregating families. Data on mean number of pupae per stem in the various generations (P_1, F_1, P_2, F_2, and $F_{2:3}$) for each cross was analysed using additive-dominance model fitting (Mather and Jinks, 1971). Chi-square was used to test the goodness of fit of the resultant models. Least square estimates were used to fit the variance components (Kearsey and Pooni, 1996).

4.2.4 Statistical and genetic analyses

4.2.4.1 Diallel analyses

The data were subject to spatial analysis using residual (or restricted) maximum likelihood (REML) procedure of GENSTAT 11th edition statistical software to fit the variance-components. Parents and crosses were considered fixed terms whereas replications, rows and columns were considered random terms. Due to the interactions between seasons and genotypes (parents and crosses) and also between seasons and combining ability effects, data from each season were analyzed separately. In the event of significant variations among genotypes, diallel analyses were carried out following Griffing's (1956) model 1 (fixed effects model) and method 2 (parents and crosses, no reciprocals). Parental lines and the F_2 populations (crosses) were considered fixed effects while cropping season was considered a random effect. The analysis allowed for estimation of general combining ability (GCA) and specific combining ability (SCA) effects. The general predictability ratio, GPR = 2GCA / (2GCA + SCA) was estimated as suggested by Baker (1978).

4.2.4.2 Generation means and variances analyses

Components of the means for five generations (P_1, P_2, F_1, F_2 and $F_{2:3}$) (Wang and Gai 2001) were estimated using weighted least square estimates (Mather and Jinks, 1971) of three parameters viz., *m* (average effect), *d* (additive) and *h* (dominance effects) according to Hayman (1958). Additive-dominance and additive-environmental components of the variation were obtained by least square estimates and the best fitting model established (Kearsey and Pooni, 1996). Narrow sense heritability was estimated as, $h^2_n = V^*_a / (V^*_a + V_e)$, where V^*_a = additive genetic component of variance and V_e = additive environmental variance (Kearsey and Pooni, 1996).

4.3 Results

4.3.1 Parents and F_2 progenies

Studies on the assessment of genotypic variation among the common bean parents under natural infestation of bean fly (*Ophiomyia phaseoli*) revealed significant differences at p ≤ 0.05 for long rains (LR) 2009 and p ≤ 0.01 for short rains (SR) 2008/09 among genotypes for all traits studied (Appendix 2c and 2d). The level of variation for resistance to bean fly was measured and quantified by evaluating the genotypes in terms of stem damage, plant mortality and pupae in stem (Tables 4.2 and 4.3). Generally, low values for these characters

indicated the degree of resistance of the genotype while high values indicated the degree of susceptibility. However, there was a significant interaction between genotype and season. Due to these interactions, data were analysed separately within seasons. Looking at the parental lines in Table 4.2 there was consistency in the trend observed in the three parameters which provided a reasonable assessment of the host-parasite interactions (resistance/susceptibility). However, there were a few exceptions where for certain genotypes variation occurred between LR and SR for given parameters. For example, genotype GBK 047858 had a moderate damage score in both cropping seasons which apparently was close to susceptible genotypes Kat B1 and KAT B9 in 2009. On the other hand, the same genotype GBK 047858 suffered 29% plant mortality while Kat x 56, a moderately susceptible genotype, had high stem damage but low plant mortality in the same cropping season 2009. In the subsequent season GBK 047858 recorded a low plant mortality, of despite having a moderate damage score of 5.5. Similarly, a moderately resistant GBK 047821 had the highest number of pupae, while relatively susceptible genotype Kat B9 had a low number of pupae. In spite of having the largest number of pupae in the stem in 2009, genotype GBK 047821 gave the highest seed yield. Conversely, Kat x 56 had the lowest plant mortality but gave low seed yield.

Variation between seasons was observed among crosses for all traits studied (Table 4.3). Generally, the F_2 population derived from crosses involving susceptible parents (Kat B1 and Kat B9) had high stem damage scores. Percent mortality was consistently high among the progenies in SR than LR, with crosses involving susceptible parents similarly exhibiting relatively high mortality. No clear pattern was observed in the other seasons but crosses involving resistant and moderately resistant parents generally showed consistent low to moderate mortality. Pupae in stem varied from cross to cross and across seasons. Crosses involving resistant parent CC 888 (G15430) and Macho (G22501), such as [GBK 047858 x CC 888 (G15430), CC 888 (G15430) x Kat x 56 and Macho (G22501) x Kat x 69], had comparably low pupae in stem for both SR and LR. There were also crosses that had consistently low pupae in stem in LR but not in SR and vice versa. Examples of such crosses were, GBK 047821 x GBK 047858, Kat B9 x Kat x 56 and Kat x 56 x Kat B1. Seed yield performance varied among crosses. Three crosses performed consistently high for seed yield across seasons and these were GBK 047821 x Kat x 69, GBK 047858x Kat x 69 and Kat B9 x Kat B1 hence showing broad adaptability. Similarly, progenies of GBK 047821 x Macho (G22501) and GBK 047821x Kat B1 showed comparatively good seed yield performance during the LR while CC 888 (G15430) x Kat x 56 performed well during SR hence displaying specific adaptability.

Table 4.2 Means for stem damage, plant mortality, pupae in stem and seed yield for parents grown under natural field infestation of bean fly (*Ophiomyia phaseoli*) for two cropping seasons at Kiboko, Kenya.

Genotype	Stem damage[a]		Plant mortality (%)		Pupae in stem		Seed yield (kg ha^{-1})	
	LR 2009	SR 2009/10	LR 2009	SR 2009/10	LR 2009	SR 2009/10	LR 2009	SR 2009/10
GBK 047821	3.8	4.4	8.9	36.9	1.35	0.87	977.9	265.8
GBK 047858	5.6	5.5	29.4	9.9	0.67	0.46	791.8	688.3
CC 888 (G15430)	4.7	3.1	24.8	34.8	0.83	0.57	704.8	557.6
Macho (G22501)	3.4	4.9	31.0	54.5	1.08	0.45	864.0	218.9
Kat x 69	3.6	5.1	34.5	53.5	0.82	0.92	949.0	383.3
Kat B9	6.5	8.9	46.3	94.8	0.89	0.85	564.2	20.0
Kat x 56	5.7	7.4	3.5	66.2	1.19	1.14	580.2	38.4
Kat B1	5.1	9.0	43.9	96.5	1.15	1.24	854.6	44.0
Mean	5.5	6.2	37.7	69.9	0.93	0.96	805.0	451.3
SE	1.2	1.3	13.2	10.9	0.22	0.22	112.0	236.6
LSD (0.05)	2.0	2.2	22.4	18.5	0.38	0.38	190.0	401.3

[a] Stem damage scores: 1 = immune and 9 = extremely susceptible.

Table 4.3 Means for stem damage, plant mortality, pupae in stem and seed yield for F_2 populations grown under natural field infestation of bean fly (*Ophiomyia phaseoli*) for two cropping seasons [long rains (LR 2009) and short rains (SR 2010)] at Kiboko, Kenya.

Cross/family	Stem damage[a]		Plant mortality (%)		Pupae in stem		Seed yield (kg ha^{-1})	
	LR 2009	SR 2009/10	LR 2009	SR 2009/10	LR 2009	SR 2009/10	LR 2009	SR 2009/10
GBK 047821 x GBK 047858	4.2	6.7	37.8	74.4	1.16	0.72	877.5	742.9
GBK 047821 x CC 888 (G15430)	3.3	7.1	37.0	79.6	0.70	1.14	995.1	352
GBK 047821 x Macho (G22501)	3.3	7.7	33.5	73.8	0.82	1.11	1007.8	311.2
GBK 047821 x Kat x 69	4.5	2.8	38.6	41.1	1.02	0.78	909.3	1092.3
GBK 047821 x Kat B9	4.4	8.6	42.9	92.6	0.91	1.33	837.0	93.2
GBK 047821 x Kat x 56	3.6	6.6	64.8	78.4	1.02	1.04	889.1	472.0
GBK 047821 x Kat B1	2.9	7.4	23.6	79.3	1.05	0.78	1117.9	290.5
GBK 047858 x CC 888 (G15430)	4.3	3.5	39.8	63.6	0.65	0.69	924.6	882.4
GBK 047858 x Macho (G22501)	3.8	7.3	48.9	81.1	0.58	1.21	903.1	157.5
GBK 047858 x Kat x 69	4.2	1.8	12.5	40.9	1.03	0.80	907.7	2475.9
GBK 047858 x Kat B9	4.0	7.6	14.4	68.4	1.11	1.25	861.4	268.8
GBK 047858 x Kat x 56	5.3	5.4	33.6	64.5	0.64	0.83	700.7	494.2
GBK 047858 x Kat B1	4.2	7.5	41.2	83.9	0.82	1.03	904.4	355.1
CC 888 (G15430) x Macho (G22501)	4.8	5.9	32.8	72.2	0.84	0.94	825.1	548.9
CC 888 (G15430) x Kat x 69	6.1	7.4	33.5	85.0	0.81	1.03	538.0	318.2
CC 888 (G15430) x Kat B9	6.9	7.4	58.9	87.6	1.14	1.44	620.8	167.0
CC 888 (G15430) x Kat x 56	5.8	4.2	41.0	69.4	0.48	0.75	563.4	926.0
CC 888 (G15430) x Kat B1	3.8	5.3	35.7	75.0	1.01	1.11	902.2	517.0
Macho (G22501) x Kat x 69	5.5	5.8	53.4	76.2	0.60	0.89	718.7	435.1
Macho (G22501) x Kat B9	5.5	8.1	34.2	84.0	0.83	1.20	738.2	138.3
Macho (G22501) x Kat x 56	6.5	5.0	34.8	73.2	1.25	0.94	641.6	614.7
Macho (G22501) x Kat B1	5.3	5.8	58.2	75.5	1.08	1.00	630.5	253.0
Kat x 69 x Kat B9	4.5	6.8	53.2	78.2	1.24	1.24	770.9	351.5
Kat x 69 x Kat x 56	3.3	6.2	34.2	57.9	1.25	1.00	961.1	146.5
Kat x 69 x Kat B1	4.5	8.1	63.4	88.9	0.81	0.86	780.4	92.3
Kat B9 x Kat x 56	5.2	8.2	55.4	81.5	0.59	1.10	582.5	44.6
Kat B9 x Kat B1	6.3	9.0	48.1	93.1	0.82	1.36	682.1	14.1
Kat x 56 x Kat B1	3.0	1.8	29.4	50.0	1.11	0.52	900.6	1475.8
Mean	5.5	6.2	37.7	69.9	0.93	0.96	805.0	451.3
SE	1.2	1.3	13.2	10.9	0.22	0.22	112.0	236.6
LSD (0.05)	2.0	2.2	22.4	18.5	0.38	0.38	190.0	401.3

[a] Stem damage scores: 1 = immune and 9 = extremely susceptible.

4.3.2 Gene action

4.3.2.1 Combining ability analyses

Mean squares due to general combining ability (GCA) and specific combining ability (SCA) were significant (p ≤ 0.05) for all traits studied except SCA for stem damage during LR 2009 (Table 4.4) (See also appendix 2e and 2f). Therefore, both GCA and SCA effects were relevant in controlling resistance and seed yield. However, general predictability ratio (GPR) values ranging from 0.63 to 0.90 obtained indicated the predominance of additive gene effects (fixable variation) over the non-additive effects in controlling the traits.

Among the parents, GBK 047821, GBK 047858, CC 888 (G15430) and Kat x 69 were found to be good general combiners for most of the traits in given seasons (Table 4.5). Particularly, GBK 047858 had overall good GCA for all characters in both LR and SR cropping seasons except during LR 2009. On the other hand, Kat B9 and Kat B1 displayed low potential and were poor general combiners for most traits across seasons. Despite Kat B1 being a poor combiner, it was a good combiner for seed yield in LR 2009 indicating specific adaptability. The genotypes with high GCA effects could provide a good potential for developing resistant cultivars with high yield performance and preferred bean market classes in the region.

4.3.2.2 Components of the means and variances for number of pupae in stem

The additive-dominance model was adequate for the analysis of variation in all crosses for number of pupae as shown by the non-significant chi-squares (Table 4.6). Among the four crosses studied, the estimates of *d* (additive) and *h* (dominance) were significantly different from zero and they all had negative values. Thus, both additive and dominance components were found to be important in controlling the inheritance of resistance to bean fly. Additive gene effects were relatively larger than dominance components among all crosses thereby confirming the results reported under combining ability analysis. The negative dominance present showed gene distribution in the direction of genes restricting the number of pupae present in the stems. Low to moderate narrow sense heritability values ranging from 0.22 to 0.45 were obtained for pupae in stem.

Table 4.4 Combining ability mean squares for stem damage, plant mortality, pupae in stem and seed yield under natural bean infestation for two cropping seasons [long rains (LR 2009) and short rains (SR 2010)] at Kiboko, Kenya.

Source	df	Stem damage[a]		Plant mortality (%)		Pupae in stem		Seed yield (kg ha^{-1})	
		LR 2009	SR 2009/10	LR 2009	SR 2009/10	LR 2009	SR 2009/10	LR 2009	SR 2009/10
GCA	7	2.12**	7.67**	184.50*	806.95**	0.055*	0.087**	59553.89**	306992.94**
SCA	28	0.94	2.99**	215.16**	267.53**	0.050*	0.057**	12808.32*	206048.34**
Error	31	0.63	0.82	69.45	57.2	0.024	0.021	5616.50	27191.00
GPR[b]		0.82	0.84	0.63	0.86	0.69	0.75	0.90	0.75

*,** Significant at p ≤ 0.05 and 0.01, respectively.

[a] Stem damage scores: 1 = immune, and 9 = extremely susceptible.

[b] General predictability ratio = 2GCA/(2GCA + SCA) (Baker, 1978).

Table 4.5 General combining ability (GCA) effects of eight parents for stem damage, plant mortality, pupae in stem and seed yield under field infestation of bean fly during the long rains (LR) 2009 and short rains (SR) 2009/10.

Parents	Stem damage rating		Plant mortality (%)		Pupae in stem		Seed yield (kg ha^{-1})	
	LR 2009	SR 2009/10	LR 2009	SR 2009/10	LR 2009	SR 2009/10	LR 2009	SR 2009/10
GBK 047821	-0.81**	-0.02	-4.31*	-3.61	0.104*	-0.001	134.50**	-17.62
GBK 047858	-0.07	-0.51*	-5.24*	-13.25**	-0.101*	-0.121**	41.85*	269.16**
CC 888 (G15430)	0.25	-0.89**	-1.10	-2.71	-0.105*	-0.0420	-46.58*	76.49
Macho (G22501)	-0.03	-0.04	1.84	1.59	-0.018	-0.044	-5.16	-116.54*
Kat x 69	-0.20	-0.67**	1.86	-5.38*	0.004	-0.020	23.96	161.66**
Kat B9	0.80**	1.78**	6.04**	14.57**	0.008	0.198**	-102.32**	-294.43**
Kat x 56	0.23	-0.38	-3.92	-2.18	0.038	-0.020	-84.52**	18.88
Kat B1	-0.16	0.73**	4.82*	10.97**	0.068	0.049	38.28*	-97.60*

*,** Significant at p ≤ 0.05 and 0.01, respectively.

Table 4.6 Genetic parameter estimates for pupae in stem among common bean crosses.

Genetic parameter	GBK 047858 x Kat B9	GBK 047858 x Kat B1	CC 888 (G15430) x Kat B9	CC 888 (G15430) x Kat B1
m	2.05±0.122**	2.613±0.148**	2.54±0.119**	2.59±0.135**
[d]	-1.05±0.158**	-1.19±0.179**	-1.41±0.138**	-1.32±0.164**
[h]	-0.82±0.257*	-1.01±0.329*	-1.08±0.252*	-1.26±0.239*
X^2	6.34	4.45	7.76	7.07
V^*_a	0.992	1.135	0.966	0.668
V_e	1.799	2.307	2.536	2.691
$h^2_{(ns)}$	0.36	0.45	0.22	0.30

*,** Significant at $p \leq 0.05$ and 0.01, respectively.

4.4 Discussion

The amount of genetic variation for resistance to bean fly can be measured and quantified based on resistance parameters. In the current study, a number of traits including percent plant mortality, pupae in stem and stem damage were used. Clearly, the level of resistance among the parents varied between cropping seasons and was revealed by low values while high values signified the degree of susceptibility. Comparatively, the superiority of parental genotypes GBK 047821, GBK 047858, CC 888 (G15430) and Macho (G22501) for resistance to bean fly was manifested on the basis of low to moderate values for the resistance parameters studied across the seasons (Table 4.2). However, interactions between seasons and genotypes occur under bean fly infestation and in semi-arid conditions (Ojwang et al., 2010). Such interactions could cause changes in genotypic performance between seasons as was observed for certain genotypes in the current study e.g. GBK 047858, GBK 047821, Kat x 56, Kat x 69 and Kat B9. This implies that selection of resistant genotypes based on multiple seasons should rely upon a combination of parameters namely, pupae in stem, plant mortality, stem damage and seed yield. Overall performance of parents was better during the long rainy (LR) season when the bean fly attack was moderate in relation to short rainy season (SR), which had high bean fly pressure. Even susceptible genotypes such as Kat B1 showed good seed yield in the LR. Two parents (GBK 047858 and CC 888 (G15430)) demonstrated stable yield performance across cropping seasons and thus had broad adaptability.

According to Mather and Jinks (1982), resorting to the use of F_2 diallel due to cost implications as was the option in the current study is often as a result of difficulty in obtaining sufficient F_1 seed. This alternative is also associated with another problem. Mainly the halving of the heterozygotes in the F_2 generation, which in turn reduces the dominance contributions of the genes involved, even though additive components remain unchanged. However, this could be addressed by raising a large F_2 population as was done in this study.

The genetic analysis of F_2 diallel data showed the importance of additive gene action in controlling bean fly resistance and seed yield under bean fly attack (Tables 4.4 and 4.6). These results agree with earlier results obtained by Mushi and Slumpa (1998), who found that GCA effects were dominant over the SCA effects in controlling resistance to bean fly, based on percent plant survival for the parents. The prediction of the success of a cross in a hybridization programme may be based on the *per se* performance of the parents and their

respective GCA effects. Besides parents GBK 047821, GBK 047858, CC 888 (G15430) and Kat x 69 showing good mean performance for most resistance and yield characters, they were also good general combiners. A high GCA effect is largely attributed to additive gene effects or additive x additive interactions which correspond to fixable genetic variance. Inclusion of parents with favourable alleles in a breeding programme should assist in effective improvement of particular traits, thereby enhancing the resistance as well as yield in target agro-ecologies.

Overall, the crosses involving parents (GBK 047821 GBK 047858 and Kat x 69), with high negative GCA for resistance traits and high positive GCA for seed yield recorded good performance, though with exceptions. A few examples of crosses showing good performance across cropping seasons in the presence of bean fly infestation were GBK 047821 x Kat x 69 and GBK 047858 x Kat x 69. These crosses showed high and stable performance. The performance of crosses involving parents with good GCA effects for given traits could be attributed to additive gene action as well considerable amount of additive x additive gene interaction. The use of elite x elite (high x high) crosses provides faster progress in selection for yield accumulation (Kelly et al., 1998). Progenies of Kat x 56 x Kat B1 showed good and stable performance and hence displayed broad adaptability. The superiority shown by such a cross may have resulted from additive x dominance or dominance x dominance gene action and thus could be non-fixable. This is because one parent (Kat B1) had significant positive GCA effects for seed yield in one cropping season while both parents were poor combiners in at least one season. It is probable that such a hybrid would be expressing favourable additive genetic effects from the better parent apart from also displaying complimentary non-additive genetic effect. If the gene action involved is additive x dominance then progenies obtained from such a cross would be valuable. As the subsequent selfing would break the dominance (Kearsey and Pooni, 1996), and in so doing lead to accumulation of positive resistance genes arising from increased homozygosity, eventually superior progenies could be obtained.

When a simple additive-dominance model fits the data (Table 4.6), then theoretically we discount the presence of complicating factors such as interactions between the genes (Mather and Jinks, 1971). Therefore, in the present study genetic analysis for five basic generations means revealed that additive gene action contributed significantly to the genetic control of pupae in stem. These results confirmed those obtained from diallel analysis.

According to Kearsey and Pooni (1996) the most appropriate model for fitting basic generation variances requires just two parameters, V_e and V^*_a both of which should be statistically significant. If $F_{2:3}$ generation is used, it may not possible to estimate all the genetic and environmental components of variation as we have four parameters V_a (additive-genetic variance), V^*_d (variance due to dominance), V_{ec} (variance due to common environment) and V_e (additive-environmental variance), but only two statistics available for estimation. The dominance contribution is likely to be small and ignoring it will cause little bias.

Arguably one of the most useful statistics that can be derived from the variance components is heritability (Kearsey and Pooni, 1996). Narrow sense heritability is more important because it measures the proportion of the variation which is due to the additive effects of genes in a specific population. From this study, low to moderate heritability values ranging from 0.22 to 0.45 were obtained for pupae in stem in different crosses involving resistant and susceptible parents (Table 4.6). Heritability estimates showed that although additive genetic components were critical in the inheritance of resistance, the non-additive gene action were also important in addition to the environmental effects. Such heritability values suggest that it would be difficult to predict progeny performance due to the presence of non-heritable variation. The implication to breeding is that a selection procedure that could result in positive accumulation of resistance genes should be adopted.

4.5 Conclusions

The present study revealed the predominance of additive over the non-additive gene action in conferring resistance to bean fly as well as seed yield accumulation. The present data, apart from being a starting point for further investigation of the genetic control of resistance to bean fly in common bean, could be useful for the development of an effective breeding programme that might develop resistant cultivars. Bean lines GBK 047858 and Kat x 69 and their crosses had good genetic potential for resistance to bean fly and yield attributes, and were consistent across cropping seasons. Such lines should be exploited in bean improvement programmes for areas prone to bean fly attack.

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Chapter 5

Varital evaluation of common bean (*Phaseolus vulgaris* L.*)* for resistance to bean fly (*Ophiomyia phaseoli*) in controlled choice and no-choice environments

Abstract

Bean fly (*Ophiomyia* spp.) is one of the most destructive insect pests of common bean (*Phaseolus vulgaris* L.) in eastern Africa. A major challenge in screening for resistance to bean fly in common bean under controlled conditions other than natural open-field tests, has been the lack of a method for artificially generating adequate fly populations. In view of this problem, a simple procedure for raising sufficient populations of adult bean flies required for screening has been described in the current study. Through this procedure, up to 62 % emergence of the adult fly was achieved and the flies retained their ability to infest bean plants. Due to the importance of host-plant resistance as part of an integrated pest management strategy, a study was undertaken to determine the presence of antibiosis and antixenosis mechanisms of resistance in common bean. Five genotypes [CC 888 (G15430), GBK 047821, GBK 047858, Ikinimba and Macho (G22501)] and two local check varieties (Kat B1 and Kat B9) were screened under free-choice in outdoor net cages and no-choice conditions in net cages placed in a shade-house. All the five resistant genotypes tested had significantly longer internodes compared to susceptible genotypes. Long internode was established as the morphological trait contributing to antixenosis component of resistance. Both ovipositional non-preference and antibiosis mechanisms were found to exist in three genotypes namely CC 888 (G45430), GBK 047858 and Macho (G22501). These genotypes retained their resistance when they were subjected to bean fly under both free-choice and no-choice conditions. They had fewer feeding/oviposition punctures, low number of pupae in the stem, reduced damage to the stems and low percent plant mortality. The remaining genotypes (Ikinimba and GBK 047821) only expressed antixenosis. Genotypes possessing multiple resistance (antibiosis, antixenosis and/or tolerance) represent an important basis for obtaining genes for the development of cultivars resistant to bean fly.

5.1 Introduction

Bean fly (*Ophiomyia* spp.) is a major insect pest of common bean in Africa. Among the species that attack beans, *Ophiomyia phaseoli* (Tyron) is reported as the most important (Greathead, 1968; Kornegay and Cardona, 1991). The pest causes widespread damage to beans and crop loss can reach up to 100% (Greathead, 1968; Abate and Ampofo, 1996; Ojwang' et al., 2010). Despite the high crop losses incurred, the majority of bean farmers who practice subsistence agriculture still rely upon traditional pest control strategies (Abate and Ampofo, 1996). On the other hand, application of insecticides is expensive and could lead to the development of resistant insect biotypes. One strategy to combat the bean fly problem is for the farmers to incorporate resistant varieties in their cropping systems.

Bean flies have been reported to be widely distributed in tropical and sub-tropical countries including Africa, Asia Australia and the Pacific Islands (Karel, 1985; Talekar and Lee, 1989). However, of all these regions, bean fly is a major limiting factor in successful cultivation of economically important legumes such as common and snap beans (*Phaseolus vulgaris* L.) in most areas of eastern Africa and soybean (*Glycine max* (L.) Merill) largely in South East Asia (Talekar and Lee, 1989). Apart from common bean and Soybean the host range of bean fly includes other *Phaseolus* spp. (*Phaseolus coccineus* and *P. lunatus*), Cowpea (*Vigna unguiculata*), Mungbean (*Vigna radiata* and *V. Mungo*), Pigeonpea (*Cajanus cajan*), *Lalab niger* and *Crotaleria juncea* (Greathead, 1968).

Until recently, most reports on crop improvement against bean fly and related agromyzids have been on soybean [*Glycine max* (L.) Merril] (Talekar and Tengkano, 1993) and mungbean [*Vigna radiata* (L.) Wilczek] (Talekar et al., 1988), but little information is available on common bean (*Phaseolus vulgaris* L.). Breeders have limited access to a full range of germplasm to source for resistance (Miklas et al., 2006). Besides, screening for resistance has largely been based on open-field tests (Clement et al., 1994; Abate et al., 1995). However, relying on natural field populations for screening germplasm or breeding populations could have its own challenges. This is because environmental factors such as rainfall (drought) (Greathead, 1968; Davies, 1998; Songa and Ampofo, 1999) and sudden increase of natural enemies at certain times of the year, could reduce bean fly populations under field conditions which would therefore render resistance screening in the field untenable due to low insect pressure (Talekar and Lee, 1989). Selection of resistant plants across generations will possibly require more reliable approaches. Hence, cage screening techniques that would ensure uniformity of pest population may enhance efficiency in obtaining resistant genotypes.

Plant defence mechanisms are known to arise from biochemical and morphological characteristics (Norris and Kogan, 1980; Clement et al., 1994). According to Norris and Kogan (1980), both these resistance mechanisms provide protection to host plants by hindering selection of the host plants by insects, affect feeding, ingestion, digestion or oviposition. Kornegay and Cardona (1991), in their review article, suggested two modes of resistance in common bean, namely antibiosis and antixenosis. Generally, limited studies have been conducted on the mechanisms of resistance to insect pests of common bean (Miklas et al., 2006).

A mass rearing procedure for bean fly has been documented in soybean (Talekar and Lee, 1989). However, the procedure has limited or no application to common bean. This is supported by the fact that the bean fly reported from Asia lay their eggs and feed on the cotyledons of soybean unlike those reported from Africa which lay their eggs either on leaves or stems of beans. It could be possible that different bean fly biotypes exist in Africa compared with those in Asia. The major challenge in breeding for insect resistance is lack of a comprehensive procedure that could enable identification and differentiation of resistant lines provided by a uniform insect pest attack (Hillocks et al., 2006). Such uniformity can only be achieved under artificial conditions. However, open-field tests are still useful especially when dealing with large numbers of genotypes. In a situation where open-field test has been used, further testing and confirmation could be necessary under artificial screening in cages.

The objectives of the study were to (1) describe a simple and inexpensive procedure for generating optimal bean fly populations for artificial cage screening, (2) explain cage screening techniques useful for distinguishing resistant from susceptible genotypes and (3) determine the nature of resistance operating in common bean against bean fly.

5.2 Materials and methods

The study was conducted at the Kenya Agricultural Research Institute (KARI), Katumani research centre, located at 1^0 35' S, 37^0 15' E; 1611 m above sea level, in semi-arid eastern Kenya. Common bean genotypes (Table 5.1) used in the study included 3 lines selected from Centro Internacional de Agricultura Tropical (CIAT), East and Central Africa Bean Research Network (ECABREN) regional nursery and 2 pure lines identified from landraces. These lines showed resistance to bean fly in earlier screening trials (Ojwang' et al. 2010). The 2 local varieties Kat B1 and Kat B9 were included as susceptible checks.

Table 5.1 Description of selected common bean genotypes used to screen for resistance against bean fly (*Ophiomyia phaseoli*).

Genotype	Source[a]	Growth habit[b]	Seed size	Seed colour	Reaction to bean fly
GBK 047821	NGBK	I	Large	Red	Resistant
GBK 047858	NGBK	III	Large	Navy blue	Resistant
Ikinimba	CIAT	II	Medium	Black	Resistant
CC 888 (G15430)	CIAT	II	Medium	Grey + cream speckles	Resistant
Macho (G22501)	CIAT	I	Medium	Light yellow	Resistant
Kat B9[c]	KARI	I	Medium	Red	Susceptible
Kat B1[c]	KARI	I	Medium	Yellow	Susceptible

[a]NGBK, National Gene Bank of Kenya; CIAT, Centro Internacional de Agricultura Tropical; KARI, Kenya Agricultural Research Institute.

[b]I, determinate growth; II, semi-determinate; III, indeterminate.

[c]Local check.

5.2.1 Raising bean fly populations

A susceptible bean variety (Kat B1) was planted in a field adjacent to the screening area to trap the bean fly in a plot measuring 10 x 10 m (Fig 5.1 a). In order to achieve a high plant population, close spacing was adopted. The rows were spaced 0.25 m apart and the intra-row spacing was 0.05 m. In order to maintain a constant supply of pupae during the screening period, it was necessary to stagger the plantings accordingly, with a gap of two weeks before the subsequent planting. At four weeks after emergence, plants were uprooted and taken to a room for removal of pupae from bean stems. Careful removal of the pupae was done using an ordinary toothpick to avoid injury to the pupae. An average of 3 pupae per plant was obtained. Since the study focused on *Ophiomyia phaseoli*, only the brown pupae were retained and black ones (*O. spencerella*) discarded. A Petri dish was used for placement of pupae during the removal. The pupae were then paced in ordinary cylindrical plastic jars with a diameter of 0.15 m and a height of 0.20 m at normal room conditions. A round hole was created on the lids of the jars and covered with a fine insect net. Thirty jars were used per experiment. Each jar was lined with moist tissue paper at the bottom and about 30 pupae placed inside. Subsequently, a normal hand sprayer was used to apply a light spray of tap water into the jars every morning and evening until the flies emerged. The flies emerged within 2 - 3 days. The experiment was repeated four times (in September

2009, November 2009, January 2010 and April 2010). Data were collected on the number of adults emerging per jar and used to calculate percent emergence.

Fig. 5.1 Screening for resistance: (a) susceptible check variety (Kat B1) showing symptoms of damage caused by bean fly (*Ophiomyia phaseoli*); (b) Large cages for free-choice tests; (c) susceptible genotype (Kat B9) in small cages under no-choice tests; (d) resistant genotype [G22501 (Macho)] in small cages under no-choice tests.

5.2.2 Free-choice tests

Cages measuring 4 m long x 2 m wide and 1.8 m high were used for the free choice study (Fig 5.1 b). The metal frames were covered with a fine insect net and a door created on the side with an ordinary zip. The seven bean genotypes were planted in the net cages in single row plots 1.5 m long, spaced 0.50 m apart and 0.10 m between plants within the rows, giving a total of 15 plants per plot. A path of 0.25 m was made on either side of the plot within the cage. Three net cages were used, each representing a replication in a randomized complete block design (RCBD). About 50 freshly emerged adult flies were introduced in each cage 1 day after plant emergence. The sex ratio assumed was that of a normal random bean fly population, which is usually 1:1 (Greathead, 1968).

Morphological data were collected by measuring the leaf area of unifoliate leaves and internode length at 2 weeks after emergence. Plant mortality was recorded cumulatively for a period of 4 weeks beginning soon after adult fly introduction. At 4 weeks after emergence, five plants were randomly sampled from each plot and uprooted to count number of pupae and for stem damage rating. A rating scale of 1 – 9 was used (1 = immune and 9 = extremely susceptible) (Kornegay and Cardona, 1991) (Appendix 2a).

Data were subjected to analysis of variance using GENSTAT 11th edition computer programme. Due to significant differences among the genotypes within experiments (repeats), a combined analysis of variance using a two way or factorial (genotypes and screenings) in a randomized complete block design was conducted. The differences were determined by *F*-test, while the significant differences between the varietal means were separated by least significance differences (LSD) at $p \leq 0.05$ using suitable error terms. Correlation analysis was made to establish if the plant physical characteristics had a role to play in plant defence mechanisms.

5.2.3 No-choice tests

Net cages measuring 0.60 m long x 0.60 m wide x 0.60 m high with a door created on one side were used for evaluating the seven common bean genotypes for bean fly resistance. The experimental design was a completely randomized design (CRD) replicated three times. There were seven net cages per replication giving a total of twenty-one. Four plastic pots measuring 0.25 m in diameter and 0.30 m high were placed in each cage (Fig. 5.1 c-d). Three plants were grown in each pot. This gave a total of twelve plants per plot (cage). Twenty adult flies were introduced in each cage one day after emergence. Three successive screenings were conducted.

Data were collected for number of bean fly oviposition/feeding punctures seven days after the introduction of adult flies on unifoliate leaves. The data were used to assess the bean fly activity. Plant mortality was recorded for a period of four weeks beginning immediately after introduction of the flies. After twenty-eight days five plants per cage were uprooted randomly for determination of pupae per stem and rating of stem damage. The rating for stem damage was based on a 1-9 scale (Appendix 2a).

Data were subjected to analysis of variance using GENSTAT 11th edition. In the event of the presence of significant interactions between genotype x experiments (successive

screenings), data were analysed separately for each experiment. The significant differences were tested by *F*-test, while the significant difference between the treatment means were separated by least significance differences (LSD) at $p \le 0.05$.

5.3. Results

5.3.1 Raising bean fly populations

Observations made revealed adult fly emergence ranging from 50 – 62 %. Consequently, sufficient bean fly populations could easily be raised for use in screening cages if the method illustrated in this study is applied. In addition, pupae obtained from materials being screened can also be put into jars to generate more adult flies for a subsequent screening.

5.3.2 Free-choice tests

There was significant variation among common bean genotypes in response to bean fly attack for traits measured (Table 5.2) (See also appendix 3a). Generally, greater preference was observed for the susceptible genotypes (Kat B1 and Kat B9) as shown by relatively higher pupae in bean stems, plant mortality and also plant damage (Table 5.3). The reverse was true in the case of the remaining genotypes which expressed resistance as shown by comparatively smaller values for the resistance parameters.

Simple correlations analysis revealed significant relationships between certain morphological and resistance parameters and also between resistance parameters separately (Table 5.4). For example, internode length was negatively correlated with pupae count (r = -0.74*), plant mortality (r = -0.92**) and stem damage (r = -0.87**). Mostly, the genotypes that had long internodes are the ones that had lower pupae number, less damage and reduced plant mortality compared to those with short internodes (Table 5.3). The number of pupae in stem was positively correlated with plant mortality (r = 0.81*) and stem damage (r = 0.89**). Similarly, stem damage was highly associated with plant mortality % (r = 0.98**).

Table 5.2 Mean squares for morphological and resistance parameter under free choice conditions.

Source	df	Internode length	Leaf area	Pupae in stem	Plant mortality (%)	Stem damage
Genotype (G)	6	0.6010 **	487.6**	5.4917**	2820.4**	23.476**
Block	2	0.0031	366.2	3.2205	347.1	6.540
Experiment (E)	2	14.7357**	17397.4**	3.9271*	1934.8**	6.873*
G x E	12	0.0842n.s	137.4n.s	0.7016n.s	305.6n.s	3.040
Error	40	0.1471	109.5	0.9008	218.5	2.206

*, ** Significant at $p \leq 0.05$ and 0.01 levels, respectively; n.s not significant.

Table 5.3 Morphological traits and infestation of selected common bean genotypes by bean fly (*Ophiomyia phaseoli*) under free choice conditions.

Genotype	Morphological characteristics		Resistance parameters		
	Internode length (cm)	Leaf area (cm^2)	Pupae in stem	Plant mortality (%)	Stem damage[a]
CC 888 (G15430)	2.81	52.2	0.94	13.5	3.44
GBK 047821	2.95	68.8	0.70	10.5	3.11
GBK 047858	2.98	59.3	1.02	14.0	3.33
Ikinimba	2.87	45.1	2.04	18.8	4.76
Macho (G22501)	2.88	54.5	2.16	23.6	4.78
Kat B1	2.51	55.1	2.21	52.6	7.11
Kat B9	2.28	59.9	2.72	49.9	6.67
LSD (0.05)	0.37	9.97	0.90	14.08	1.42

[a]Based on a scale of 1-9 (1 = immine and 9 = extremely susceptible).

Data are mean of three replications combined over three successive screenings.

Table 5.4 Correlations between morphological and resistance parameters under free choice conditions.

Parameter	IL	LA	PS	PM
Internode length (IL)				
Leaf area (LA)	-0.0036			
Pupae in stem (PS)	-0.7398*	-0.3762		
Plant mortality (PM)	-0.9202**	-0.0506	0.8146*	
Stem damage	-0.8705**	-0.2167	0.8942**	0.9757**

*, ** Significant at p ≤ 0.05 and 0.01 levels, respectively.

5.3.3 No-choice tests

Leaf feeding/oviposition punctures, number of pupae in stem, percent plant mortality and stem damage varied over the successive screenings among common bean genotypes. Because of the differences, there were significant interactions between genotypes and different screenings. For that reason, data were analyzed independently for each screening trial. Analysis of variance within each screening trial showed that genotypes differed significantly for all the traits measured (Table 5.5) (See also appendix 3b). Generally, when the bean fly was forced or restricted to feed and oviposit on a single bean genotype, the trend changed (Table 5.6). Some of the genotypes that appeared resistant under free choice conditions became vulnerable. A good example of such a genotype was GBK 047821 which consistently had higher leaf punctures, higher pupae in stem, a higher mortality and considerably high stem damage in all successive screenings. Ikinimba also exhibited a similar trend when compared to the local susceptible check varieties. Three genotypes, CC 888 (G15430), GBK 047858 and Macho (G22501), consistently showed resistance during successive screenings.

Table 5.5 Mean squares for resistance parameters under no-choice conditions.

		Observation 1			
Source	df	Leaf punctures	Pupae in stem	Plant mortality (%)	Stem damage
Genotype	6	772.59**	23.196**	2518.0**	21.667**
Error	14	40.92	1.618	210.1	1.048
		Observation 2			
Source	df	Leaf punctures	Pupae in stem	Plant mortality (%)	Stem damage
Genotype	6	218.08**	5.002**	1071.43**	10.111**
Error	14	52.19	0.774	99.21	1.000
		Observation 3			
Source	df	Leaf punctures	Pupae in stem	Plant mortality (%)	Stem damage
Genotype	6	123.97**	3.785**	1041.48**	9.714**
Error	14	23.37	0.375	45.20	0.571

** Significant at $p \leq 0.01$ level.

Table 5.6 Resistance parameters of various common bean genotypes against bean fly through three successive screenings under no-choice conditions.

Genotype	Feeding/oviposition punctures	Pupae in stem	Plant mortality (%)	Stem damage[a]
Observation 1				
CC 888 (G15430)	4.6	0.67	5.6	2.0
GBK 047821	48.4	7.53	53.0	7.3
GBK 047858	25.7	0.50	8.3	2.0
Ikinimba	41.1	6.07	76.9	8.3
Macho (G22501)	7.9	1.33	11.1	2.0
Kat B1	24.1	1.80	60.8	5.3
Kat B9	19.6	2.20	49.2	5.7
LSD (0.05)	11.2	2.23	25.4	1.79
Observation 2				
CC 888 (G15430)	0.1	0.27	2.8	2.0
GBK 047821	24.4	3.53	41.7	6.0
GBK 047858	4.4	2.00	5.6	3.3
Ikinimba	10.6	3.07	38.9	6.0
Macho (G22501)	0.9	0.07	2.8	2.0
Kat B1	2.7	1.77	36.1	5.3
Kat B9	3.8	1.80	38.9	5.7
LSD (0.05)	12.7	1.54	17.4	1.75
Observation 3				
CC 888 (G15430)	1.6	0.00	0.0	1.7
GBK 047821	19.9	3.40	36.1	5.7
GBK 047858	3.0	0.93	2.8	2.7
Ikinimba	5.3	1.67	22.7	4.3
Macho (G22501)	1.4	0.27	2.8	1.3
Kat B1	5.0	1.27	38.9	5.3
Kat B9	3.7	1.67	41.7	5.0
LSD (0.05)	8.5	1.07	11.7	1.32

[a]Based on a scale of 1-9 (1 = immune and 9 = extremely susceptible).

5.4. Discussion

The procedure for raising insect pest (bean fly) populations described in this study is simple, and inexpensive. It neither requires laboratory controlled conditions nor expensive facilities. However, the open beds where a susceptible variety is grown for trapping natural bean fly populations should be sheltered from excessive rainfall particularly for areas that receive optimal amounts of rainfall. Through this procedure up to 62% adult fly emergence was recorded showing that a sufficient number can be raised but this would depend much on the environmental conditions. The flies obtained retained their natural ability to attack beans and high infestation levels were achieved.

The tests made were useful in detecting differences among common bean genotypes. A physical barrier (internode length) was responsible in slowing down growth and development of bean fly larvae thereby resulting to low pupae numbers. This trait was expressed by all the resistant genotypes CC 888 (G15430), GBK 047821, GBK 047858, Ikinimba and Macho (G22501) (Tables 5.3 and 5.6). Further evidence was demonstrated through significant negative correlations between the morphological and resistance parameters. For example internode length was associated with pupae number in stem, plant mortality and stem damage. The resistant genotypes had a low number of pupae in stem, stem damage and percent plant mortality. Results obtained here partly confirm those obtained by Maerere and Karel (1984), who reported that low pupae counts were associated with thin stems and long internodes. The antixenosis present in these genotypes could have resulted from the fact that since the larvae transcend through the stem after emergence to settle at the root-stem junction, they could get exhausted along the way and thus fail to pupate. Legumes rely upon a set of defences to protect themselves against insect pests (Edwards and Singh, 2006). Plant structures can act directly on the herbivorous pests by discouraging the herbivore feeding (antixenosis). Tolerance to stem damage in beans is a mechanism of resistance to *O. phaseoli* (Maerere and Karel, 1984). Emitting of volatile compounds in reaction to damage caused by bean fly could be implicated in bean fly ovipositional non-preference (Wei et al., 2006).

The critical period of bean fly attack is the first four weeks after germination. Soon after germination the unifoliate leaf therefore becomes the site for first instar feeding (Karel, 1985) and plays the dominant role in determining the extent of bean fly damage to the plant. However, leaf area of the unifoliate leaves was not important due to its lack of correlation with resistance parameters. From the no-choice study it was evident that leaf/oviposition punctures were an important indicator of bean fly activity. Although oviposition occurs only in

some punctures, the number of punctures that contain eggs is not well known but the punctures primarily provide an indication of bean fly activity on the bean plants (Talekar and Hu, 1993). Clearly, the resistant genotypes (CC 888 (G15430), GBK 047858 and Macho (G22501) showed a low number of punctures compared to the other genotypes. Previous studies have demonstrated a positive association between the number of leaf punctures and the agromyzid infestation in mungbean (Talekar and Hu, 1993). However, physical damage inflicted by bean fly through ovipositional/feeding punctures hardly affects plant growth and development.

The genotypes studied could be placed into two main resistance categories. The first group was composed of genotypes GBK 047821 and Ikinimba expressing only one mechanism of resistance, mainly antixenosis. The second category included genotypes that appeared to posses both antixenosis and antibiosis mechanisms of resistance and these were CC 888 (G15430), GBK 047858 and Macho (G22501). Cardona and Kornegay (1999) stated that the mechanisms of resistance to insects in common bean can be divided into antibiosis and antixenosis. Genotypes exhibiting both mechanisms are good candidates for resistance breeding. Reports from previous studies have suggested that resistance breeding should aim at developing cultivars with more than one resistance mechanism so that the resistance will be durable (Clement et al., 1994).

5.5. Conclusions

One of the major challenges in breeding for resistance against bean fly in common bean has been the lack of a comprehensive procedure that could enable identification and differentiation of resistant lines provided by a uniform insect pest attack (Hillocks et al., 2006). From this study, a simple procedure for generating optimal populations of adult bean flies that could provide a uniform insect pest pressure during screening in net cages was described.

The results confirmed that both antixenosis and antibiosis mechanisms of resistance against bean fly exist in common bean, although some bean genotypes, mainly GBK 047821 and Ikinimba, seemed to have only one mechanism. Long internode was a physical barrier accountable for interfering with larval growth and the development thereby resulting in low pupation rate. This was also manifested in reduced damage to stems and a high plant survival (reduced plant mortality). Genotypes, CC 888 (G15430), GBK 047858 and Macho

(G22501) expressed both tolerance and antibiotic factors responsible for reduced fecundity of the insect pest.

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Chapter 6

General overview

Host-plant resistance is one of the key strategies employed as a component of integrated pest management. However, limited access to germplasm resources, lack of a proper screening procedure and lack of adequate information on gene action has made breeding for resistance against bean fly difficult. This research was therefore undertaken with a view to generate information that would be useful for not only the enhancement of host-plant resistance of common bean to bean fly, but also for the improvement of other important agronomic traits, including yield. The study was divided into three main parts. The first part was a participatory plant breeding study conducted in two major bean growing agro-ecologies of the semi-arid areas, mainly dry transitional and dry mid-altitudes, in order to validate farmers' perceptions of bean varieties and pests. The second part was concerned with screening of germplasm for new sources of resistance. The last part involved a genetic study to determine the nature of gene action controlling the inheritance of resistance and a study to establish the mechanisms of resistance present in common bean.

Participatory plant breeding was conducted at four sites covering two major districts (Machakos and Kitui) in semi-arid eastern Kenya where common bean is widely grown. The study was carried out with the aim of obtaining information on farmer perceptions of bean varieties, reasons for variety choice, farmers' knowledge of bean pests and other major production constraints. The key findings were as follows:

- Intercropping is a common practice by small-scale farmers in the semi-arid areas where 90% of the farmers indicated that they intercrop beans with maize. Intercropping was underscored by farmers as a practice employed to mitigate pest problems.

- Drought tolerance, high yield, early maturity and insect pest resistance were the most important criteria for variety preference.

- Adoption of improved varieties was high with only less than 10% of the farmers growing local varieties. However, the choice of variety grown varied with location and depended on specific needs of the farmers. The drought tolerant varieties released from KARI Katumani (Kat B1, Kat x 56 and Kat x 69) were more preferred in drier areas (dry-mid altitude) while the KARI Thika varieties (GLP 2, GLP 24, GLP 1004, GLP x 92 and GLP 585) released specifically for wetter areas, were preferred for areas that receive slightly more rainfall (dry transitional agro-ecology).

- Self-sufficiency in beans is still to be realized with only 23% of the farmers in dry transitional agro-ecology indicating they were self-sufficient, while only 18% of the farmers in dry mid-altitude were self sufficient.

- Drought, insect pests, crop diseases, lack of certified seeds and low soil fertility were mentioned as the major constraints to bean production in the semi-arid areas.

- According to farmers, crop loss incurred by them due to insect pest attack ranges from 12-67% in dry mid agro-ecology and 10-50% in dry transitional agro-ecology.

- Bean fly (*Ophiomyia* spp.), African bollworm (*Helicoverpa armigera*), bean aphid (*Aphis fabae*) were the main pests of common bean according the farmers.

Common bean genotypes were screened for resistance to bean fly at Kenya Agricultural Research Institute (KARI), Kiboko sub-centre, Kenya for three cropping season. The objective was to identify lines tolerant/resistant to bean fly but adapted to semi-arid conditions. To accomplish this, 64 genotypes were subjected to natural bean fly populations under drought stressed and non-stressed conditions. The control was sprayed with chemical pesticides and also subjected to drought stressed and non-stressed conditions. The research highlights were as follows:

- Genotypes differed in their reaction to bean fly under drought stressed conditions. The significant genotype x environment interactions for seed yield indicted that genotypic performance would depend on insecticide applied, drought stress as well as seasonal variation.

- Genotypes GBK 047810, GBK 047866 and GBK G21212, GBK 047826, GBK 047818, G21212 and GBK 047880 performed well under drought stressed during the long rains, showing specific adaptability. Similarly, GBK 047815, GBK 047858 GBK 047812, CIM 9314-36 and GBK 047803 were associated with short rains. Ikinimba, GBK 047821, GBK 036488 were broadly adapted due to their stable performance in both cropping seasons.

- Seed yield loss due to drought ranged from 3%-69% during the short rains (SR) and from 6%-65% during the long rains (LR). Application of insecticide resulted in yield improvement.

- Regression analysis of number of bean fly pupae in the stem on plant mortality under DS and NS environments revealed a significant positive relationship.

Genetic analysis of common bean genotypes for resistance to bean fly was conducted in order to determine the nature of gene action controlling the inheritance. The study revealed that:

- General combining ability was more important than the specific combining ability for stem damage, plant mortality, pupae in the stem and seed yield based on Griffings' model 1 (fixed effects) and method 2 diallel analysis (Griffing, 1956) involving F_2s and parents. The high values for general predictability ratios ranging from 0.69 for pupae in stem in LR 2009 to 0.90 for seed yield during LR 2009 further confirmed the superiority of additive gene effects over the non-additive effects.
- Two genotypes GBK 047858 and Kat x 69 had good general combining ability for traits that are important for bean fly resistance i.e. stem damage, plant mortality and pupae in stem. These genotypes were also good general combiners for seed yield.
- Low to moderate narrow heritability estimates of 0.22-0.45 obtained for pupae in the stem indicated that apart from additive gene effects, non-additive gene effects and environmental effects played a role in the expression of this trait.

Screening for resistance to bean fly under controlled condition would require a reliable supply of artificially reared insects. A simple procedure for screening for resistance to bean fly under free-choice and no-choice tests were carried out on five resistant/tolerant common bean genotypes and two local checks to establish the mechanisms responsible for resistance to bean fly. The major findings reported were as follows:

- In the current study, a technique that neither requires expertise nor expensive laboratory equipment was described. Through this method, over 60% emergence of adult flies was achieved.
- Both antibiosis and antixenosis mechanisms of resistance are available in common bean. However, some genotypes for example Ikinimba and GBK 047821 appeared to have only antixenosis component of resistance operating, while others such as CC 888 (G15430), GBK 047858 and Macho (G22501) expressed both antixenosis and antibiosis.
- Long internode was a morphological trait responsible for interfering with larval growth and the development thereby resulting in low pupation rate. This was manifested in reduced damage to stems and a high plant survival (reduced plant mortality).

Breeding implications and future research needs

Participatory plant breeding revealed that farmers are aware of major insect pests of beans, particularly the bean fly and have already put in place traditional control measures. Therefore, when choosing their varieties for planting, insect pest resistance is one of the main factors considered. The key implication to breeding is that even though good genetic control for important traits such as yield, quality and resistance to important biotic and abiotic constraints, found within the bean gene pools (Andean and Mesoamerican races) should be exploited, local adaptation is an important consideration for choice of parental materials. This is because farmer fields provide a wide range of environmental conditions for testing the breeding materials which helps in avoidance of genotype x environment interactions. Therefore, varieties developed and tested widely under varied conditions such as those provided by small-scale famer fields would fit well in target agro-ecologies. Particularly, breeding for resistance to bean fly should take into account not only increased levels of resistance through recombination of lines with different resistance mechanisms and genepools, but also through the development of bean varieties that are adapted to intercropping systems. Even though the overall level of resistance could be low to moderate, it may offer some potential protection in traditional farming systems hence improve on food security. Consequently such varieties would be acceptable to the farmers. In addition, breeders in collaboration with entomologists should take advantage of integrated pest control practices that are already carried out by the farmers. Such traditional control measures include the use of resistant varieties in combination with good cultural practices, biological pest control, and the use of bio-pesticides which are sustainable and require little money and expertise.

Screening for new sources of resistance to bean fly from primary gene pools of common bean has been attempted in the past even though failures arose from lack of establishment of proper links between researchers identifying the resistance and breeders who would introduce resistance into commercial lines This study identified new sources of resistance from local landraces and also confirmed the resistance of lines earlier screened by CIAT and adapted to the semi-arid areas. These sources of resistance could be exploited in breeding programmes in the region for the development of bean fly resistant lines in order to minimize the yield losses arising from bean fly attack under drought. To enhance progress, elite x elite crosses should be attempted.

The genetic studies showed that additive gene effects played a major role in the inheritance of bean fly resistance and seed yield accumulation compared to non-additive gene action.

Besides, some bean lines (GBK 047858 and Kat x 69) expressed high general combining ability for resistance to bean fly and yield attributes. A high general combining ability effect is largely attributed to additive gene effects or additive x additive interactions which correspond to fixable genetic variance. The implication to breeding is that a selection procedure that could result in accumulation of positive attributes should be adopted. Therefore, the genetic potential of such lines could be exploited in bean improvement programmes for areas prone to bean fly attack to enhance production in target agro-ecologies.

One of the major impediments to breeding for resistance against bean fly in common bean has been the lack of a comprehensive procedure that could enable identification and differentiation of resistant lines provided by a uniform insect pest attack. From this study, a simple procedure for generating optimal populations of adult bean flies, that could provide a uniform insect pest pressure during screening in net cages, was described. The method described in this study should be employed in breeding programmes in the region aimed at developing bean fly resistant varieties. Genotypes having both antixenosis and antibiosis mechanisms of resistance especially those with long internodes could be a repository for useful genes for resistance breeding.

In summary, the deployment of insect resistance genes will require further characterization and genetic tagging either as qualitative or quantitative characters. Therefore, the deployment and adoption of marker-assisted selection in combination with conventional breeding for bean fly resistance would result in faster the progress. The potential for developing bean cultivars with high levels of resistance to bean fly appears achievable. Generally, one method of pest control may not provide a long term control because of variations arising from seasons, locations and crop management systems. An integrated approach is more sustainable and should involve multidisciplinary team of scientists, thus bringing together plant breeders and entomologists. However, the resistant genotypes developed need to be combined with high yield and consumer-preferred traits to better serve the farmers.

Appendices

Appendix 1

a) REML variance components analyses for five parameters for common bean genotypes grown under natural bean fly infestation combined over three cropping seasons (2008-2009)

i) Response variate: Seed yield

Fixed model:　　　　　Constant + Genotype + Environmentt + Year +
Genotype.Environment + Genotype.Year + Environment.Year + Genotype.
Environment.Year + lin_row + lin_col
Random model:　　　　row + col + row.col + rep

Estimated variance components

Random term	component	s.e.
row	1487.	1741.
col	9512.	4047.
rep	0.	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
row.col	Identity	Sigma2	79408.		6175.

Deviance: -2*Log-Likelihood

Deviance	d.f.
5009.98	378

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Genotype	134.94	63	2.14	<0.001
Environmentt	137.69	1	137.69	<0.001
Year	280.96	2	140.48	<0.001
Genotype.Environment	52.12	63	0.83	0.834
Genotype.Year	273.78	126	2.17	<0.001
Environment.Year	2.84	2	1.42	0.241
Genotype.Environment.Year	99.52	126	0.79	0.961
lin_row	4.01	1	4.01	0.045
lin_col	4.47	1	4.47	0.034

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
lin_col	4.47	1	4.47	0.034
lin_row	4.01	1	4.01	0.045
Genotype.Environment.Year	99.52	126	0.79	0.961

Standard errors of differences

Average:	176.6
Maximum:	189.3
Minimum:	164.2

Standard error of differences for same level of factor:

	Genotype	Environment
Average:	180.0	170.0
Maximum:	180.0	176.3
Minimum:	180.0	164.2

ii) Response variate: 100-seed weight

Fixed model: Constant + Genotype + Environment + Year +
Genotype.Environment + Genotype.Year + Environment.Year +
Genotype.Environment.Year + lin_row + lin_col

Random model: row + col + row.col + rep

Estimated variance components

Random term	component	s.e.
row	0.136	0.168
col	0.732	0.353
rep	0.094	0.525

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e
row.col	Identity	Sigma2	7.600		0.591

Deviance: -2*Log-Likelihood

Deviance	d.f.
1470.66	378

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Genotype	2959.88	63	46.98	<0.001
Environment	175.30	1	175.30	<0.001
Year	341.63	2	170.82	<0.001
Genotype.Environment	70.49	63	1.12	0.242
Genotype.Year	504.74	126	4.01	<0.001
Environment.Year	0.95	2	0.47	0.622
Genotype.Environment.Year	129.28	126	1.03	0.403
lin_row	6.59	1	6.59	0.010
lin_col	0.77	1	0.77	0.380

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
lin_col	0.77	1	0.77	0.380
lin_row	6.59	1	6.59	0.010
Genotype.Environment.Year	129.28	126	1.03	0.403

Standard errors of differences

Average: 1.767

Maximum: 1.975

Minimum: 1.606

Standard error of differences for same level of factor:

	Genotype	Environment
Average:	1.844	1.659
Maximum:	1.844	1.719
Minimum:	1.844	1.606

iii) Response variate: Days to maturity

Fixed model: Constant + Genotype + Environment + Year +

Genotype.Environment + Genotype.Year + Environment.Year +

Genotype.Environment.Year + lin_row + lin_col

Random model: row + col + row.col + rep

Number of units: 768

Estimated variance components

Random term	component	s.e.
row	0.65	0.60
col	0.66	0.71
rep	0.01	0.76

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
row.col	Identity	Sigma2	21.47		1.68

Deviance: -2*Log-Likelihood

Deviance	d.f.
1854.29	378

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Genotype	332.01	63	5.27	<0.001
Environment	86.59	1	86.59	<0.001
Year	2480.28	2	1240.14	<0.001
Genotype.Environment	22.41	63	0.36	1.000
Genotype.Year	197.79	126	1.57	<0.001
Environment.Year	15.65	2	7.82	<0.001
Genotype.Environment.Year	45.71	126	0.36	1.000
lin_row	0.49	1	0.49	0.484
lin_col	1.80	1	1.80	0.179

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
lin_col	1.80	1	1.80	0.179
lin_row	0.49	1	0.49	0.484
Genotype.Environment.Year	45.71	126	0.36	1.000

Standard errors of differences

Average:		2.833
Maximum:		3.003
Minimum:		2.692

Standard error of differences for same level of factor:

	Genotype	Environment
Average:	2.817	2.776
Maximum:	2.817	2.873
Minimum:	2.817	2.692

iv) Response variate: Plant mortality (%)

Fixed model: Constant + Genotype + Environmentt + Year +

Genotype.Environment + Genotype.Year + Environment.Year +

Genotype.Environment.Year + lin_row + lin_col

Random model: row + col + row.col + rep

Number of units: 768

Estimated variance components

Random term	component	s.e.
row	6.1	5.5
col	22.5	10.8
rep	1.5	13.7

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
row.col	Identity	Sigma2	217.9		17.0

Deviance: -2*Log-Likelihood

Deviance	d.f.
2756.03	378

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Genotype	94.48	63	1.50	0.006
Environment	113.70	1	113.70	<0.001
Year	70.99	2	35.50	<0.001
Genotype.Environment	65.34	63	1.04	0.396
Genotype.Year	182.85	126	1.45	<0.001
Environment.Year	2.05	2	1.03	0.358
Genotype.Envmt.Year	89.82	126	0.71	0.994
lin_row	2.27	1	2.27	0.132
lin_col	1.66	1	1.66	0.197

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
lin_col	1.66	1	1.66	0.197
lin_row	2.27	1	2.27	0.132
Genotype.Environment.Year	89.82	126	0.71	0.994

Standard errors of differences

Average: 9.422

Maximum: 10.39

Minimum: 8.624

Standard error of differences for same level of factor:

	Genotype	Environment
Average:	9.694	8.937
Maximum:	9.694	9.269
Minimum:	9.694	8.624

v) Response variate: Pupae in stem

Fixed model: Constant + Genotype + Environment + Year +

Genotype.Environment + Genotype.Year + Environment.Year +

Genotype.Environment.Year + lin_row + lin_col

Random model: row + col + row.col + rep

Number of units: 768

Estimated variance components

Random term	component	s.e.
row	0.0066	0.0087
col	0.0007	0.0095
rep	0.0000	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
row.col	Identity	Sigma2	0.370		0.0289

Deviance: -2*Log-Likelihood

Deviance	d.f.
291.45	378

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Genotype	153.99	63	2.44	<0.001
Environment	5.22	1	5.22	0.022
Year	165.45	2	82.73	<0.001
Genotype.Environment	123.34	63	1.96	<0.001
Genotype.Year	92.09	126	0.73	0.990
Envmt.Year	0.54	2	0.27	0.765
Genotype.Environment.Year	67.91	126	0.54	1.000
lin_row	0.00	1	0.00	0.977
lin_col	9.18	1	9.18	0.002

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
lin_col	9.18	1	9.18	0.002
lin_row	0.00	1	0.00	0.977
Genotype.Environment.Year	67.91	126	0.54	1.000

Standard errors of differences

Average:	0.3639
Maximum:	0.3812
Minimum:	0.3515

Standard error of differences for same level of factor:

	Genotype	Environment
Average:	0.3613	0.3592
Maximum:	0.3613	0.3706
Minimum:	0.3613	0.3515

b) REML variance components analyses for three parameters for common bean genotypes grown in two environments and two levels of insecticide application combined over two cropping seasons

i) Response variate: Seed Yield

Fixed model: Constant + Genotype + Insecticide + Year + Environment + Genotype.Insecticide + Genotype.Year + Insecticide.Year + Genotype.Environment + Insecticide.Environment + Year.Environment + Genotype.Insecticide.Year + Genotype.Insecticide.Environment + Genotype.Year.Environment + Insecticide.Year.Environment + lin_row + lin_col

Random model: row + col + row.col + rep

Estimated variance components

Random term	component	s.e.
row	6008.	3201.
col	4086.	2170.
rep	4677.	8851.

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
row.col	Identity	Sigma2	72110.		4521.

Deviance: -2*Log-Likelihood

Deviance	d.f.
7490.16	569

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Genotype	370.46	63	5.88	<0.001
Insecticide	361.39	1	361.39	<0.001
Year	807.66	1	807.66	<0.001
Environment	378.64	1	378.64	<0.001
Genotype.Insecticide	117.35	63	1.86	<0.001
Genotype.Year	174.02	63	2.76	<0.001
Insecticide.Year	4.98	1	4.98	0.026
Genotype.Environment	87.18	63	1.38	0.024
Insecticide.Environment	0.99	1	0.99	0.319
Year.Environment	7.51	1	7.51	0.006
Genotype.Insecticide.Year	95.39	63	1.51	0.005
Genotype.Insecticide.Environment	40.04	63	0.64	0.989
Genotype.Year.Environment	47.60	63	0.76	0.925
Insecticide.Year.Environment	0.00	1	0.00	0.965
lin_row	0.36	1	0.36	0.547
lin_col	0.03	1	0.03	0.857

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
lin_col	0.03	1	0.03	0.857
lin_row	0.36	1	0.36	0.547
Insecticide.Year.Environment	0.00	1	0.00	0.965
Genotype.Year.Environment	47.60	63	0.76	0.925
Genotype.Insecticide.Environment	40.04	63	0.64	0.989
Genotype.Insecticide.Year	95.39	63	1.51	0.005

Standard errors of differences

Average:	187.7
Maximum:	253.5
Minimum:	135.6

Standard error of differences for same level of factor:

	Genotype	Insecticide
Average:	227.9	142.8
Maximum:	227.9	150.5
Minimum:	227.9	135.6

Average variance of differences:

51951.	20411.

ii) Response variate: 100-seed weight

Fixed model: Constant + Genotype + Insecticide + Year + Environment + Genotype.Insecticide + Genotype.Year + Insecticide.Year + Genotype.Environment + Insecticide.Environment + Year.Environment + Genotype.Insecticide.Year + Genotype.Insecticide.Environment + Genotype.Year.Environment + Insecticide.Year.Environment + lin_row + lin_col

Random model: row + col + row.col + rep

Estimated variance components

Random term	component	s.e.
row	0.109	0.095
col	0.205	0.133
rep	0.000	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
row.col	Identity	Sigma2	4.580		0.288

Deviance: -2*Log-Likelihood

Deviance	d.f.
1936.84	569

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Genotype	5504.96	63	87.38	<0.001
Insecticide	115.68	1	115.68	<0.001
Year	1194.35	1	1194.35	<0.001
Environment	522.47	1	522.47	<0.001
Genotype.Insecticide	41.45	63	0.66	0.984
Genotype.Year	376.64	63	5.98	<0.001
Insecticide.Year	3.56	1	3.56	0.059
Genotype.Environment	112.24	63	1.78	<0.001
Insecticide.Environment	0.59	1	0.59	0.441
Year.Environment	5.55	1	5.55	0.018
Genotype.Insecticide.Year	42.49	63	0.67	0.978
Genotype.Insecticide.Environment	22.59	63	0.36	1.000
Genotype.Year.Environment	61.68	63	0.98	0.523
Insecticide.Year.Environment	0.08	1	0.08	0.782
lin_row	0.33	1	0.33	0.568
lin_col	2.05	1	2.05	0.152

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
lin_col	2.05	1	2.05	0.152
lin_row	0.33	1	0.33	0.568
Insecticide.Year.Environment	0.08	1	0.08	0.782
Genotype.Year.Environment	61.68	63	0.98	0.523
Genotype.Insecticide.Environment	22.59	63	0.36	1.000
Genotype.Insecticide.Year	42.49	63	0.67	0.978

Standard errors of differences

Average:	1.203
Maximum:	1.352
Minimum:	1.079

Standard error of differences for same level of factor:

	Genotype	Insecticide
Average:	1.263	1.114
Maximum:	1.263	1.154
Minimum:	1.263	1.079

iii) Response variate: Days to maturity

Fixed model: Constant + Genotype + Insecticide + Year + Environment + Genotype.Insecticide + Genotype.Year + Insecticide.Year + Genotype.Environment + Insecticide.Environment + Year.Environment + Genotype.Insecticide.Year + Genotype.Insecticide.Environment + Genotype.Year.Environment + Insecticide.Year.Environment + lin_row + lin_col

Random model: row + col + row.col + rep

Estimated variance components

Random term	component	s.e.
row	0.000	bound
col	0.117	0.064
rep	0.000	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
row.col	Identity	Sigma2	2.087		0.129

Deviance: -2*Log-Likelihood

Deviance	d.f.
1482.66	569

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Genotype	4388.79	63	69.66	<0.001
Insecticide	1111.49	1	1111.49	<0.001
Year	4998.49	1	4998.49	<0.001
Environment	517.55	1	517.55	<0.001
Genotype.Insecticide	155.49	63	2.47	<0.001
Genotype.Year	1055.50	63	16.75	<0.001
Insecticide.Year	564.31	1	564.31	<0.001
Genotype.Environment	191.25	63	3.04	<0.001
Insecticide.Environment	1.91	1	1.91	0.167
Year.Environment	4.98	1	4.98	0.026
Genotype.Insecticide.Year	155.55	63	2.47	<0.001
Genotype.Insecticide.Environment	59.24	63	0.94	0.611
Genotype.Year.Environment	241.57	63	3.83	<0.001
Insecticide.Year.Environment	2.77	1	2.77	0.096
lin_row	6.32	1	6.32	0.012
lin_col	0.13	1	0.13	0.714

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
lin_col	0.13	1	0.13	0.714
lin_row	6.32	1	6.32	0.012
Insecticide.Year.Environment	2.77	1	2.77	0.096
Genotype.Year.Environment	241.57	63	3.83	<0.001
Genotype.Insecticide.Environment	59.24	63	0.94	0.611

Standard errors of differences

Average:	0.8030
Maximum:	0.9090
Minimum:	0.7224

Standard error of differences for same level of factor:

	Genotype	Insecticide
Average:	0.8658	0.7363
Maximum:	0.8658	0.7606
Minimum:	0.8658	0.7224

c) A simple regression analysis of number of bean fly pupae in stem as a function of percent plant mortality under DS and NS environments

Response variate: Plant mortaility

Fitted terms: Constant + Pupae in stem + Environmentt + pupae in stem.Environment

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	3	35921.	11973.5	67.91	<.001
Residual	380	67002.	176.3		
Total	383	102922.	268.7		
Change	-1	-11.	11.3	0.06	0.801

Percentage variance accounted for 34.5

Standard error of observations is estimated to be 13.3.

Estimates of parameters

Parameter	estimate	s.e.	t(380)	t pr.
Constant	32.14	3.07	10.46	<.001
Pupae in stem	5.41	1.72	3.15	0.002
Environment	-16.96	4.31	-3.93	<.001
Pupae in stem.Environment	-0.63	2.49	-0.25	0.801

Correlations between parameter estimates

Parameter		ref correlations			
Constant	1	1.000			
Pupae in stem	2	-0.950	1.000		
Environment	3	-0.712	0.677	1.000	
Pupae in stem.Environment	4	0.656	-0.691	-0.949	1.000
		1	2	3	4

Appendix 2

a) A rating scale for stem damage by bean fly

Damage description	% damage of phloem tissues by larvae before pupation/pupae	Rating
Immune	< 5	1
Highly resistant	5 -25	2
Resistant	26 - 35	3
Modorately resistant	36 - 45	4
Average/tolerant	46 - 55	5
susceptible	56 - 65	6
Modorately susceptible	66 - 75	7
Highly susceptible	76 - 85	8
Extremely susceptible	> 85	9

b) Generation mean and variance component analysis procedure for a five-parameter model based on P_1, P_2, F_2 and $F_{2:3}$

i) Generation mean analysis procedure

Generation means and their expectation based on additive dominance model in common bean for a cross GBK 047858 x Kat B9.

Generation	Mean	Variance	df	Var-mean	Wt	m	d	h	se
P1	0.95	0.997368	19	0.049868	20.05277	1	1	0	0.223312
F1	1	0.947368	19	0.047368	21.11111	1	0	1	0.217643
F2	1.99	2.757475	99	0.027575	36.26506	1	0	0.5	0.166056
F2:3	1.73	1.956667	99	0.019567	51.10733	1	0	0.25	0.139881
P2	3.05	0.997368	19	0.049868	20.05277	1	-1	0	0.223312

Weight (Wt) = family size (n_i)/Variance e.g. for P1 Wt = 20/0.997369 = 20.05277 and same applies for other families.

The five eqauations and their weights would be combined to give three equations mainly weighted least square estimates of the three parameters (*m*, *d*, and *h*). Each equation is multiplied through by m and by its weight and the generated columns of five etires for the three parameters and their totals are then summed.

m	d	h		
20.0528	20.0528	0.0000	=	19.0501
21.1111	0.0000	21.1111	=	21.1111
36.2651	0.0000	18.1325	=	72.1675
51.1073	0.0000	12.7768	=	88.4157
20.0528	-20.0528	0.0000	=	61.1609
148.5890	0.0000	52.0205	=	261.9053

The remaining two equations are worked out in the same way using the coeffients of *d* and *h* in turn and their weights as multipliers as given below.

m	d	h		
20.0528	20.0528	0.0000	=	19.05013193
0.0000	0.0000	0.0000	=	0.0000
0.0000	0.0000	0.0000	=	0.0000
0.0000	0.0000	0.0000	=	0.0000
-20.0528	20.0528	0.0000	=	-61.1609
0.0000	40.1055	0.0000	=	-42.1108

m	d	h		
0.0000	0.0000	0.0000	=	0
21.1111	0.0000	21.1111	=	21.1111
18.1325	0.0000	9.0663	=	36.0837
12.7768	0.0000	3.1942	=	22.1039
0.0000	0.0000	0.0000	=	0.0000
52.0205	0.0000	33.3716	=	79.2988

The equations then yiled three simultaneous equations which may be solved to give the estimates of m, d and h.

The approach used to solve the simultenous equations was by way of matrix inversions. The three equations written in the form given below.

$$\begin{bmatrix} 148.5890 & 0.0000 & 52.0205 \\ 0.0000 & 40.1055 & 0.0000 \\ 52.0205 & 0.0000 & 33.3716 \end{bmatrix} \quad \begin{matrix} m \\ d \\ h \end{matrix} \quad \begin{bmatrix} 261.9053 \\ -42.1108 \\ 79.2988 \end{bmatrix}$$

$$\mathbf{J} \qquad\qquad \mathbf{M} \qquad \mathbf{S}$$

Where **J** is the information matrix, **M** is the estimate of parameters and **S** is the matrix scores.

The solution takes the general form

$$\mathbf{M} = \mathbf{J^{-1}S}$$

A 3 x 3 matrix inversion procedure.

A11	40.1055	0.0000	1338.385	A21	0.000000	52.020475	0	A31	0.0000	52.0205	-2086.31
	0.0000	33.3716			0.000000	33.371585			40.1055	0.0000	
A12	0.0000	0.0000	0	A22	148.589042	52.020475	2252.522	A32	148.5890	52.0205	0
	52.0205	33.3716			52.020475	33.371585			0.0000	0.0000	
A13	0.0000	40.1055	-2086.309	A23	148.589042	0.000000	0	A33	148.5890	0.0000	5959.244
	52.0205	0.0000			52.020475	0.000000			0.0000	40.1055	

	1338.385	0	-2086.30928
Cofactor matrix			
C	0	2252.522	0
	-2086.31	0	5959.243903

Adj A=	transpose of C	1338.385	0	-2086.30928
		0	2252.522	0
		-2086.31	0	5959.243903

Also,   **IAI**   =

148.5890	0.0000	52.0205	=	a11*A11+a12*A12+a13*A13
0.0000	40.1055	0.0000	=	90338.61586
52.0205	0.0000	33.3716		

Therefore A^{-1}/**IAI** =

	1338.385	0	-2086.30928
1.10695	0	2252.522	0
	-2086.31	0	5959.243903

Plaese note that a computer could be used to carry out the matric inversion.

The inversion then leads to the following solution

$$
\begin{matrix} m \\ d \\ h \end{matrix} \quad = \quad
\begin{bmatrix} 0.014815 & 0.000000 & -0.023094 \\ 0.000000 & 0.024934 & 0.000000 \\ -0.023094 & 0.000000 & 0.065966 \end{bmatrix}
\quad = \quad
\begin{bmatrix} 261.905344 \\ -42.110818 \\ 79.298769 \end{bmatrix}
$$

The estimate of m is then given by

m = 0.014815 x 261.905344 + 0.0000 x -42.110818 - 0.023094 X79.298769   = 2.048831

The standard error of m = √0.014815 = 0.122

d = 0.000000 x 261.905344 + 0.024934 x -42.110818 + 0.000000 x 79.298769 = -1.05

The standard error of d = √0.024934= 0.158

h = -0.023094 x 261.905344 + 0.000000 x -42.110818 + 0.065966 x 79.298769 = -0.81753

The standard error of d = √0.065966= 0.257

Therefore:

m   =   2.048831   s.e. of m =   0.122
d   =   -1.05   s.e. of d   =   0.158
h   =   -0.81753   s.e. of h   =   0.257

Expected generation mean

P1= m+d   0.998831
F1 = m+h   1.231297
F2 = m+0.5h   1.640064
F2:3 = m+0.25h   1.844448
P2 = m-d   3.098831

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A χ^2 test for the the goodness of fit for the model

Generation	Wt	m	d	h	Observed mean	Expected mean		$\chi^2 = (\text{exp} - \text{obs})^2 * Wt$
P1	20.05277	1	1	0	0.95	0.999		0.047815
F1	21.11111	1	0	1	1	1.231		1.129413
F2	36.26506	1	0	0.5	1.99	1.640		4.440841
F2:3	51.10733	1	0	0.25	1.73	1.844		0.669416
P2	20.05277	1	-1	0	3.05	3.099		0.047815
							$\chi^2(2)$	6.3353 NS
							$P(0.05)$	5.99
							$P(0.01)$	9.21

NS = non-significant

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ii) Generation variance component analysis procedure

Expectaton of the within-family variances in the terms of the additive dominance genetic and the additive environmental components of the variation in common bean from a cross GBK 047858 x Kat B9.

Generation	Within-family Variance	Expectation		
		V_e	V^*_a	V^*_d
P1	0.997368	1	0	0
P2	0.997368	1	0	0
F1	0.947368	1	0	0
F2	2.757475	1	0.5	0.25
F2:3	1.956667	1	0.25	0.125

The data from the table above is keyed into the computer and the least square estimates obtained using a multiple regression model fitted. The response variate being the with with-family variances while the fitted terms being the V_a and V_d. But since the generations used here were five generations (P_1, P_2, F_1, F_2 and $_{F2:3}$), V_d could not be included in the model because it is aliased to V_a.

Regression analysis output from the model fitting

Response variate: Variance

Fitted terms: Constant, V_a

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	2.588164	2.588164	1046.55	<.001
Residual	3	0.007419	0.002473		
Total	4	2.595583	0.648896		

Percentage variance accounted for 99.6

Standard error of observations is estimated to be 0.0497.

Estimates of parameters

Parameter	estimate	s.e.	t(3)	t pr.
Constant	0.9916	0.0278	35.67	<.001
V_a	1.7987	0.0556	32.35	<.001

Note: The estimate of the constant from the regression gives us the environmental component of the variation denoted as V_e whiel the estimate of V_a gives the additive genetic variance.

c) REML variance components analyses for four parameters for the diallel experiment for long rains 2009

i) Response variate: Stem damage_rating

Fixed model: Constant + Trt_ID + lin_row + lin_col

Random model: Row + Col + Row.Col

Estimated variance components

Random term	component	s.e.
Row	0.361	0.339
Col	0.000	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Row.Col	Identity	Sigma2	1.252		0.338

Deviance: -2*Log-Likelihood

Deviance	d.f.
83.68	31

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	68.44	35	1.97	26.3	0.038
lin_row	1.33	1	1.33	8.3	0.281
lin_col	8.21	1	8.21	27.4	0.008

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	59.60	35	1.71	26.3	0.078
lin_row	1.33	1	1.33	8.3	0.281
lin_col	8.21	1	8.21	27.4	0.008

Standard errors of differences

Average:	1.186
Maximum:	1.242
Minimum:	1.119

ii) Response variate: Pupae in stem

Fixed model: Constant + Trt_ID + lin_row + lin_col

Random model: Row + Col + Row.Col

Estimated variance components

Random term	component	s.e.
Row	0.00202	0.00694
Col	0.00000	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Row.Col	Identity	Sigma2	0.0477		0.01286

Deviance: -2*Log-Likelihood

Deviance	d.f.
-31.98	31

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	70.84	35	2.05	21.5	0.041
lin_row	1.48	1	1.48	9.0	0.255
lin_col	32.37	1	32.37	27.6	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	68.90	35	2.00	21.5	0.047
lin_row	1.48	1	1.48	9.0	0.255
lin_col	32.37	1	32.37	27.6	<0.001

Standard errors of differences

Average:	0.2247
Maximum:	0.2410
Minimum:	0.2184

iii) Response variate: Plant mortality (%)

Fixed model: Constant + Trt_ID + lin_row + lin_col

Random model: Row + Col + Row.Col

Estimated variance components

Random term	component	s.e.
Row	70.8	56.3
Col	49.6	46.7

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Row.Col	Identity	Sigma2	138.9		43.0

Deviance: -2*Log-Likelihood

Deviance	d.f.
252.13	31

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	77.69	35	2.25	20.3	0.028
lin_row	0.04	1	0.04	7.5	0.845
lin_col	2.97	1	2.97	4.3	0.155

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	77.81	35	2.25	20.3	0.028
lin_row	0.04	1	0.04	7.5	0.845
lin_col	2.97	1	2.97	4.3	0.155

Standard errors of differences

Average: 13.19

Maximum: 13.86

Minimum: 12.19

iv) Response variate: Seed yield

Fixed model: Constant + Trt_ID + lin_row + lin_col

Random model: Row + Col + Row.Col

Number of units: 72

Estimated variance components

Random term	component	s.e.
Row	2788.	2854.
Col	0.	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Row.Col	Identity	Sigma2	11233.		3043.

Deviance: -2*Log-Likelihood

Deviance	d.f.
392.58	31

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	143.30	35	4.12	25.7	<0.001
lin_row	1.22	1	1.22	8.2	0.301
lin_col	20.05	1	20.05	27.3	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	118.73	35	3.42	25.7	<0.001
lin_row	1.22	1	1.22	8.2	0.301
lin_col	20.05	1	20.05	27.3	<0.001

Standard errors of differences

Average: 112.0

Maximum: 117.6

Minimum: 106.0

d) REML variance components analyses for four parameters for the diallel experiment for short rains 2009/10

i) Response variate: Stem damage rating

Fixed model: Constant + Trt_ID + lin_row + lin_col

Random model: Row + Col + Row.Col

Number of units: 72

Estimated variance components

Random term	component	s.e.
Row	0.172	0.318
Col	0.000	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Row.Col	Identity	Sigma2	1.636		0.437

Deviance: -2*Log-Likelihood

Deviance	d.f.
89.32	31

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	157.62	35	4.56	22.5	<0.001
lin_row	1.46	1	1.46	3.0	0.314
lin_col	0.23	1	0.23	28.1	0.632

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	140.31	35	4.06	22.5	<0.001
lin_row	1.46	1	1.46	3.0	0.314
lin_col	0.23	1	0.23	28.1	0.632

Standard errors of differences

Average: 1.334

Maximum: 1.430

Minimum: 1.279

Average variance of differences: 1.780

ii) Response variate: Pupae in stem

Fixed model: Constant + Trt_ID + lin_row + lin_col

Random model: Row + Col + Row.Col

Estimated variance components

Random term	component	s.e.
Row	0.00157	0.00671
Col	0.01927	0.01625

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Row.Col	Identity	Sigma2	0.0422		0.01310

Deviance: -2*Log-Likelihood

Deviance	d.f.
-28.64	31

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	89.07	35	2.63	14.7	0.025
lin_row	5.97	1	5.97	2.3	0.118
lin_col	0.46	1	0.46	7.2	0.519

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	89.82	35	2.65	14.7	0.024
lin_row	5.97	1	5.97	2.3	0.118
lin_col	0.46	1	0.46	7.2	0.519

Standard errors of differences

Average: 0.2219

Maximum: 0.2319

Minimum: 0.2071

iii) Response variate: Plant mortality (%)

Fixed model: Constant + Trt_ID + lin_row + lin_col

Random model: Row + Col + Row.Col

Number of units: 72

Estimated variance components

Random term	component	s.e.
Row	0.0	bound
Col	0.0	bound

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Row.Col	Identity	Sigma2	114.4		27.8

Deviance: -2*Log-Likelihood

Deviance	d.f.
231.39	31

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	223.86	35	6.40	34.0	<0.001
lin_row	0.68	1	0.68	34.0	0.417
lin_col	0.27	1	0.27	34.0	0.610

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	219.59	35	6.27	34.0	<0.001
lin_row	0.68	1	0.68	34.0	0.417
lin_col	0.27	1	0.27	34.0	0.610

Standard errors of differences

Average: 10.87

Maximum: 11.77

Minimum: 10.70

iv) Response variate: Seed yield

Fixed model: Constant + Trt_ID + lin_col

Random model: Row.Col

Number of units: 72

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Row.Col	Identity	Sigma2	54382.	13000.	

Deviance: -2*Log-Likelihood

Deviance	d.f.
447.05	34

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	295.85	35	8.45	35.0	<0.001
lin_col	0.15	1	0.15	35.0	0.705

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Trt_ID	274.17	35	7.83	35.0	<0.001
lin_col	0.15	1	0.15	35.0	0.705

Standard errors of differences

Average: 236.6

Maximum: 256.3

Minimum: 233.2

e) Diallel analyses: Griffing's method 2; model 1 for four parameters during long rains 2009

i) Variate: Stem damage

Source	d.f.	s.s.	m.s	v.r	F tab (α=0.05)	F tab(α=0.01)
gca	7	14.88722215	2.126746021	3.397357862	2.323	3.281
sca	28	26.36185016	0.941494648	1.503985061	1.842	2.386
error	31		0.626			

ii) Variate: Pupae in stem

Source	d.f.	s.s.	m.s.	v.r	F tab (α=0.05)	F tab(α=0.01)
gca	7	0.384814574	0.054973511	2.304968994	2.323	3.281
sca	28	1.398748256	0.049955295	2.09456163	1.842	2.386
error	31		0.02385			

iii) Variate: Plant mortality (%)

Source	d.f.	s.s.	m.s.	v.r	F tab (α=0.05)	F tab(α=0.01)
gca	7	1291.523915	184.5034164	2.656636666	2.323	3.281
sca	28	6024.499382	215.1606922	3.098066123	1.842	2.386
error	31		69.45			

iv) Variate:Seed yield

Source	d.f.	s.s.	m.s.	v.r	F tab (α=0.05)	F tab(α=0.01)
gca	7	416877.222	59553.88886	10.60338091	2.323	3.281
sca	28	358632.888	12808.31743	2.280480269	1.842	2.386
error	31		5616.5			

f) Diallel analyses: Griffing's method 2; model 1 for four parameters during short rains 2009/10

i) Variate: Stem damage

Source	d.f.	s.s.	m.s.	v.r	F tab (α=0.05)	F tab(α=0.01)
gca	7	53.68534855	7.669335507	9.375715779	2.323	3.281
sca	28	83.70702242	2.989536515	3.654690116	1.842	2.386
error	31		0.818			

ii) Variate: Pupae in stem

Source	d.f.	s.s.	m.s.	v.r	F tab (α=0.05)	F tab(α=0.01)
gca	7	0.608642076	0.086948868	4.120799431	2.323	3.281
sca	28	1.609944483	0.057498017	2.725024514	1.842	2.386
error	31		0.0211			

iii) Variate: Plant mort (%)

Source	d.f.	s.s.	m.s.	v.r	F tab (α=0.05)	F tab(α=0.01)
gca	7	5648.65362	806.9505171	14.10752652	2.323	3.281
sca	28	7490.80628	267.5287957	4.677076848	1.842	2.386
error	31		57.2			

iv) Variate:Seed yield

Source	d.f.	s.s.	m.s.	v.r	F tab (α=0.05)	F tab(α=0.01)
gca	7	2148950.566	306992.938	11.29024081	2.323	3.281
sca	28	5769353.65	206048.3446	7.577814153	1.842	2.386
error	31		27191			

Appendix 3

a) Combined analyses of variance for six parameters in free-choice screening experiments for bean fly resistance repeated three times

i) Variate: Internode length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0062	0.0031	0.02	
Rep.*Units* stratum					
Genotype	6	3.6061	0.6010	4.09	0.003
Experiment	2	29.4714	14.7357	100.19	<.001
Genotype.Experiment	12	1.0102	0.0842	0.57	0.851
Residual	40	5.8832	0.1471		
Total	62	39.9771			

Standard errors of differences of means

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
s.e.d.	0.1808	0.1184	0.3131

Least significant differences of means (5% level)

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
l.s.d.	0.3654	0.2392	0.6329

ii) Variate: Stem diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.8636	1.4318	4.96	
Rep.*Units* stratum					
Genotype	6	1.4460	0.2410	0.84	0.550
Experiment	2	147.7908	73.8954	256.07	<.001
Genotype.Experiment	12	2.4153	0.2013	0.70	0.744
Residual	40	11.5429	0.2886		
Total	62	166.0585			

Standard errors of differences of means

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
s.e.d.	0.2532	0.1658	0.4386

Least significant differences of means (5% level)

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
l.s.d.	0.5118	0.3351	0.8865

iii) Variate: Leaf area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	732.4	366.2	3.34	
Rep.*Units* stratum					
Genotype	6	2925.6	487.6	4.45	0.002
Experiment	2	34794.8	17397.4	158.85	<.001
Genotype.Experiment	12	1648.5	137.4	1.25	0.283
Residual	40	4380.8	109.5		
Total	62	44482.1			

Standard errors of differences of means

Table	Genotype	Experiment	Genotype Expriment
rep.	9	21	3
d.f.	40	40	40
s.e.d.	4.93	3.23	8.54

Least significant differences of means (5% level)

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
l.s.d.	9.97	6.53	17.27

iv) Variate: Pupae in stem

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.4410	3.2205	3.58	
Rep.*Units* stratum					
Genotype	6	32.9505	5.4917	6.10	<.001
Experiment	2	7.8543	3.9271	4.36	0.019
Genotype.Experiment	12	8.4190	0.7016	0.78	0.668
Residual	40	36.0324	0.9008		
Total	62	91.6971			

Standard errors of differences of means

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
s.e.d.	0.447	0.293	0.775

Least significant differences of means (5% level)

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
l.s.d.	0.904	0.592	1.566

v) Variate: Plant mortality (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	694.3	347.1	1.59	
Rep.*Units* stratum					
Genotype	6	16922.5	2820.4	12.91	<.001
Experiment	2	3869.7	1934.8	8.86	<.001
Genotype.Experiment	12	3667.2	305.6	1.40	0.207
Residual	40	8738.9	218.5		
Total	62	33892.6			

Standard errors of differences of means

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
s.e.d.	6.97	4.56	12.07

Least significant differences of means (5% level)

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
l.s.d.	14.08	9.22	24.39

vi) Variate: Stem damage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	13.079	6.540	2.96	
Rep.*Units* stratum					
Genotype	6	140.857	23.476	10.64	<.001
Experiment	2	13.746	6.873	3.12	0.055
Genotype.Experiment	12	36.476	3.040	1.38	0.217
Residual	40	88.254	2.206		
Total	62	292.413			

Standard errors of differences of means

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
s.e.d.	0.700	0.458	1.213

Least significant differences of means (5% level)

Table	Genotype	Experiment	Genotype Experiment
rep.	9	21	3
d.f.	40	40	40
l.s.d.	1.415	0.926	2.451

b) Analyses of variance for four parameters in no-chioce screening experiments for bean fly resistance repeated three times

Experiment 1

i) Variate: Leaf punctures

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	4635.53	772.59	18.88	<.001
Residual	14	572.85	40.92		
Total	20	5208.38			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	5.22

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	11.20

ii) Variate: Plant mortality (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	15108.1	2518.0	11.99	<.001
Residual	14	2940.9	210.1		
Total	20	18049.0			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	11.83

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	25.38

iii) Variate: Pupae in stem

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	139.176	23.196	14.34	<.001
Residual	14	22.647	1.618		
Total	20	161.823			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	1.038

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	2.227

iv) Variate: Stem damage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	130.000	21.667	20.68	<.001
Residual	14	14.667	1.048		
Total	20	144.667			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	0.836

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	1.792

Experiment 2

i)   Variate: Leaf punctures

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	1308.48	218.08	4.18	0.013
Residual	14	730.62	52.19		
Total	20	2039.10			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	5.90

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	12.65

ii) Variate: plant mortality (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	6428.57	1071.43	10.80	<.001
Residual	14	1388.89	99.21		
Total	20	7817.46			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	8.13

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	17.44

iii) Variate: Pupa in stem

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	30.0124	5.0021	6.46	0.002
Residual	14	10.8333	0.7738		
Total	20	40.8457			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	0.718

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	1.540

iv) Variate: Stem damage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	60.667	10.111	10.11	<.001
Residual	14	14.000	1.000		
Total	20	74.667			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	0.816

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	1.751

Experiment 3

i) Variate: Leaf punctures

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	743.80	123.97	5.30	0.005
Residual	14	327.20	23.37		
Total	20	1071.00			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	3.95

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	8.47

ii) Variate: Plant mortality (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	6248.85	1041.48	23.04	<.001
Residual	14	632.84	45.20		
Total	20	6881.70			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	5.49

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	11.77

iii) Variate: Pupae in stem

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	22.7124	3.7854	10.09	<.001
Residual	14	5.2533	0.3752		
Total	20	27.9657			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	0.500

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	1.073

46.6

iv) Variate: Stem damage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	58.2857	9.7143	17.00	<.001
Residual	14	8.0000	0.5714		
Total	20	66.2857			

Standard errors of differences of means

Table	Genotype
rep.	3
d.f.	14
s.e.d.	0.617

Least significant differences of means (5% level)

Table	Genotype
rep.	3
d.f.	14
l.s.d.	1.324