OCCURRENCE OF COWPEA APHID-BORNE MOSAIC VIRUS AND PROSPECTS OF IMPROVING RESISTANCE IN LOCAL COWPEA LANDRACES IN UGANDA

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GENERAL ABSTRACT

Viral diseases are a major limiting factor to cowpea production in many countries of Africa. In Uganda, studies indicated that the cowpea aphid-borne mosaic virus (CABMV) is common and a potential threat to cowpea production in the region. There have been no efforts to develop cowpea cultivars with resistance to CABMV in Uganda. This work focused on the development of cultivars resistant to CABMV.

Production of cowpea in Uganda is constrained by several factors, including a lack of awareness of diseases among the majority of farmers. A participatory rural appraisal (PRA) was conducted to elicit farmers' indigenous knowledge of cowpea production and also to gain insight into their understanding of viral diseases affecting cowpea in Uganda. PRA tools such as group discussions, transect walks, problem listing and ranking were used to gather information. Insect pests, diseases, low yielding cultivars and the high cost of pesticides were perceived to be the most important production constraints. Farmers were not aware of the problem of virus diseases, but provided descriptive names of symptoms. Only three cowpea cultivars (Ebelat, Ecirikukwai and Blackcowpea) were produced in the area. Seed size and colour were seen as important traits in new varieties.

Information about the occurrence, distribution and identity of cowpea viruses is limited in Uganda. The objective of this study was to identify the important cowpea virus diseases occurring naturally in the major cowpea growing regions of Uganda. Surveys were conducted to determine the incidence and severity of virus symptoms in four districts (Soroti, Kumi, Pallisa and Tororo) in 2004 and 2005. The incidence ranged from 40.5 to 94.4% and severity ranged from 15.0 to 30.6% (for Kumi and Pallisa districts, respectively) during the 2004 surveys. In 2005, the incidence ranged from 55.9 to 85.4% and severity ranged from 4.7 to 14.5% (for Tororo and Soroti districts, respectively). The CABMV, cowpea mild mottle virus (CPMMV), cowpea severe mosaic virus (CPSMV) and cucumber mosaic virus (CMV) were serologically detected by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

Fifty four improved cowpea genotypes were screened for resistance to CABMV during the first season of 2004 at Serere Agricultural and Animal Production Research Institute in Uganda. Further screening was conducted in the second season of 2004 using 27 genotypes. The genotypes were planted in single rows between the rows of the

susceptible cultivar, Ebelat. This was to provide high pressure of aphid vector (*Aphis craccivora* Koch) and CABMV inoculum. In addition, the test genotypes were artificially inoculated with a CABMV extract on fully expanded primary leaves of fourteen day-old seedlings. The CABMV incidence and severity was assessed. Disease severity was assessed on a 0-60% visual estimation scale where 0 = with no symptoms and 60 = with severe symptoms. Serological analysis was conducted using DAS-ELISA. Five genotypes showed good levels of resistance to CABMV, namely MU-93, IT82D-889, IT82D-516-2, IT85F-2841 and SECOW-2W. These resistant lines were crossed with three susceptible local landraces, namely Ebelat, Ecirikukwai and Blackcowpea in a North Carolina II mating design.

The F_1, F_2 and BC_1F_1 populations and the parents were evaluated in the field to assess the response to CABMV and to study the inheritance of resistance to CABMV. The general combining ability (GCA) and specific combining ability (SCA) effects were significant, indicating that both additive and non-additive genetic factors are important in determining the control of CABMV in cowpea. The proportions (%) of the sum of squares for crosses attributable to GCA and SCA for CABMV severity were 51.4% for GCA due to females, 8.4% for GCA due to males and 40.2% for the SCA. The narrow-sense heritability estimates, obtained by regressing F_1 on mid-parents was 0.87 and 0.84, F_2 on F_1 progenies 0.49 and 0.48, and F_2 progenies on mid-parents 0.63 and 0.79, for AUDPC and final disease severity, respectively. Single gene conditioned resistance in seven populations, but resistance was quantitatively inherited and involved many genes in eight populations. Observation of transgressive segregation and moderate to high heritability suggests a quantitative mode of gene action and the importance of additive effects. The predominance of GCA variance, high heritability estimates and observation of transgressive segregation suggested that resistance could be improved by selection.

DECLARATION

The work presented in this thesis for the award of the Doctor of Philosophy is my original work and has not been submitted for a degree in any university.

Signed …… Date……

MARTIN ORAWU

This thesis has been submitted for examination with our approval as University of KwaZulu-Natal supervisors.

Signed …… Date……

Prof. Rob Melis

Signed …… Date……

Prof. Mark Laing

DEDICATION

To my wife Sarah, my children Patricia, Vivian and Paul, and my brother the late Dr. Samuel. K. Nabulere.

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ABBREVIATIONS

AUDPC	Area under disease progress curve
BC_1F_1	First backcross of F_1
bp	Base pairs
C	Centigrade
CABMV	Cowpea aphid-borne mosaic virus
CCMV	Cowpea chlorotic mottle virus
CMV	Cucumber mosaic virus
CPCMV	Cowpea chlorotic mosaic virus
CPMMV	Cowpea mild mottle virus
CPMV	Cowpea mosaic virus
CPSMV	Cowpea severe mosaic virus
CV	Coefficient of variation
d	Days
DAI	Days after inoculation
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
DF	Degree of freedom
DNA	Deoxyribose nucleic acid
dsDNA	Double single DNA
F_1	First filial generation
F_2	Second filial generation
FAO	Food and Agriculture Organisation
GCA	General combining ability
ha	Hectare
hr	Hour
ICIPE	International Centre for Integrated Pest Ecology
IgG-AP	Immunoglobulin-alkaline phosphatase
IITA	International Institute of Tropical Agriculture
KCl	Potassium chloride
KH_2PO_4	Monobasic potassium hydrogen phosphate
LSD	Least Significant Difference
min	Minutes
ml	Millilitre
mm	Millimetre

mm mo-1	Millimetre per month
mo	Months
NaCl	Sodium chloride
Na_2HPO_4	Dibasic sodium hydrogen phosphate
NaN_3	Sodium azide
nM	nanometer
RNA	Ribose nucleic acid
P	Probability
Pnpp	P-nitrophenyl phosphate
PRA	Participatory rural appraisal
PSB	Phosphate saline buffer
PSB-T	Phosphate saline buffer-Tween20
PVP	Polyrinylpyrrolidone
R	Resistant
R^2	Coefficient of multiple determinations
S	Susceptible
SAARI	Serere Agricultural and Animal Production Research Institute
SBMV	Southern bean mosaic virus
SCA	Specific combining ability
SE	Standard error
t	Tons
t y^{-1}	Tons per year
µl	Microlitre
wk	Week
X^2	Chi-square
y	Years

GENERAL INTRODUCTION

1 Origin of cowpea

Cowpea (*Vigna unguiculata* (L.) Walp.) is indigenous to Africa, with a centre of origin in the former Transvaal region (now Gauteng and Mpumalanga provinces) of South Africa (Cobley and Steele, 1975; Padulosi and Ng, 1997). Although some authors have suggested that the cowpea originated in Asia, much of the recently published evidence suggests that it originated in Africa (Rachie and Roberts, 1974; Ng and Marechal, 1985; Fery, 1990). Nevertheless, the centre of greatest diversity of cultivated cowpea is in the savannah regions of northern Guinea in West Africa (Ng, 1995). Ng and Marechal (1985) reported that germplasm accessions from Nigeria, Niger, Burkina Faso, and Ghana show greater diversity than accessions from East Africa. This supports the theory that West Africa was the primary centre of cowpea domestication (Ng and Padulosi, 1988; Fery, 1990; Ehlers and Hall, 1997). Southeast Asia appears to be a secondary centre of cowpea diversity since significant genetic variability occurs on the subcontinent (Pant *et al*., 1982; Baudoin and Marechal, 1985). The primary centre of diversity of the wild *Vigna* species is in Southern Africa and East Africa (Ng and Padulosi, 1988).

Ehlers and Hall (1997) suggested that *Vigna unguiculata* is thought to be the immediate progenitor of the cultivated cowpea. This, however, shows that natural hybrids between cultivated and wild cowpea species occur and form weedy populations in some parts of West Africa. Despite the numerous reports of introgression and extensive variation in morphological and phenological traits among cultivated cowpea accessions, genetic variability in the cultivated gene pool appears to be limited. In recent studies assessing the genetic variability based on isozymes (Panella and Gepts, 1992; Vaillancourt *et al*., 1993), seed storage protein diversity (Panella *et al*., 1993), and chloroplast DNA (Vaillancourt and Weeden, 1992), the cultivated cowpea has been shown to have a narrow genetic base.

2 Taxonomy of cowpea

The cowpea has several distinctive forms and close affinities to *Phaseolus* and *Dolichos*, and this has led to an increase in scientific and common names used for the crop. Botanists agree that the cultivated cowpea belongs to the botanical species, but there

has been debate on the classification and nomenclature of taxa at the intra-specific level. Most institutes, like the US Department of Agriculture, adopted the classification scheme by Verdcourt (1970) and subdivided the cultivated forms of *Vigna unguiculata* into three subspecies, namely cowpea, *Vigna unguiculata* subspecies *unguiculata* (formerly *Vigna sinensis* (L.) Savi ex Hassk.); catjang, *Vigna unguiculata* subspecies catjang (formerly *Vigna cylindrical* (L.) Skeels); and yardlong bean, *Vigna unguiculata* subspecies *sesquipendalis* (formerly *Vigna sesquipendalis* (L.) Fruw., and *Vigna sinensis* (L.) Savi ex Hassk. The wild forms were also subdivided into the subspecies *dekindtiana* and *mensis* (Verdcourt, 1970).

However, Fery (1990), Ehlers and Hall (1997) did not consider Verdcourt's three cultivated subspecies as being distinct, but considered the subspecies *unguiculata* and *sesquipendalis* as cultigroups of cowpea, recognised as *unguiculata.* This is the common form of biflora or catjang, characterised by small erect pods, found in Asia. The *Vigna sesquipendalis*, or yardlong bean, found in Asia has been characterised by its long pods, which are consumed as a green snap bean (formerly *Vigna sinensis* var. *textiles* A. Cheval). It is grown for its fibre in West Africa and has long peduncles (Baudoin and Marechal, 1985; Fery, 1990; Ehlers and Hall, 1997). Fery (1990) indicated that all subspecies of *Vigna unguiculata* cultigroups and subspecies of *Vigna dekindtiana* varieties are inter-fertile with the cultivated subspecies *unguiculata*. Furthermore, Fery (1980) argued that *Vigna unguiculata* has not been hybridised successfully with any other species, but Fatokun and Singh (1987) reported the successful inter-specific hybridisation of *Vigna pubescens* and *Vigna unguiculata*.

3 Cowpea production in the world and Africa

Cowpea is grown in more than 60 countries occupying most parts of Asia and Oceania, the Middle East, southern Europe, Africa, southern USA, Central and South America (Singh *et al*., 2003). World cowpea production is estimated at 3.6 million t from 11.3 million ha, and 80% of the world production comes from Africa (FAOSTAT, 2000; Singh *et al*., 2003). Nigeria accounts for 75% of production in Africa (FAOSTAT, 2000). In Uganda, mean yield of cowpea is less than 400 kg ha^{-1} (Sabiti *et al*., 1994) and it is estimated to be at 20,000 t y^{-1}, with northern and eastern regions accounting for most of the production in the country (FAO, 1997).

4 Significance of cowpea

Cowpea is high in protein and the essential amino acids, lysine and methionine, that are deficient in cereals (Singh *et al*., 2000). It therefore makes an important supplement to low protein cereal-based staple diets common in developing countries. This nutritious and balanced food ensures good health and enables the body to resist infectious diseases and slow down their development (Singh *et al*., 2000). It is a good source of dietary fibre and complex carbohydrates and is consumed in various forms as dry seeds, green pods and leaves (Muleba *et al*., 1997). The mature cowpea pods are harvested and the green, as well as the dry, haulms are fed to livestock, particularly in the dry season when animal feed is scarce (DeVries and Toenniessen, 2001; Singh *et al*., 2003). The nutritional quality and high consumption levels make cowpea an important food crop contributing to human nutrition, especially in Africa.

Cowpea is a drought tolerant crop, curbs soil erosion and fixes atmospheric nitrogen, while the decaying residues contribute to soil fertility in the tropics of Africa (Wrigley, 1981; Shetty *et al*., 1995; Singh *et al*., 2003). Like many other legumes, the nodule bacteria in the soils reduce the atmospheric nitrogen into compounds for assimilation by the cowpea plants (Mulongoy, 1985). Furthermore, the crop is tolerant of low soil fertility, due to its high rates of nitrogen fixation and effective symbiosis with mycorrhizae, which enable it to withstand acid and alkaline soil conditions (Kwapata and Hall, 1985; Elowad and Hall, 1987; Fery, 1990). Effective cowpea-rhizobium symbiosis fixes up to 150 Kg N^{-1} ha^{-1} and supplies 80-90% of the host plant nitrogen requirement (Mulongoy, 1985).

Cowpea is an important component of cropping systems in the tropics, particularly in sub-Saharan Africa (Olufajo and Singh, 2000). It is mainly grown in mixtures with other crops and a great diversity of crop mixtures has been reported (Perrin and Phillips, 1978; Henrient *et al*., 1997; Mortimore *et al*., 1997). The principal reasons why farmers intercrop cowpea are flexibility, profit maximisation, reduction in risks, soil conservation, weed control and nutritional advantages (Shetty *et al*., 1995). The demand for cowpea in urban settlements is increasing. This has led farmers to change from intercropping to sole cropping of cowpeas in order to increase total production of the crop. In such areas, some horse-drawn peanut seeders and cultivators have been modified for use with cowpeas (Thiaw *et al*., 1993). The International Institute of Tropical Agriculture (IITA) has made a concerted effort to improve cowpea varieties, as well as improve cropping systems to increase total productivity, with limited use of purchased inputs (Singh, 1993; Singh and Ajeigbe, 2000).

The crop has a considerable ability to adapt to high temperatures and drought compared to other crop species (Ehlers and Hall, 1997), and hence can produce significant dry grain yields of up to 1000 kg ha^{-1} with available moisture averaging at 181 mm of rainfall. Cowpea is a deep-rooted crop and can do well in a variety of soils, but it is commonly considered to do best in well-drained, sandy loam soils. However, breeding for resistance to drought has been successful and has focused on the levels of rooting or earliness that are optimal, depending upon the environment and genetic background. The development of short-cycle cowpeas is focused on the selection of strains with a shorter vegetative stage, through selecting for earlier flowering, rather than selection for a shorter reproductive stage, because the grain yield of cowpea is far more dependent upon the amount and activity of leaves present during the reproductive stage than during the vegetative stage (Turk and Hall, 1980).

5 Production constraints of cowpea

Despite its importance, cowpea farmers face several adverse factors in growing the crop and throughout the tropics, diseases and insect pests are major production constraints (Rusoke and Rubaihayo, 1994; Edema and Adipala, 1996; Omongo *et al*., 1998; Tarawali *et al*., 2000; Singh *et al*., 2003). Virus diseases, besides other biological agents such as insect pests, bacteria, fungi and nematodes, have long been associated with yield losses ranging from 10-100% in field grown cowpea crops (Shoyinka *et al*., 1997), depending on the virus-host vector relationships, as well as prevailing epidemiological factors. In Uganda, where the crop is intensely grown in the northern and eastern regions, cowpea viruses are becoming a major problem for cowpea production. It is estimated that up to 100% losses in grain yields can occur due to virus infections.

The major viruses affecting cowpea in Africa include cowpea chlorotic mottle virus (CCMV), cowpea aphid-borne mosaic virus (CABMV), cowpea mild mottle virus (CPMMV), southern bean mosaic virus (SBMV), cowpea mosaic virus (CPMV), cucumber mosaic virus (CMV), cowpea chlorotic mosaic virus (CPCMV) and cowpea severe mosaic virus (CPSMV) (Thottappilly and Rossel, 1985; Hampton *et al*., 1997). Other diseases such as anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magnus) Bri. & Car.), zonate leaf spot (*Ascochyta phaseolorum* Sacc.), white zonate leaf spot (*Dactuliophora tarri* Leakey), Fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum* (E.F. Sm.) W.C. Snyder & H.N. Hans.), foot rot (*Fusarium solani* (Mart.). Sacc.) rust

(*Uromyces phaseoli* (Pers.) Wint.), scab (*Sphaceloma* sp.), yellow blister (*Synchytrium dolichi* (Cooke) Gaum), gray leaf mold (*Cercospora canescens* Ellis & G. Martini), powdery mildew (*Erysiphe polygoni* DC.) bacterial blight (*Xanthomonas campestris* par. v*ignicola* and *Pseudomonas syringae*) are also very important in cowpea production (Emechebe, 1975; Singh and Allen, 1979; Edema *et al*., 1997; Emechebe and Florini, 1997; Wydra and Singh, 1998; Singh *et al*., 2003).

Insect pests represent the most serious constraint to cowpea production throughout Africa. In many areas, losses due to insect pests are so high that yields seldom rise above 100-150 kg ha^{-1} on farmers' fields (Rusoke and Rubaihayo, 1994; Sabiti *et al*., 1994; Kitch *et al*., 1997). Cowpea is attacked by several insect pests, but those of most economic importance include aphids (*Aphis craccivora* Koch), flower thrips (*Megalurothrips sjostedti* Trybom), pod borers (*Maruca vitrata* Geyer), a complex of pod-sucking bugs, especially (*Clavigralla* spp.), and storage bruchids (*Callosobruchus* spp.) (Edema and Adipala, 1996; Murdock *et al*., 1997; Omongo *et al*., 1997; IITA, 1998; Nampala *et al*., 1999; Karungi *et al*., 2000a,b; Singh *et al*., 2003).

Other factors contributing to low cowpea production in sub-Saharan Africa include parasitic weeds such as *Striga* spp., susceptible local cultivars, low plant population, poor agronomic practices and a lack of improved varieties (Sabiti *et al*., 1994; Lane *et al*., 1995).

6 Justification of the study

In Uganda, the cowpea improvement programme was initiated at Makerere University and started with the collection of local and exotic accessions, which were screened for yield potential (Rubaihayo *et al*., 1973). Although the promising selections were evaluated under different management practices for control of diseases and insect pests (Edema and Adipala, 1996; Karungi *et al*., 2000a, b), an attempt to improve resistance to viruses in the existing locally grown susceptible cowpea varieties has to date not been done in Uganda.

Among the viral diseases that affect cowpea crops, the important ones include CABMV, CPSMV, CPMV, CPCMV, CPMMV, CMV and SBMV. These are the most prevalent cowpea viruses in Africa (Singh and Allen, 1979; Rossel and Thottappilly, 1985; Hampton *et al*., 1997). However, CABMV of the potyvirus group is one of the important

viral pathogens of cowpea reported in major cowpea growing districts in Uganda (Edema *et al*., 1997). The CABMV is transmitted by aphids *(Aphis craccivora* Koch*)* in a non-persistent manner. This has been shown to cause a significant infection of severe mosaic and the diseased cowpea plants show variable amounts of dark green vein banding or interveinal chlorosis, plant stunting and leaf distortion (Rybicki and Pietersen, 1999).

The use of CABMV resistant cultivars has been cited as one of the major strategies, among an array of options, to increase cowpea yields (IITA, 1998). The potential success is premised on the availability of sources of resistance to CABMV and the incorporation of resistance into the local germplasm to develop resistant cultivars.

8 Objectives of the study

The main objective of this study was to improve resistance in local cowpea varieties to CABMV to be of great benefit to Ugandan farmers. Therefore, the specific objectives were to:

1) Determine the level of indigenous knowledge on cowpea virus diseases among local farmers in Uganda;
2) Identify the important viruses infecting cowpea in Uganda;
3) Identify new sources of resistance to CABMV for use in cowpea varietal improvement; and
4) Determine the inheritance of resistance to CABMV in cowpea.

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CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

In this chapter, the literature on cowpea is reviewed in ten sections. The sections cover cultivation and utilisation, marketing and trait preferences, reproduction, genetics, viruses, aphid vector, management practices, breeding for virus resistance, methods for detecting viruses and mating design scheme.

1.2 Cultivation and utilisation of cowpea in Africa

Cowpea has a long history of use in Africa as both an agronomic and horticultural crop. Many types of cowpea cultivars are grown on a large scale as a vegetable crop and for dry grains. The crop has long been popular in home gardens and is marketed in the form of young fresh leaves, green pods and dry grains. The cowpea has been reviewed by several authors based on the genetics, physiology, production and breeding strategies of cowpeas (Rachie and Roberts, 1974; Summerfield *et al*., 1974; Rachie and Silvestre, 1977; Wien and Summerfield, 1984; Steele *et al*., 1985; Summerfield *et al*., 1985; Singh *et al*., 1997).

Despite the limitation in genetic diversity among the cultivated species, cowpea still remains a widely grown legume in many regions of Africa. It is grown as a food crop and as a cash crop (Rachie and Roberts, 1974; Davis *et al*., 1991). It is one of the major grain legumes cultivated throughout the tropics of Africa (Singh and van Emden, 1979; Bressani, 1985; Rachie, 1985; Nkongolo, 2003). The grain and leaves are rich sources of high quality protein and vitamins, which provide an excellent supplement to the low protein staple cereal, root and tuber crops in many African countries (Bressani, 1985; Kitch *et al*., 1998). The daily diet of cowpea impacts positively on the health of people, as the bulk of the diet of rural and urban poor, especially in Africa, consists of low protein foods derived from cassava, yam, plantain, banana, millet, sorghum and maize (Singh *et al*., 2003).

1.3 Marketing and economics of cowpea

Cowpea is grown for home use as well as for sale in the market. It is important for breeding programmes to have knowledge of local consumer preferences for both uses of cowpea. Cowpea developed for the market should have attractive pods and seeds, which remain harvestable for an extended period of time (Fery, 1990). In the traditional cowpea growing countries of Africa, there is a well developed network of village buyers, who assemble small quantities of cowpea grains from farmers into bags. Merchants transport and store the bags, ready for export or processing. Understanding cowpea marketing within countries, and trade linkages across regions, helps the breeder to select for a wide range of characteristics, such as seed size, colour and taste, which are preferred by the consumers.

The increase in cowpea production is linked to the use of improved technologies, including high yielding varieties, improved crop protection and good production practices, which lead to greater profitability. However, the profitability may substantially decrease if hidden costs, such as opportunity costs of capital, health hazards and environmental costs are taken into consideration. Coulibaly and Lowenberg-DeBoer (2000) conducted impact assessment studies in Senegal, Cameroon and Mali and showed that research on cowpea reached a large number of people and a substantial economic benefit was generated. Through the adoption of technologies, with the integration of biological and social sciences in cowpea research, farmers may be able to help alleviate food insecurity and reduce poverty.

1.4 Reproduction of cowpea

The cowpea is a self-pollinated crop. It has a cleistogamous flower that exhibits synchronous pollen shedding and stigma receptivity (Singh and Rachie, 1985; Ehlers and Hall, 1997). The flowers are large, about 20 mm in length and width, and typically purple or white. The style and stigma are surrounded by anthers tightly enclosed in a straight keel. Anther dehiscence and pollination of a particular flower normally occurs in the early morning on the day the flower opens. The flowers open only once and remain open for several hours. The stigmas become receptive for about 12 hr before anther dehiscence, which is useful in making artificial hybrids. Ehlers and Hall (1997) reported that occasionally there could be significant, but low levels of out-crossing in breeding nurseries and seed production fields, due to visitation by large bees. The cowpea

inflorescence consists of 4 to 12 pairs of flowers, formed on the distal ends of 50 to 600 mm long peduncles arising from leaf axils (Summerfield *et al*., 1974; Ehlers and Hall, 1997). Floral buds complete their development in 1-2 wk and usually only the first two flower pairs develop into pods. After two to four pods are set, further development of other floral buds on the apex of the peduncle (raceme) is arrested, until the first set pods become mature. The length of peduncles typically doubles after anthesis (Ehlers and Hall, 1997).

The initiation of flowering ranges from 30 to 90 d after planting, and the attainment of the dry seed maturity stage ranges from 55 to 240 d after planting (Wien and Summerfield, 1984). Cowpea cultivars that flower early have a shorter or more concentrated flowering period than cultivars that flower late (Wien and Summerfield, 1984). Mak and Yap (1980) reported that early maturity is inherited quantitatively, and that early maturing is conditioned by two dominant genes. Zaveri *et al*. (1980) indicated that additive gene action is responsible for much of the genetic variation for earliness in crops.

Photoperiod and air temperature are the major regulatory factors in the reproductive ontogeny and most cowpea genotypes respond to photoperiod as a quantitative short-day plant, but some are insensitive to a wide range of photoperiods. Fery (1985) reported that photoperiodic response is conditioned by a pair of major genes and that short-day response is dominant over the photoperiod-insensitive response. Warmer temperatures generally hasten flowering in both photoperiod sensitive and insensitive genotypes (Fery, 1990). Very high temperatures can affect reproductive development and cause abortion of the floral buds and reduction in pod set (Patel and Hall, 1990; Ismail and Hall, 1998).

1.5 Genetics of cowpea

Cowpea (*Vigna unguiculata* (L.) Walp) is grown extensively in the tropics of Africa. The wild and weedy forms exist in many regions of Africa (Rawal, 1975). The wild types grow in the secondary forests and woodland savannahs of the humid and sub-humid regions of Africa (Rawal *et al*., 1976). The wild forms of plants, namely *Vigna unguiculata,* are perennial climbers with distinct characteristics such as large, aromatic flowers and black dehiscent pods (Rawal *et al*., 1976). The weedy types, belonging to annual creepers within the var. *dekindtiana*, are widely adapted to the lowlands of tropical Africa and they morphologically resemble the cultivated form of *Vigna unguiculata* in growth habits such

as being erect, semi-erect and climbing types (Rawal, 1975). The wild and weedy subspecies of cowpea (*Vigna unguiculata* subsp. *dekintiana*) hybridise easily with the cultivated forms and produce viable hybrids (Rawal, 1975; Ng, 1990). Rawal *et al*. (1976) established that the wild forms could only be used as male parents for crossing, as attempts to use these as female parents were not successful. However, in a recent study using the wild varieties as female parents, the hybridisation was successful when open flowers that failed to produce pollen were cross-pollinated (Aliboh *et al*., 1996). Aliboh *et al*. (1996) further indicated that the differences obtained as a result of crossing the two cowpea types could be due to the degree of sensitivity of the flowers to the disturbances of the flowers during the process of emasculation, and environmental conditions. The use of the embryo rescue technique has been used in interspecific hybridisation between cowpea (*Vigna unguiculata* (L.) Walp.) and a hairy wild relative (*Vigna pubescens*) to produce hybrid embryos, which would otherwise have failed (Fatokun and Singh, 1987). The wild types such as the *Vigna vexillata*, which provide sources of genes for resistance and are incompatible with the cultivated cowpea, can probably be manipulated by the embryo rescue technique for the successful transfer of desirable traits into the cultivated cowpea (Adetula *et al*., 2005).

The cowpea crop is highly self-pollinated under most environmental conditions (Williams and Chambliss, 1980; Saccardo *et al.,* 1992). The cowpea is diploid, with 2n = 2x = 22 chromosomes (Faris, 1964; Barone and Saccardo, 1990; Pignone *et al*., 1990; Saccardo *et al*., 1992). Barone and Saccardo (1990) described the karyotype of cowpea as being composed of one very long chromosome and one very short chromosome, with the remaining nine chromosomes being allocated to three size groups. Pignone *et al*. (1990) described 11 chromosome pairs falling into three size groups: five long, five medium and one short. Advanced cytogenetic techniques such as fluorescent staining of chromosomes and silver staining of nucleolar organising regions are being employed and promise to be useful for plant breeding programmes in the future (Galasso *et al*., 1997). Knowledge of genome organisation is important for understanding how genomes function and evolve, as this is likely to provide information that can be useful in plant breeding programmes involving hybridisation and genetic manipulation (Galasso *et al*., 1995). Cytogenetic studies indicate that in *Vigna* species, there is often resistance to diseases in wild ancestors and a possibility of transferring characters depends on the phylogenetic distances among the species (Ladeinde *et al*., 1980).

1.6 Cowpea viruses

In this study, the major focus is on CABMV; hence the rest of the viruses will not be reviewed in great detail. Symptoms of plant virus diseases have been recognised for many hundreds of years, although it has only recently become possible to identify and study the causal pathogens themselves. The most damaging diseases of cowpea crops are caused by viruses and they represent a very significant proportion of losses of the potential value of the entire crop in sub-Saharan Africa (Thottappilly and Rossel, 1992). Cowpea plants are often infected by more than one virus simultaneously with resultant yield losses (Thottappilly and Rossel, 1992). Of the 20 important viruses recorded on cowpea from different areas of the world, eight economically important viruses are reported in Africa (Thottappilly and Rossel, 1985; Mali and Thottappilly, 1986; Thottappilly and Rossel, 1992). The important viruses of cowpea include cowpea mosaic virus (CPMV), southern bean mosaic virus (SBMV), cucumber mosaic virus (CMV), cowpea aphid-borne mosaic virus (CABMV), cowpea severe mosaic virus (CPSMV), cowpea mild mottle virus (CPMMV) and cowpea chlorotic mottle virus (CPCMV) (Taiwo and Shoyinka, 1988; Thottappilly and Rossel, 1992).

In Uganda, information on virus diseases and their identity is still scanty and only limited studies on the diagnosis of a few viruses have been done. Studies on the occurrence of cowpea diseases in different seasons and cropping systems have shown that cowpea aphid-borne mosaic virus is a common disease in cowpea growing regions in Uganda (Edema *et al*., 1997).

1.6.1 Cowpea aphid-borne mosaic virus (CABMV)

The CABMV is a potyvirus, characterised by filamentous particles ranging from 727 to 765 nm in length and 11 nm wide (Lovisolo and Conti, 1966; Bock, 1973; Kaiser and Mossahebi, 1975; Behncken and Malveesky, 1977; Singh and Rachie, 1985; Rybicki and Pietersen, 1999; Bashir *et al*., 2002), with modal length ranging from 725 to 750 nm (Mali *et al*., 1988; Bashir and Hampton, 1995). The molecular weight of CABMV coat protein has been reported as being 29000, 31000 and 34000 KDa (Taiwo *et al*., 1982; Bashir *et al*., 2002). Similarly, Dijkstra *et al*. (1987) reported that the molecular mass of the protein of five isolates of blackeye mosaic virus including cowpea aphid-borne mosaic-Moroccan isolate is also found within the range of 28000-34000 KDa. The CABMV is one of the potyviruses, whose complete nucleotide sequence has yet to be determined, but the 3'-

terminal 1221 nucleotide of a Zimbabwe isolate of CABMV has been sequenced (Bashir *et al*., 2002), and the sequence comprises an open reading frame of 990 nucleotides and a 3' non-coding region of 231 nucleotides.

1.6.1.1 Epidemiology and transmission of CABMV

Plant viruses cannot penetrate the intact cuticle of a host plant unaided, but they can be transmitted from one plant to another with the help of a wound-causing agent, the vector, thereby aiding the viruses into the plant cells (Russell, 1978). The seed transmissibility of CABMV reflects its wide geographical distribution in the off-season crop (Rossel and Thottappilly, 1990). Weeds, volunteer crops and wild legumes may act as reservoirs of CABMV, and there is evidence that the use of irrigation in addition to perennially damp areas provides reservoirs for CABMV in the semi-arid savannah regions of Africa (Raheja and Leleji, 1974; Rossel, 1977). Infection from infected seeds plays a vital role in the disease initiation, and aphid infestations are secondary in the spread of the disease under field conditions (Bashir *et al*., 2002). In addition, the cultivation of susceptible cowpea cultivars is also an important factor that favours the spread of the virus diseases in the cowpea growing regions of Africa (Thottappilly, 1992). The CABMV was described by Lovisolo and Conti (1966) infecting cowpea crops under field conditions depending upon crop susceptibility, virus strain and environmental conditions (Bashir *et al*., 2002). The nature and severity of the symptoms induced by the virus vary with the cowpea genotype, virus strain and the time of infection (Rossel and Thottappilly, 1985). Symptoms of CABMV include severe mosaic, mottling, interveinal chlorosis, vein-banding, leaf distortion, blistering and stunting, with the severity dependent on host genotype and virus strain (Bock and Conti, 1974; Thottappilly and Rossel, 1992; Bashir *et al*., 2002).

In the inoculated plants of cowpea, the vein-banding strain of CABMV from Nigeria induces vein prominence, which follows on the next trifoliate leaf at least by characteristic vein-banding symptoms (Bashir *et al*., 2002). The infected plants are sometimes killed, and some may become severely infected with necrotis on the stem and leaves, resulting in extreme yield reduction (Fischer and Lockhart, 1976; Shoyinka *et al*., 1997). Mazyad *et al*. (1981) and Bashir and Hampton (1996a) observed varying expression of symptoms including severe leaf mosaic, leaf mottling, distortion of leaflets, vein-banding, interveinal chlorosis, blistering and stunting of leaves in field-grown cowpea plants. Generally, when the disease intensity progresses, leaf cupping occurs and later leaves become distorted

with necrotic lesions and the infected plants remain stunted and bushy with retardation or inhibition of flowering. Studies by Pappu *et al*. (1997) and Thottappilly and Rossel (1997) reported similar findings with other crops such as bambara-groundnut (*Vigna subterranean* (L.) Verdc) and sesame (*Sesamum indicum* L.), when inoculated with CABMV.

1.6.1.2 Variability of CABMV

The viral nucleic acid initiates infection and carries the genetic code of the virus, which gives the appropriate instructions to the host cell to replicate viral nucleic acid. In the virus, there can be a re-arrangement of equivalent genes between the genomes of different particle types, resulting in greater genetic variability of the virus (Russell, 1978). In addition, genetic variability can be increased by mutation (Russell, 1978). The potential genetic variability in the virus is of great importance to the plant breeder, because of the danger that new resistance-breaking virus strains may arise. The virus is known to exist in a number of genetically distinct strains:

- The European strain, which causes severe distorting mosaic symptoms in cowpea plants (Lovisolo and Conti, 1966);
- The African strain, which induces irregular angular broken mosaic symptoms, while the African mild strain induces very mild mottle with little or no effect on plant growth, and the African vein-banding strain, which induces broad dark-green and vein-banding symptoms (Bock, 1973);
- The Brazilian strain is a severe pathogen of peanut in Brazil and induces symptoms consisting of ringspots and blotches (Gillaspie *et al*., 2001);
- The Moroccan strain induces severe mosaic, necrosis and leaf deformation in cucurbits, and it has been found to be a distinct potyvirus species. This distinction was established after comparison of the coat protein to the other cucurbit potyviruses using serological techniques (Baum *et al*., 1979; Purcifull and Hiebert, 1979).

1.6.2 Other cowpea viruses

Cowpea severe mosaic virus (CPSMV) is one of the important pathogens of cowpea in the Caribbean region (Haque, 1979; Fulton and Allen, 1982). It is characterised by the expression of typical leaf symptoms consisting of severe green yellow mosaic, leaf puckering of varying degrees and severe reduction of leaf size (Pathmanathan *et al*., 1997; Umaharan *et al*., 1997a). Early infection in plants results in severely stunted plants and seed yield losses of up to 80% (Lima and Nelson, 1977). The virus is transmitted by the beetle (Fulton *et al.,* 1987), and successful control is dependent upon adoption of a regular programme of insecticide application (Umaharan *et al*., 1997a). However, this kind of control measure proves to be costly, cumbersome, and often impracticable, thus breeding for cultivar resistance to cowpea severe mosaic virus was suggested. It provides a better alternative for stabilising yields in cowpea because of reduced persistence of the virus, thus more farmers are involved in cowpea production with less dependence on pesticide usage (Umaharan *et al*., 1997b).

Cowpea mild mottle virus (CPMMV) was first reported as a minor virus in Ghana, but subsequently became important on crops such as soybean in Nigeria (Jeyanandarajah and Brunt, 1993). The virus has now been shown to have a very extensive geographical distribution and a wide natural host range. The virus is readily transmitted by sap in a semi-persistent or non-persistent manner by whiteflies depending on the virus isolate (Iwaki *et al*., 1982; Muniyappa and Reddy, 1983). Naturally infected cowpea plants may exhibit a mild systemic mottle, but plants are mostly symptomless (Thottappilly and Rossel, 1992). On artificial inoculation, infected cowpea plants develop necrotic lesions on primary and trifoliate leaves, and sometimes severe systemic chlorosis on trifoliate leaves may occur (Thottappilly and Rossel, 1992). However, the virus has been considered to be of little significance to cowpea because very few cowpea genotypes are susceptible to it (IITA, 1981).

The cucumber mosaic virus (CMV) causes systemic symptoms which are mild mottle, mosaic and leaf distortion, with characteristic ring-spots (Thottappilly and Rossel, 1992). The virus is sap-transmissible by aphids in a non-persistent manner, and has also been shown to be transmitted by seed at a rate of 15% in certain cowpea genotypes (IITA, 1982). Despite its common and widespread occurrence, either through the seed or by aphid transmission, it is considered as a mild cowpea pathogen, except in infection of sensitive genotypes or when combined with other viruses (Hampton *et al*., 1997).

Cowpea mosaic virus (CPMV) is one of the most commonly reported virus diseases of cowpea, in which it causes chlorotic spots with diffuse borders in primary leaves, and trifoliate leaves develop bright yellow, light green mosaic in younger leaves (Pouwels *et al*., 2002). The host range has been shown to be rather limited, and few hosts are known outside the leguminosae (Fulton *et al*., 1987). The control methods have been investigated and the most practical method is the use of resistant cultivars. Nagaraju and Keshavamurthi (1998) reported eight cowpea lines to be resistant to cowpea mosaic virus.

Cowpea chlorotic mottle virus (CPCMV) produces a T-strain that induces an extensive systemic chlorosis in cowpea, and continuous propagation of attenuated variant (CPCMV-M) induces mild green mottle symptoms (Kuhn and Wyatt, 1979; de Assis-Filho *et al*., 2002). Although the virus has been isolated from two weed species in Nigeria (Thottappilly *et al*., 1993), CPCMV infection of cowpea appears to be confined to North and South America (Hampton *et al*., 1997).

1.7 Effects and transmission by aphid vector

Aphis craccivora Koch has been reported to be the most efficient vector of CABMV in sub-Saharan Africa (Bock, 1973). Aphids cause direct damage to plants by sucking sap from the young terminal growth, but can be found infesting leaves, stem tissues, flowers and pods (Bata *et al*., 1987; Pathak, 1988; Schreiner, 2000). At high population levels of aphid infestation, plants of susceptible cultivars have reduced vigour, distorted leaves, and small, poorly nodulated root systems and in extreme cases, the susceptible plants are killed (Singh and Allen, 1980).

The CABMV is readily transmitted in cowpea by sap inoculation by the aphid (*Aphis craccivora* Koch) vector. The virus is reported to be transmitted by several aphid species such as *Aphis craccivora, Aphis gossypii, Aphis spiraecola, Aphis medicaginis, Aphis fabae, Aphis citricola, Aphis sesbanie, Marcrosiphum euphorbia, Myzus persicae, Rhopalosiphum maidis, Cerataphis palmae* and *Acyrthosiphon pisum* (Bock, 1973; Bock and Conti, 1974; Mazyad *et al*., 1981; Dijkstra *et al*., 1987; Mali *et al*., 1988; Thottappilly, 1992; Thottappilly and Rossel, 1992) in a stylet-borne non-persistent manner. The transmission level of CABMV in cowpea plants has been shown to vary when different aphid species are feeding on the same plant. For instance, when *Aphis craccivora* and

Aphis gossypi are interacting together on the cowpea plant, the transmission level has been reported to be 57%, and sometimes in the range of 64-71% (Bashir and Hampton, 1994). However, both colonising and non-colonising aphid species are important in the epidemiology of CABMV, but colonising aphid species are mainly responsible for the secondary spread of the virus (Bashir *et al*., 2002). The feeding behaviour and damage of *Aphis craccivora*, the most important species on the cowpea crop in Africa, is influenced by the cowpea cultivars, population size and environmental conditions (Bashir *et al*., 2002).

Studies have shown that two viral gene products determine aphid transmissibility. These are the coat protein and helper component protein (Pirone and Thornbury, 1983). Different hypotheses have been proposed to explain the mechanism of transmission by the aphid vectors: the stylet-borne hypothesis, where the virus particles get attached to the tip of the stylet of the mouthparts and the virions mechanically attach to or detach from the aphid's stylets (Martin *et al*., 1997). The second mechanism of transmission by the aphids is by the ingestion-egestion hypothesis, which suggests that aphids contribute more actively to the acquisition and release of the virus (Harris, 1977; Martin *et al*., 1997). Martin *et al*. (1997) further emphasised that virions are acquired when aphids ingest cell contents in the process of food selection and later inoculated during intracellular regurgitation on a healthy plant. Aphids moving to healthy plants after acquisition would release virions during regurgitation, which may be functional in removing blocking cell organelles (chloroplasts) from the food canal entrance, or injecting noxious plant components (Martin *et al*., 1997).

The cowpea aphid, a cosmopolitan, and a serious insect pest of cowpea, is widely distributed in the cowpea growing areas of the world (Singh and Rachie, 1985; Bata *et al*., 1987; Pathak, 1988). The aphid is a major pest of cowpea in Africa, Asia, Latin America and USA (Chalfant, 1985; Daoust *et al*., 1985; Singh, 1985; Singh and Jackai, 1985). In the tropics, the female aphids reproduce parthenogenetically and the eggs develop within the mother and nymphs are born live (Singh and Rachie, 1985; Schreiner, 2000). Within a few days, nymphs mature into reproductive adults and population density can increase very rapidly, and in the early stages of an infestation, adult aphids have no wings, but winged forms appear in subsequent generations and disperse to other plants. Madden *et al*. (2000) indicated that the aphid vector is said to be viruliferous from the time it acquires a virus until the virus is lost. Nault (1997) reaffirmed that as long as the aphid vector is feeding and moving among plants, the period of transmission of the virus can be within minutes.

1.8 Management practices for control of CABMV and aphid vector

The CABMV infection can be enhanced in the cowpea field in several ways: a) infestation by the aphid vector, b) planting of susceptible cowpea cultivars, and c) infected seeds. A range of control measures can be employed, such as the use of insecticides to control the vector at the right time, and breeding for host plant resistance to CABMV and its vector (Rossel and Thottappilly, 1985; Thottappilly and Rossel, 1992; Bashir and Hampton, 1996b; Bashir *et al*., 2002).

The vector can furthermore be controlled through cultural practices, which include early planting, close spacing and intercropping of cowpea with other component crops (Ogenga-Latigo *et al*., 1993; Kannaiyan and Haciwa, 1993; Edema *et al*., 1997; Karungi *et al*., 2000a, b). The production of virus-free seed is an additional measure for the control of the virus, particularly if certified seed is produced in areas where the virus is not known to occur. Field inspection and roguing of diseased plants may ultimately help to eliminate seed-borne inoculum. There is evidence that CABMV may occasionally be seed transmitted in symptomless plants (Aboul-Ata *et al*., 1982; Bashir and Hamptom, 1996a), and there is potential value in implementing a rapid indexing procedure for its detection in seed lots.

Seed certification is a quality assurance system, whereby the seed produced for marketing is subject to official control and inspection so as to provide a guarantee to the purchaser. In governing protocols, the seed is produced, multiplied and marketed according to predetermined standards and systems while maintaining the genetic integrity of the product. Seed certification ensures supply of high quality seed to farmers that are true to type, high in purity and germination capacity and free from pests and diseases. The seed certification programme should be started at the basic level of the germplasm collection available to the plant breeders and continue through the subsequent development of varieties. The monitoring of the presence of seed-borne viruses is conducted on actively growing crops. A seed certification programme for CABMV has been practiced at IITA and samples are examined both visually and with the use of ELISA to detect the presence of seed-transmitted viruses (Hamilton, 1983; Bashir *et al*., 2002).

1.9 Breeding for resistance to CABMV

The lack of immunity in plants due to weak defence mechanisms usually leads to invasion by the pathogens. Thus, the pathogen succeeds and invades the plant due to the possession of the virulent gene called Avr-gene that causes the pathogen to produce signals to trigger infection in the host lacking the corresponding R-gene for defence (Dangl and Jones, 2001). Most significantly, the function of R-gene is dependent on the genotype of the pathogen (Keen, 1990; De Wit, 1992; Crute and Pink, 1996; Dangl *et al*., 1996; Hammond-Kosack and Jones, 1996; Knogge, 1996).

Plant resistance is often correlated with the activation of specific defence responses to the pathogen and this results in the failure of the pathogen to cause infection in the host plant. In some instances, the pathogen may fail to establish itself in the host because of the following:

- either the plant becomes unable to support the niche requirements for a potential pathogen and is thus a non-host
- or the plant possesses preformed structural barriers or toxic compounds that confine successful infections to specialised pathogen species (Hammond-Kosack and Jones, 1996).

Upon recognition of the invading pathogen by the host plant, defence mechanisms are instituted and the invasion remains localised, but this may depend on induced responses in the host plant. However, incompatible responses are frequently associated with the appearance of necrotic flecks containing dead plant cells at the sites of the invading pathogen. The result of the hypersensitive response in the host cells can be phenotypically diverse, ranging from hypersensitive response in a single cell spreading necrotic areas accompanying limited pathogen colonisation (Agrios, 1988; Holub *et al*., 1994). In extreme hypersensitivity, virus multiplication is limited to the initially infected cells, because of an ineffective viral-coded movement protein (Matthews, 1992). Heath (1980) proposed that the hypersensitive response seems to play a causal role in disease resistance in plants attributed by the plant cell death, which deprives the pathogen of access to further nutrients (Hammond-Kosack and Jones, 1996).

Other features of plant mechanisms of resistance response include induced synthesis of antimicrobial metabolites, often referred to as phytoalexins, and synthesis of enzymes that are harmful to the pathogen, such as chitinases and glucanases. These are produced in the plant cell walls in response to the pathogen invasion in the infected

areas (Dixon and Lamb, 1990; Dixon *et al*., 1994). The responses due to chitinase expression or phytoalexin biosynthesis make incremental contributions that slow down pathogen development (Maher *et al*., 1994; Zhu *et al*., 1994).

The use of resistant varieties is a practical and inexpensive method of controlling both CABMV and its vector (Pathak, 1988). Identification of several resistant lines has been used in the breeding programmes to develop aphid and virus resistant varieties (Singh and Natare, 1985; Singh *et al*., 2003). Significant efforts have been made at the IITA to develop high-yielding cowpea varieties resistant to CABMV (Bata *et al*., 1987; Singh *et al*., 2003). Several cowpea breeding lines, including IT86D-880, IT82D-889, IT83S-818, IT86D-1010, IT96D-659, IT97K-1068-7 and IT95K-52-34, have been developed with multiple virus resistance (Singh *et al*., 1997; Singh and d'Hughes, 1999; Singh *et al*., 2003).

An efficient breeding programme for insect resistance requires not only the availability of sources of resistance, but also knowledge of inheritance and genetic control systems (Pathak, 1988). At the International Institute of Tropical Agriculture (IITA) and International Centre for Integrated Pest Ecology (ICIPE), crosses between resistant and susceptible varieties indicated that resistance to aphid is simply inherited, with resistance being the single dominant gene (Pathak, 1988). Similarly, inheritance of resistance to CABMV has been reported to be governed by a single dominant or recessive gene (Taiwo *et al*., 1981; Fisher and Kyle, 1994; Fisher and Kyle, 1996), sometimes in association with minor or modifier genes (Patel *et al*., 1982). Provvidenti *et al*. (1983) reported that resistance to CABMV in common bean (*Phaseolus vulgaris* (L.) was conferred independently by a single dominant gene.

A heritable tendency not to become infected when exposed to infection with a virus, to which it is susceptible, can be a useful characteristic in virus resistant varieties. This is because it significantly slows down the rate of development of an epidemic in the field. This type of resistance has been widely used in conjunction with other forms of resistance in breeding for resistance to viruses. Moreover this type of control measure does not displace other options, but is compatible with other management practices. In addition, resistance minimises dependence on pesticide usage and alleviates the negative effects on the environment. Bashir *et al*. (2002) described three types of resistance to CABMV:

- immunity, resistance involving rapid death of infected tissues (hypersensitivity),
- formation of chlorotic or necrotic local lesions at the inoculation site and

- virus tolerance.

Plants that are immune to a particular virus show no reaction whatsoever when inoculated with the virus, and the virus does not multiply and this type of resistance is considered to be the most common (Bashir and Hampton, 1996b). Resistance may also be expressed as the development of very mild mosaic, without adverse effects on plant growth, as well as by latent infection in which systemic infection occurs without the appearance of symptoms (Ladipo and Allen, 1979; Patel *et al*., 1982; Bashir and Hampton, 1996b).

Cowpea improvement programmes in Africa received a great deal of attention at IITA as a centre of training, germplasm collection, screening, improvement, maintenance and breeding for disease resistance (Singh and Rachie, 1985; Singh *et al*., 2003). Sources of resistance to CABMV have been identified among cowpea germplasm (Williams, 1977; Lapido and Allen, 1979; Mali *et al*., 1981; Patel *et al*., 1982; Taiwo *et al*., 1982; Kannaiyan *et al*., 1987). The work of Cisse *et al*. (1997) indicates that the late maturing cowpea line PI596353 was not only resistant to CABMV, but also to the aphid vector and other diseases. Bashir and Hampton (1996b) evaluated 51 cowpea lines by mechanical inoculation under greenhouse conditions against seven strains of CABMV isolates of geographically diverse origin and identified TVU-410, TVU-1582 and TVU-1593 being immune to all seven isolates. Similar findings were reported by Singh and d'Hughes (1999) on cowpea breeding lines, namely IT96D-659, IT96D-660, IT97K-1068-7 and IT95K-52-34, being completely resistant to several cowpea viruses including CABMV. Sources of CABMV resistance, including those with multiple resistance to several distinct viruses (Allen, 1983), have been widely utilised in cowpea breeding and elsewhere in Africa (Singh *et al*., 1987; Kannaiyan and Haciwa, 1993).

1.10 Methods for detecting viruses

Different strains of a virus can be isolated using a number of different methods based on comparison of the type and severity of symptoms on a range of test plants, either by serology, immuno-electrophoresis or by enzyme-linked immunosorbent sandwich assay (ELISA) tests. Immunosorbent assays for instance are widely applied in the detection of numerous plant viruses because of their sensitivity (Hampton, 1983).

Application of the diagnostic method of the polymerase chain reaction (PCR) provides the most sensitive method for detecting a number of plant viruses, including CABMV in

cowpea. The method utilises a pair of synthetic oligonucleotides or primers, each hybridising to one strand of double stranded DNA (dsDNA) target, with the pair spanning a region that reproduces exponentially (Mackay *et al*., 2002). The hybridisation primer is a substrate for a DNA polymerase, which creates a complementary strand via sequential addition of deoxyribonucleotides (Mackay *et al*., 2002). The PCR process occurs in three cycles: dsDNA separation at >90°C, primer annealing at 50-75°C and optimal extension at 75-78°C (Mackay *et al*., 2002). In comparison to ELISA, it is not laborious, and the method is very sensitive in detecting viruses even when the sample concentration is too dilute. It is up to 10^5 times more sensitive than the ELISA method such as direct antigen coating enzyme-linked immunosorbent assay (Gillaspie *et al*., 1999). However, the combinations of PCR and detection assays have been used to obtain quantitative data with promising results, but these approaches suffer from laborious post-PCR handling steps (Guatelli *et al*., 1989).

1.11 Mating design scheme

In developing crosses to determine gene action governing resistance in various crops to a particular virus, mating design schemes are adopted in breeding programmes. The mating designs are used to generate crosses in order to make measurements on the progeny and parents amenable to certain types of analyses. Breeders usually use simple and complex designs for generating crosses (Stuber, 1980). There are four types of simple designs namely hybrid cross, backcross, topcross and polycross. The complex designs include diallel (full and half diallel designs) and North Carolina. Complex designs are widely used to estimate genetic variances and to generate families or use in either full-sib or half-sib recurrent selection schemes (Stuber, 1980). Comstock and Robinson (1948, 1952) developed three designs known as North Carolina mating designs I, II and III. Each of them provides estimates for the two most important genetic parameters namely: additive genetic variance and variance due to dominance. Cockerham (1963) showed that North Carolina mating designs are meant to provide plant breeders with information regarding the traits being investigated for a reference population. This knowledge allows plant breeders to determine whether selection, aiming at cultivar development, can be feasible from the source population (Ortiz and Golmirzaie, 2002). Full and half-sib progenies are produced by attempting biparental matings in the F_2 generation of a cross between two pure lines (Singh and Chaudhary, 1985).

The mating design I is used to estimate additive and dominance variances and also to generate families for evaluation in full-sib or half-sib recurrent selection. The costly, time consuming effort required to produce sufficient seed for replicated trials by utilising drill-row plots, has essentially made impossible the use of this design and similar designs in self-pollinating species such as small grains and soybeans (Stuber, 1980). Furthermore, Ortiz and Golmirzaie (2002) pointed out that the very low precision of digenic variance by design I makes this mating scheme less acceptable than others, if this kind of genetic variation is important in the crop species for which inheritance is under investigation. The North Carolina mating design II is essentially a factorial mating design and is used to estimate genetic variances and to evaluate inbred lines for combining ability (Stuber, 1980; Ortiz and Golmirzaie, 2002). In this design, each member of a group of parents used as male is mated to each member of another group of parents used as female (Singh and Chaudhary, 1985). This design is well suited to multi-flowered plants because each plant can be used repeatedly as both male and female. In experimental designs, the progenies are normally blocked so that all of the families from the mating of a single group of males to a single group of females remain as an intact unit. The mating design III involves backcrossing of F_2 plants to the two inbred lines from which the F_2 was derived (Comstock and Robinson, 1948, 1952). In this design, the number of inbred plants crossed to each F_2 should be large enough to ensure sufficient seed for evaluations. However, design III is used rather infrequently and primarily to estimate the average dominance of genes (Stuber, 1980).

1.12 Summary

Cowpea is an important food legume crop in Africa and has the ability to grow under adverse weather conditions. Information on cowpea viruses, virus vectors and genetics has been documented. Eight cowpea viruses CCMV, CABMV, CPMMV, SBMV, CPMV, CMV, CPSMV and CPCMV have been documented as posing a threat to cowpea production in Africa. Of particular interest to this study is the occurrence of CABMV and inheritance of resistance in the susceptible cowpea cultivars in Uganda. Four strains of CABMV have been reported and they seem to cause variable infections in cowpea. The transmission of CABMV is mainly done by an aphid (*Aphis craccivora* Koch) vector. Several management practices have been employed to control CABMV including cultural practices, insecticides and seed certification. Breeding for resistance is generally preferred and is part of the focus in this study. The survey of literature in the subsequent chapter also identified some gaps in farmers' preferences, perceptions of cowpeas, and

identification of resistance sources and inheritance of resistance. These gaps are covered in this study but in different chapters (Chapters 2 through to 5).

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CHAPTER TWO

FARMERS' PERCEPTIONS OF COWPEA PRODUCTION AND CONSTRAINTS IN EASTERN UGANDA

Abstract

A participatory rural appraisal (PRA) was carried out to elicit farmers' perceptions about cowpea production and constraints in eastern Uganda. Four sub-counties, namely Malera, Bukedea, Kapir and Ngora, in Kumi district were selected for interviews with farmers. An open-ended discussion with a group of farmers, guided by a checklist and with direct participant observation, was undertaken to obtain detailed information during the PRA. The main points addressed were: major crops cultivated, cropping systems, cowpea landraces, cowpea production, cowpea marketing and constraints. The important crops grown by farmers were cassava, groundnuts, cowpea and sweet potatoes. The focus group methods allowed farmers to assess the major crops. They ranked cowpea as the third in importance after cassava and groundnuts. The three main local cowpea types mentioned by the farmers were Ebelat, Ecirikukwai and Blackcowpea. Farmers indicated that for market value, cowpea is selected for production according to market acceptance, early maturity, high yield, good palatability, and tolerance to diseases and insect pests. The major constraints mentioned by farmers were insect pests, high cost of pesticides, poor agronomic practices, diseases, poor storage, price fluctuation, drought and low yielding varieties. In this study, the farmers pointed out during the interviews that they were not aware of the diseases, but provided descriptive names of symptoms, such as stunted plants, leaf deformation, mosaic leaves, yellowing of leaves, leaf spots, death of plants, leaf rust and formation of yellow powder on leaves. The farmers' inability to recognise and identify the diseases could be attributed to their inability to associate symptoms with respective pathogens. The results showed that farmers still cultivate susceptible low yielding cowpea cultivars, which in most cases may be even more susceptible to the emerging new virulent strains of viruses. Therefore, there is a need for improvement of resistance to viruses among the cowpea cultivars available to farmers. In this way farmers will be in a position to increase substantially the area of land under cowpea production and consequently, increase yields.

2.1 Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is an important grain legume that contributes a substantial amount of dietary protein for low-income rural and urban populations in Uganda (Sabiti *et al*., 1994). The crop forms an integral part of cropping systems in Africa (Olufajo and Singh, 2002). It is cultivated for home consumption as well as for cash in Uganda. Furthermore, the crop is of critical importance in eastern and northern Uganda as its leaves provide a source of vegetable that helps to offset early season famine (Isubikalu *et al*., 2000). In spite of its significance, the mean yield of cowpea in Uganda has gradually decreased over the years to less than 400 kg ha^{-1} (Adipala *et al*., 1997). It is estimated that about 20,000 t y^{-1} of cowpea grain yields are produced (FAO, 1997). The low mean yields have been due to several factors, among which are the upsurge in insect pests and diseases. A case in point is the persistence of infection of the cultivated cowpea varieties by virus diseases in the cowpea growing regions of Uganda.

Studies have shown that the process of adoption of new improved production technology tended to be low in marginal areas, where there was limited farmer involvement in the research process (Tripp, 1982; Maurya *et al*., 1988). A decade ago, the approach to development tended not to analyse and understand farmers' real needs (Hagmann *et al*., 1999). The adoption of improved technology was consequently low because the rural farming clientele lacked a sense of ownership of the ideas imposed on them (Hagmann *et al*., 1999). These days, government and non-government institutions are increasingly recognising the need to move away from giving instructions towards more participatory approaches, which support communities in their capacity to set and fulfill their own development goals (Hagmann *et al*., 1999). Since the early 1980s, development-oriented scientists have focused attention on improving methodological approaches for generating information from the village communities, with whom they work, by participating in problem identification, and determination and execution of planned action. This is likely to address the real needs of the farmers (Vabi, 1996). Chambers (1992) indicated that participatory tools and techniques, such as semi-structured and key informant interviews, transect walks, matrix scoring and ranking, can promote dialogue between research teams and village communities. The valuable insights of breeders in developing a product/variety could be complimented by the indigenous knowledge of farmers (Sperling *et al*., 1993). This is critical when determining which traits are valued or preferred by farmers. Participation of farmers is being advocated by many researchers or development partners to promote acceptance and adoption of technology. This is

intended to enable farmers to become the co-owners of the research and development process as well as its outcomes (Maurya *et al*., 1988; Prain *et al*., 1992; Franzel *et al*., 1995; Witcombe *et al*., 1996). The importance of farmer participation in the research is the provision of the demand-pull necessary to ensure that the effort in breeding work is focused on key issues of value to the farmer and consumer (Rhoades and Booth, 1982). Technology development without farmer participation has limited chances of being adopted. The participatory approach improves adoption of improved technology and enhances farmers' knowledge, and enables indigenous knowledge and innovations to be integrated in the research. This study undertook a PRA to elicit farmers' indigenous knowledge of cowpea production, with the purpose of integrating this into the breeding work and also to gain insight into their understanding of cowpea virus diseases affecting cowpea in Uganda.

2.2 Materials and methods

2.2.1 Selection of study area and farmers

The study was conducted in Kumi district located 33°7'E, 1°5'N and is 914-1600 m above sea level. The study covered four sub-counties in the district namely Malera, Bukedea, Ngora and Kapir during the second season of 2004. Much of the soil is characterised as being sandy loam, while the land has a gentle slope. It receives a bimodal rainfall, with heavy rainfall occurring in the first season around March-May and during the second season, starting around August-September (Figure 1), and the annual rainfall is about 1000 mm. The district experiences a minimum and a maximum temperature of 17.5°C and 27.5°C, respectively (Figures 2 and 3). Selection of farmers was done at community level through key informants such as the agricultural extension officers and local chiefs. The participants included farmer leaders, innovative farmers, women and men, poor farmers with limited resources, stakeholders and traditional leaders.

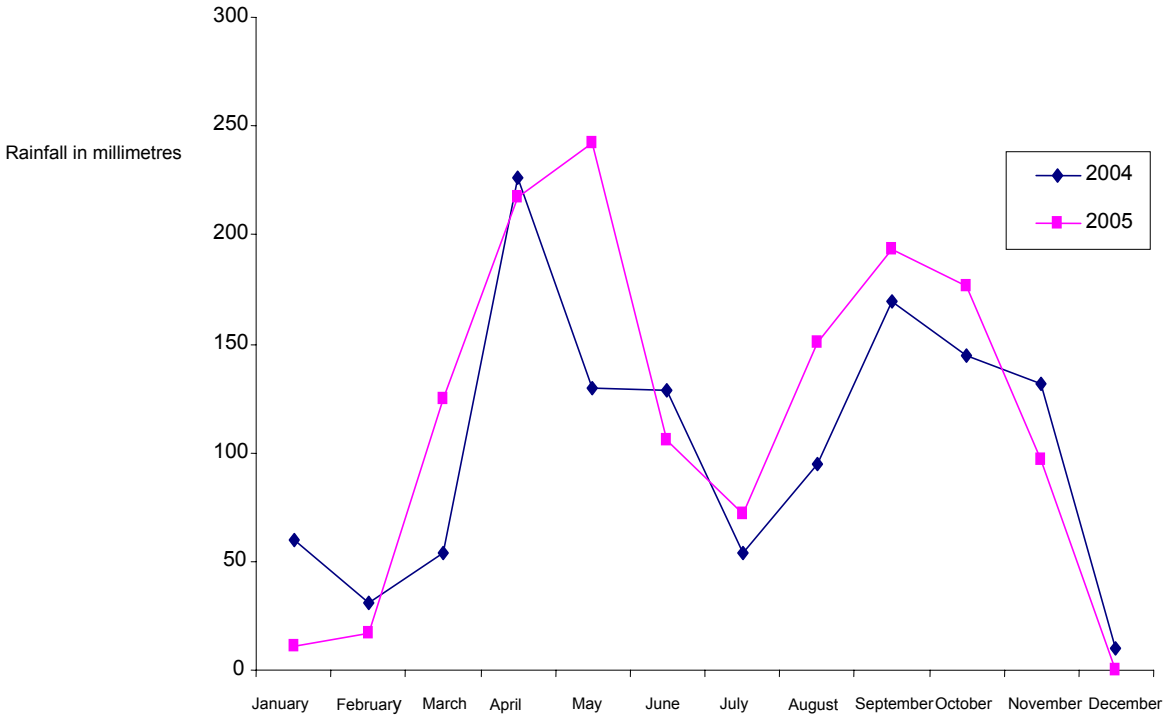

Figure 1: Monthly rainfall distribution in millimetres during 2004 and 2005

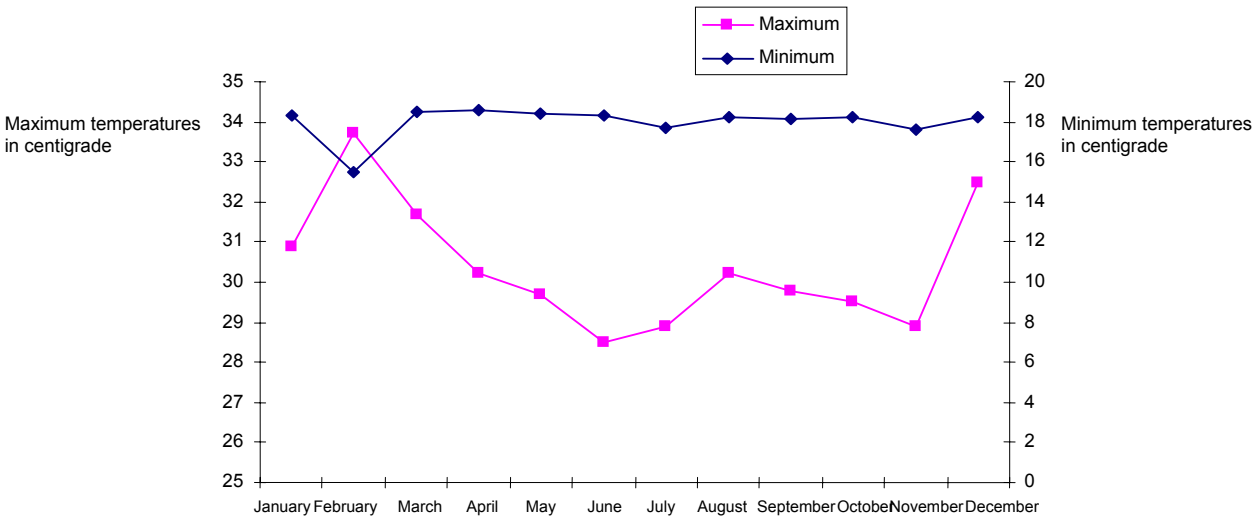

Figure 2: Maximum and minimum temperature distribution during 2004

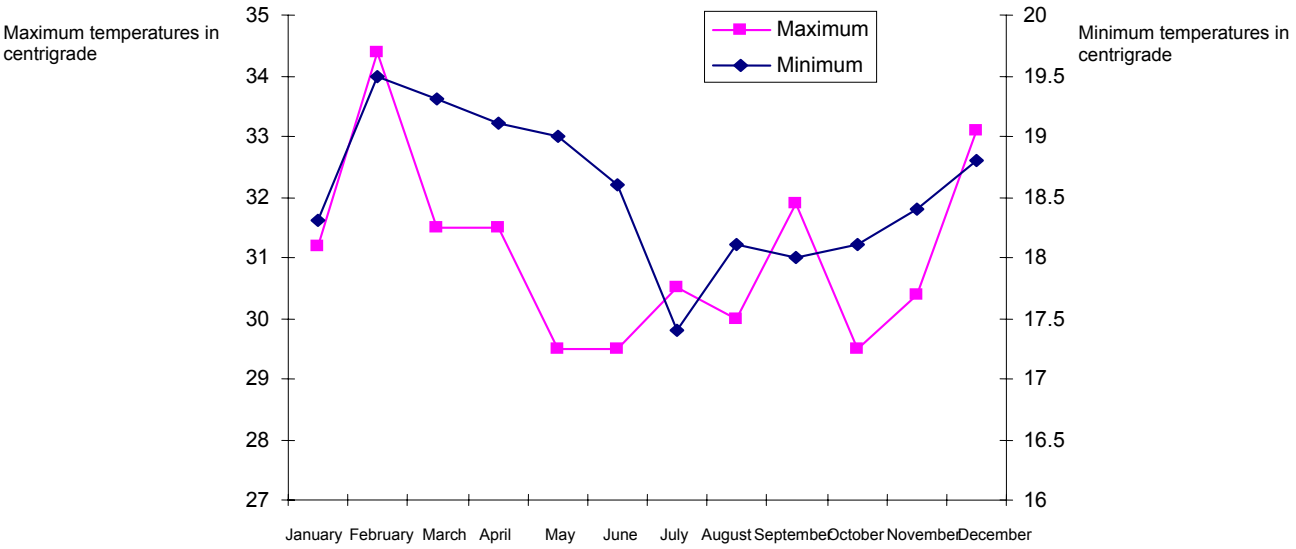

Figure 3: Maximum and minimum temperature distribution during 2005

2.2.2 Interview techniques and data collection

PRA tools such as group discussions, problem listing and ranking were used to gather information during the study in Kumi district. An open-ended interview, guided by a checklist and direct participant observation, was undertaken to obtain detailed information during the session. A representative sample of not less than three farmers from each village was selected and this constituted an estimated group of 20 farmers of both sexes. The socio-economic classes, as perceived by the community, were assumed to be adequately represented. The farmers were interviewed with the help of lead questionnaires, during which probing questions were asked. The views from a cross-section of farmers were discussed and a consensus reached on the issues mentioned. In case of a farmer presenting his or her opinion in the local language, which the researcher could not understand, the technical officer (the agricultural extension officer) would interpret in English. A transect walk was conducted in a few fields planted with cowpea after the session to promote discussions amongst farmers about cowpea production and the associated constraints. These walks allowed individual farmers to asses the status of cowpea in the field and also to observe the virus symptoms. The exercise involved the whole survey team, constituting a socio-economist, agricultural extension officer and research scientist. This study planned to involve a total of 80 farmers from the four sub-counties. Qualitative and quantitative data on dominant crops, cowpea production, cultivars grown, planting season, land size, cropping systems, crop growth characteristics, maturity, yield potential, seed colour, viral diseases and other constraints were collected. A pair-wise ranking system was used for ranking of the problems. A direct matrix ranking method was used to assess cowpea varieties with multiple traits such as growth vigour, growth characteristics, earliness, yield potential, disease and insect pest resistance or tolerance.

2.3 Results

2.3.1 Crops and cropping systems

The results of group discussions (Figure 4) revealed that farmers grow a variety of crops of their choice during the year, with a large number of crops allocated in the first season due to availability of rainfall and fewer crops were grown in the second season (Table 1). The high frequency of activities in the first season suggests that farmers diversify

cultivation of crops during this season in order to enhance food production and increase the level of household income. The two major production seasons were characterised in the study. The first rainy season starts around March and ends in July and has sufficient rainfall between April and May. The second rainy season has short rains starting around August and ending in December. In the first rainy season, the major crops included cassava (*Manihot esculenta* Crantz), groundnuts (*Arachis hypogea* L.), finger-millet (*Eleusine coracana* L. Gaertn.), sorghum (*Sorghum bicolor* L. Moench), maize (*Zea mays* L.), and to a lesser extent, sim-sim (*Sesamum indicum* L.), sun-flower (*Heliathus anuus*), beans (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.) and tomatoes (*Lycopersicon esculentum* Miller). In the second rainy season, the major crops included cowpea (*Vigna unguiculata* L. Walp.), sweet-potatoes (*Ipomea batatas* L. Lam.), cotton (*Gossypium hirsutum*), green-gram (*Vigna radiata* L. Wilczek) and to a lesser extent, bambara-groundnuts (*Vigna subterranea* Thouars Verdc). Although cowpea crop is cultivated mostly in the second season, its cultivation is picking up, in addition to the other major crops grown as a means of generating income by most farmers in this region of Uganda.

Figure 4: Farmers' group discussion during PRA session

Table 1: Percentage distribution of main crops grown by respondents in the sub-counties of Kumi district

Crop	Sub-county			
	Malera (n = 35)	Bukedea (n = 26)	Ngora (n = 21)	Kapir (n = 22)
First rainy season				
Cassava	100	100	76.2	77.3
Groundnuts	100	92.3	71.4	77.3
Finger-millet	100	100	52.4	54.5
Sorghum	100	100	52.4	59.1
Maize	100	53.8	57.1	59.1
Rice	8.6	38.5	----	----
Sun-flower	34.3	----	----	----
Sim-sim	42.9	----	----	----
Beans	31.4	46.2	47.6	----
Cucumber	25.7	----	----	36.4
Tomatoes	11.4	----	----	----
Second rainy season				
Bambara-groundnuts	45.7	----	----	36.4
Cotton	100	34.6	57.1	54.5
Cowpea	100	100	52.4	77.3
Sweet-potato	100	61.5	61.9	77.3
Green-gram	100	30.8	47.6	59.1

The figures are percentage responses, n = number of respondents and ---- = crop not reported

Overall, it was noted that cassava, groundnuts, cowpea and sweet-potatoes were mentioned as the most important crops grown for cash income and food security, while sim-sim, bambara-groundnuts, cucumber and tomatoes were less cultivated by farmers (Table 2). The study showed that in Malera and Bukedea sub-counties in Kumi district, cowpea was ranked as the third most important crop after cassava and groundnuts. In contrast, farmers in Kapir and Ngora sub-counties ranked cowpea as being the first and fourth crop among the cultivated crops, respectively. Ranking of cowpea showed farmers' strong interest and willingness to grow it. This was probably because of the increasing demand for cultivation to target markets, as well as alleviating poverty and

famine at household levels. The results of the study showed that farmers cultivate cowpea in association with other crops. Cowpea is intercropped by most farmers (94.3%) and with a few farmers growing it as a monocrop (0.3%), while 5.4% of the farmers, practiced both monocropping and intercropping.

Table 2: Direct matrix ranking of the dominant crops in the sub-counties of Kumi districts

Sub-county	Crop	Cowpea ranking	Comment
Malera	Cassava, groundnuts, cowpeas, finger-millet, maize, sorghum, green-gram, sweet-potato, sunflower, cotton, rice, beans, sim-sim, bambaranuts, cucumber, tomatoes	3	Cassava and groundnuts are the main crops
Bukedea	Cassava, groundnuts, cowpeas, finger-millet, sorghum, sweet-potato, maize, beans, rice, cotton, green-gram,	3	Cassava and groundnuts are the main crops
Ngora	Cassava, groundnuts, sorghum, finger-millet, sweet-potato, cowpeas, maize, beans, green-gram, cotton	4	Cassava, groundnuts and sweet-potatoes are most leading crops, respectively
Kapir	groundnuts, sorghum, cowpeas, green-gram, cassava, maize, sweet-potato, finger-millet, bambaranuts, cucumber, cotton	1	Cowpea is main crop together with cassava, groundnuts and sweet-potatoes

2.3.2 Cowpea production and marketing

The farmers mentioned that the proportion of land allocated for cowpea varied from 0.5 to 2.5 ha, depending on the availability of land, labour, finance and rainfall (Table 3). Cowpea landraces grown by farmers included Ebelat, Ecirikukwai and Blackcowpea. The Ecirikukwai variety was described as an early maturing and takes between 75-85 d to mature compared with 90 d for Ebelat and over 90 d for Blackcowpea. The farmers reported that on average, total yield of each cultivar grown differed significantly. For instance, Ebelat varied between 100 and 400 kg ha^{-1}, Blackcowpea between 75-500 kg ha^{-1} and Ecirikukwai between 75-200 kg ha^{-1}. Considerable quantities are sold to NGOs, schools, local markets and fellow farmers. Farmers were able to sell their cowpea at prices ranging between 65,000-75,000 Uganda shillings per 70 kilogram weight of cowpea grains at wholesale price.

Table 3: Cowpea varieties and associated yield in the sub-counties of Kumi district

Sub-county	Varieties	Cultivated area (ha)	Maturity period (days)	Yield kg ha^{-1}
Malera	Ebelat	2-3	90	100-200
	Blackcowpea	„	>90	75-150
	Ecirikukwai	„	75-80	75-100
Bukedea	Ebelat	1-5	90	100-400
	Ecirikukwai	„	80-85	100-200
	Blackcowpea	„	120	200-500
Ngora	Ebelat	1-2	90	100-200
	Blackcowpea	„	100	100-200
	Ecirikukwai	„	90	75-100
Kapir	Ebelat	1-3	90	100-200
	Ecirikukwai	„	75	50-100
	Blackcowpea	„	>90	100-300

2.3.3 Preferred varieties and associated characteristics of cowpea

Farmers reported that their selection of cowpea varieties for commercial production is based on consumer preference (Table 4). In this way, farmers mentioned that they have to select cowpea varieties for production on the basis of seed colour, market price, yield potential, palatability, earliness and tolerance to pests and diseases. For instance, farmers ranked Ebelat as the most preferred variety because of its good taste, seed colour (white), hilium colour (black), market acceptance, and high yield potential, while the Blackcowpea variety was less preferred because of price fluctuation, poor palatability and unpleasant seed colour, but it has a high yield potential. The variety Ecirikukwai was reportedly popular because of its earliness and tasty leafy parts for consumption, in spite of its low yields. However, Ebelat, Ecirikukwai and Blackcowpea were all reported to be vulnerable to insect pests and diseases.

Table 4: Characteristics of cowpea varieties preferred by farmers in the sub-counties of Kumi district in Uganda

Variety	Preference			Ranking	Attributes
	Growth characteristics	Seed colour	Hilium colour		
Ebelat	Semi-bushy	White	Black	1	Market acceptance, good taste, early maturity and high yielding , but susceptible to pests and diseases
Ecirikukwai	Semi-bushy	Cream	White	2	Sweet spinach, low yielding, early maturity, but susceptible to pests and diseases
Blackcowpea	Bushy	Black	White	3	Poor taste, market uncertainty, high yielding, late maturity, but susceptible to pests and diseases

1 = Excellent, 2 = good and 3 = slightly moderate

2.3.4 Constraints in cowpea production

Several constraints were mentioned and ranked by the farmers (Table 5). The most important constraints reported were insect pests, diseases and low yielding varieties. The farmers reported that these constraints drastically reduced yields. Farmers also noted that price fluctuation for cowpea tended to be a problem. Whenever there was a bumper harvest cowpea prices dropped, forcing them to keep their produce in storage, until such a time that the price increased in the off-season. Because of poverty, farmers were often forced to sell their product at low prices.

Table 5: Pair-wise ranking of the most important constraint in cowpea production in the sub-counties of Kumi district

Constraint	Scores by farmers per sub-county					
	Malera	Bukedea	Ngora	Kapir	Total score	Ranking
Insect pests	2	1	1	1	5	1
Diseases	3	2	-	-	5	1
Poor storage	5	3	-	3	11	5
Weed sp.	-	5	4	5	14	6
Hailstorm	-	6	4	5	15	7
High costs of pesticides	-	3	1	2	6	2
Poor agronomic practices	1	4	-	3	8	4
Drought	4	-	2	-	6	2
Low yielding varieties	5	-	-	-	5	1
Price fluctuation	-	-	3	4	7	3

1 = very serious problem and 7 = minor; - = not reported

The most important insect pests mentioned by farmers were aphids, pod-sucking bugs, thrips, pod borers and flower beetles (Table 6). Overall, the aphids, pod-sucking bugs, thrips and pod borers were the most important insect pests ranked. These key insect pests were considered to be devastating on cowpea, because they attack the crop at all stages during active growth. The insect pests inflict on the plants loss of plant nutrients, reduced growth and a decline in yields. Farmers noted that although the aphid incidence is sporadic, they have severe effects on cowpea plants leading to the death of most infested plants. The sucking insects such as pod-sucking bugs, thrips and pod borers are widespread and can occur throughout the seasons, whenever cowpea is grown.

Table 6: Pair-wise ranking of field insect pests reported attacking cowpea in the sub-counties of Kumi district

Sub-county	Insect pest	Ranking	Occurrence
Malera	Aphids	1	Seasonal and in patchy distribution, especially during dry spell
	Pod-sucking bugs	2	Common and widespread
	Thrips	3	Common and widespread
	Pod borers	4	Common and widespread
	Flower beetles	5	Occasional
Bukedea	Aphids	1	Seasonal, but not well distributed in the field grown cowpea during dry spell
Ngora	Aphids	1	Seasonal
	Pod-sucking bugs	2	Common and widespread
	Flower beetles	4	Occasional
	Pod borers	3	Common and widespread
Kapir	Aphids	1	Common and widespread during favourable weather conditions
	Pod-sucking bugs	1	Common and widespread in all weather conditions
	Flower beetles	3	Occasional

1 = severe and 5 = minor damage

The majority of the farmers interviewed were not aware of the diseases, but used descriptive names for symptoms, such as stunted plants, leaf deformation, mosaic leaves, yellowing of leaves, leaf spots, leaf rust, formation of yellow powder on leaves and death of plants (Table 7). The inability of the farmers to identify the diseases is simply due to their lack of information or knowledge about the causal agents of the diseases they apparently associated with the symptoms on their cowpea crops. Although they provided descriptions of the symptoms, such as mottling, stunted growth, mosaic leaves and chlorotic leaves (Figure 5), they lacked the knowledge to recognise and identify the viruses.

Table 7: Disease symptoms reported to occur on cowpea in the fields in sub-counties of Kumi district in Uganda

Sub-county	Disease symptom	Occurrence
Malera	Stunted plants	Common and widespread
	Leaf deformation	Common and widespread
	Mosaic leaves	Common and widespread
	Yellowing of leaves	Occasional
Bukedea	Not aware of symptoms	-------
Ngora	Leaves covered with yellow powder	Occasional
	Leaf spot	Common in warm seasons
	Leaf rust	Occasional
	Leaf mottling	Common and widespread
Kapir	Leaves covered with yellow powder	Occasional
	Leaf spot	Occasional
	Viral infection	Common and widespread

Figure 5: Farmers identify disease symptoms on cowpea plants with the guidance of research student to the right

2.4 Discussion and conclusion

The study clearly demonstrated that a participatory approach, as an aid to farmer involvement in research, was an efficient and effective method in utilising local farmers' knowledge. The approach provided a friendly atmosphere for farmers to engage in discussions on a number of issues floated by them. The issues raised by farmers were noted down, discussed and ranked according to the order of importance following the open-ended questionnaires. This kind of response shown by farmers during the PRA sessions, suggests that farmers have valuable knowledge of issues that affect their crop production. Biggs (1978), Rhoades and Booth (1982) and Kitch *et al*. (1998) confirmed that farmers have valuable knowledge and they can do agricultural research on their own.

The most important food crops mentioned by farmers were cassava, groundnuts, sweet-potatoes, cowpea, finger-millet, sorghum, maize and green-gram, while cotton was the crop grown as the main cash crop. According to the farmers, food crops were regarded as important because they serve both as a food crop and a source of cash income. The ranking of cowpea, in comparison to other crops, was an indication that farmers were developing a strong interest in cowpea production, because of the increasing demand for cowpea from other towns and neighbouring countries, and due to a premium price paid at the market level. Besides the income that was appreciated, farmers acknowledge the importance of cowpea being compatible with other crops when grown in mixtures. This increases production not only of cowpea, but also of other crops, and farmers appreciated the intercropping system, as it provides more food and cash, especially in the rural areas. Olufajo and Singh (2002) indicated that intercropping practices lead to profit maximisation, risk reduction in case of failure of one of the crops, soil fertility improvement and better weed control. Growing several crops in a season puts farmers in a better position to meet their household needs. For instance, farmers indicated that growing crops that mature at different times enables them to have a constant food supply throughout the year, and this was seen as a way to improve food security and reduce poverty.

Knowledge of the attributes of cowpeas preferred by farmers is essential when developing an improved cowpea (Coulibaly and Lowenberg-DeBoer, 2002). Breeders need to know what characteristics farmers want, such that when an improved variety is availed to them it possesses the preferred traits. Traits of interest to farmers in this study were white seed colour, earliness, yield potential, good taste, and tolerance to insect

pests and diseases. Kitch *et al*. (1998) indicated that farmers seek varieties with particular traits, such as large white seeds that command a premium price. Coulibaly and Lowenberg (2002) observed that market studies are useful in indicating varieties with characteristics prefered by consumers, which sell for a premium price.

The results showed that farmers demonstrated a deep understanding of the constraints affecting their agricultural production. Farmers were aware of the major constraints such as insect pests, diseases and lack of high yielding varieties that limited production. Interestingly, the most striking thing elicited from farmers was the ability to name the key insect pests such as aphids, pod sucking bugs, thrips and pod borer, which they thought were the only factors contributing to low yields. However, it was not possible for them to mention any diseases, except for the descriptive names of symptoms. This showed a lack of awareness among the farmers of the problems associated with the incidence of cowpea diseases. This indicated a need for awareness of diseases and causal agents in cowpea. Sensitising farmers about disease identification will enable them to become aware of the problem and know how best to deal with it.

Although cowpea is becoming an increasingly important crop in Uganda, its average yields are relatively low. For instance, average yields of 75-200 kg ha^{-1} for Ecirikukwai, 100-400 kg ha^{-1} for Ebelat and 100-500 kg ha^{-1} for Blackcowpea, are far below average yield attainable at on-stations of over 2500 kg ha^{-1} (Bationo *et al*., 2002; Singh, 2002). The findings confirmed those of Sabiti *et al*. (1994) and Adipala *et al*. (1997), who reported yields of less than 400 kg ha^{-1} in farmers' fields in Uganda. The important factors such as increased insurgence of the complexes of insect pests (Adipala *et al*., 1997; Omongo *et al*., 1997; Omongo *et al*., 1998) and diseases (Edema *et al*., 1997) have been reported to affect cowpea yields.

The PRA approach showed the importance of cowpea in the region. The results indicated that farmers demonstrated a clear understanding of the major constraints involved in cowpea production, which include insect pests, diseases and low yielding cultivars. Farmers expressed the need for better traits that can enhance commercial cultivation of cowpea. Integrating farmers' knowledge and priorities is essential in cowpea variety development in Uganda and will lead to a more rapid adoption of new varieties.

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CHAPTER THREE

OCCURRENCE AND PREVALENCE OF COWPEA VIRUS DISEASES IN UGANDA

Abstract

The study was carried out to identify the economically important cowpea viruses in the cowpea growing areas in Uganda. Two surveys were conducted to determine the incidence and severity of virus symptoms in the four major cowpea growing districts between 2004 and 2005. Field samples were obtained from 5-6 wk old cowpea plants from 60 locations in eastern Uganda. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to test the 220 virus symptomatic leaf samples collected. The virus symptoms were observed in all the districts surveyed, but with varying levels of incidence and severity. The virus incidence ranged from 40.5 to 94.4%, and severity ranged from 15.0 to 30.6% (for Kumi and then Pallisa districts, respectively) during 2004 surveys. In 2005, the virus incidence ranged from 55.9 to 85.4%, and severity ranged from 4.7 to 14.5% (for Tororo and then Soroti districts, respectively). The cowpea aphid-borne mosaic virus (CABMV), cowpea mild mottle virus (CPMMV), cowpea severe mosaic virus (CPSMV) and cucumber mosaic virus (CMV) were serologically detected by DAS-ELISA. CPMMV and CPSMV were detected in three and four districts, respectively while CABMV was detected only in two districts during 2004 surveys. In 2005, the four viruses were detected in all four districts surveyed. Mixed infection of viruses was observed, with CPSMV being common in all the samples tested during 2004 and 2005.

3.1 Introduction

Cowpea is one of the most widely adapted and nutritious food legume crops. The crop shows considerable adaptation to drought compared to other crop species. Dry grain for human consumption is the principal product of the cowpea plant, but leaves, fresh peas and fresh pods are consumed (Ehlers and Hall, 1997). Ehlers and Hall (1997) showed that farmers in California can achieve up to 4000 kg ha^{-1} of dry grain yields of cowpea as long as the crop exhibits resistance to abiotic and biotic stresses (Ehlers and Hall, 1997). In the case of Uganda, where the crop is intensely cultivated in the northern and eastern

regions, farmers achieve less than 400 kg ha^{-1} (FAO, 1997). While farmers are developing a strong interest in cowpea production, they still face several adverse factors, among which is the prevalence of diseases and insect pests (Rusoke and Rubaihayo, 1994; Edema and Adipala, 1996; Omongo *et al*., 1998; Tarawali *et al*., 2000; Singh *et al*., 2003). Virus diseases, besides other biological agents such as insect pests, bacteria, fungi and nematodes, have long been associated with yield losses ranging from 10-100% in field grown cowpea crops (Shoyinka *et al*., 1997), depending on the virus-host vector relationships, as well as prevailing epidemiological factors. In Uganda, cowpea viruses have become a major problem to cowpea production. It is estimated that up to 100% losses in grain yields can occur due to virus infections alone.

Symptoms of plant virus diseases have been recognised for many decades, although it has only recently become possible to identify and study the causal pathogens. The most damaging diseases for cowpea crops are caused by viruses and they represent a very significant proportion of losses regarding the potential value of the crop in sub-Saharan Africa (Thottappilly and Rossel, 1992). Cowpea plants are often infected by more than one virus disease, and this can cause serious economic losses in agricultural production (Byoung-Cheorl *et al*., 2005). Worldwide, up to 20 viruses have been recognised in cowpea, but only eight viruses are important in Africa (Thottappilly and Rossel, 1992). The economically important viruses in Africa include cowpea chlorotic mottle virus (CPCMV), cowpea severe mosaic virus (CPSMV), southern bean mosaic virus (SBMV), cowpea aphid-borne mosaic virus (CABMV), cowpea mild mottle virus (CPMMV), cowpea mosaic virus (CPMV), cucumber mosaic virus (CMV) and cowpea chlorotic mosaic virus (CPCMV) (Thottappilly and Rossel, 1992; Alegbejo and Kashina, 2001). The occurrence of viral diseases varies from region to region depending on factors such as population dynamics of virus vectors, climatic conditions, cropping systems, cultivar types and virus inoculum levels (Wisler *et al*., 1998). Disease symptoms caused by viruses vary in nature, but the most common symptoms include mosaic, systemic chlorosis, leaf distortion, leaf mottling and stunting of plants.

Despite the significance of cowpea in enhancing food security as well as a cash crop for the majority of farmers, only limited information is available about the occurrence, distribution and identity of cowpea viruses in Uganda. Only limited studies on the diagnosis of a few viruses have been done. The information obtained on viruses is needed as the first step towards the search for control strategies for viruses in cowpea. The present study aimed to collect information on the occurrence of viruses in cowpea in Uganda.

3.2 Materials and methods

3.2.1 Survey areas and sampling

Surveys for virus incidence and severity were carried out in four districts, namely Soroti, Kumi, Pallisa and Tororo districts (Figure 6). Field surveys were conducted on farmers' fields in two consecutive years, during the second rainy season between October and November in eastern Uganda. The second season was selected for the study because cowpea is grown predominantly during this season by the majority of farmers. The surveys were conducted in 2004 and 2005. In 2004, four distant fields approximately 5 km apart were randomly selected for the study in each district. In 2005, seven distant fields approximately 5 km apart were randomly selected for the study in each district. Fields and districts were taken into consideration to determine whether there were variations in virus occurrence amongst districts, fields across districts and fields within each district. Plant samples for analysis were taken from 1 x 1 m quadrants from the fields. These were treated as replicates from each field. A diagonal sampling pattern was carried out and the total number of plants within each quadrant was counted to estimate the percentage of the diseased plants. This survey was carried out when the cowpea crops were estimated to be 5 – 6 wk old. Two trifoliate leaves from each plant within the quadrant were sampled for virus symptoms.

In 2004, 108 virus symptomatic leaf samples were collected from 32 locations in the districts of Soroti, Kumi, Tororo and Pallisa (Figure 6). Thirty two leaf samples of cowpea with virus symptoms (mostly leaf mottling and leaf mosaic were observed in the fields surveyed) were collected from Soroti, 32 from Kumi, 22 from Tororo and 22 from Pallisa. For the purpose of verifying the variability and occurrence of viruses between the years, a second survey was conducted in 2005 during which 112 virus symptomatic leaf samples were collected from 28 locations in the same districts already mentioned above. Twenty eight leaf samples with virus symptoms were collected from each district. The surveys and collection of samples were carried out in the same location, but not necessarily from the same field sites surveyed previously. The total number of plants per quadrant was counted. In each quadrant, the number of plants with virus symptoms was counted.

3.2.2 Data assessement

Disease incidence was calculated by expressing the number of plants with virus symptoms as a percentage of the total number of plants in each quadrant. Disease severity was assessed visually as the percentage of leaf area exhibiting virus symptoms according to the rating scale (Table 8).

Table 8: Rating scale used for scoring disease severity

Percentage score	Disease symptoms
0	No virus symptoms
<10	Symptoms just beginning to manifest on one plant leaf
10-20	Minor symptoms on leaves
20-30	Moderate symptoms on leaves
30-40	Third of the plant leaves with symptoms
40-45	Three quarter of plant leaves with symptoms
45-50	Quite severe symptoms on plant leaves
50-60	Severe symptoms beginning to intensify
>60	Very severe symptoms and death of the plant

The analysis of variance was carried out using the model:

$\mathbf{Y_{ijk} = \mu + r_i + d_j + f_k + (d/f)_{jk} + \varepsilon_{ijk}}$ in a Genstat computer package and the Least Significant Difference (LSD) mean separation procedure was calculated.

Where Y_{ijk} is the level of the virus symptoms observed at the ijk^{th} location

μ is the overall mean observed for virus symptoms

r_i is the level of virus symptom observation at i^{th} quadrant

d_j is the level of virus symptom observation at j^{th} district

f_k is the level of virus symptom observation at k^{th} field

d/f_{jk} is the level of virus symptom observation at jk^{th} field nested in district

εijk is the error term associated with each observation

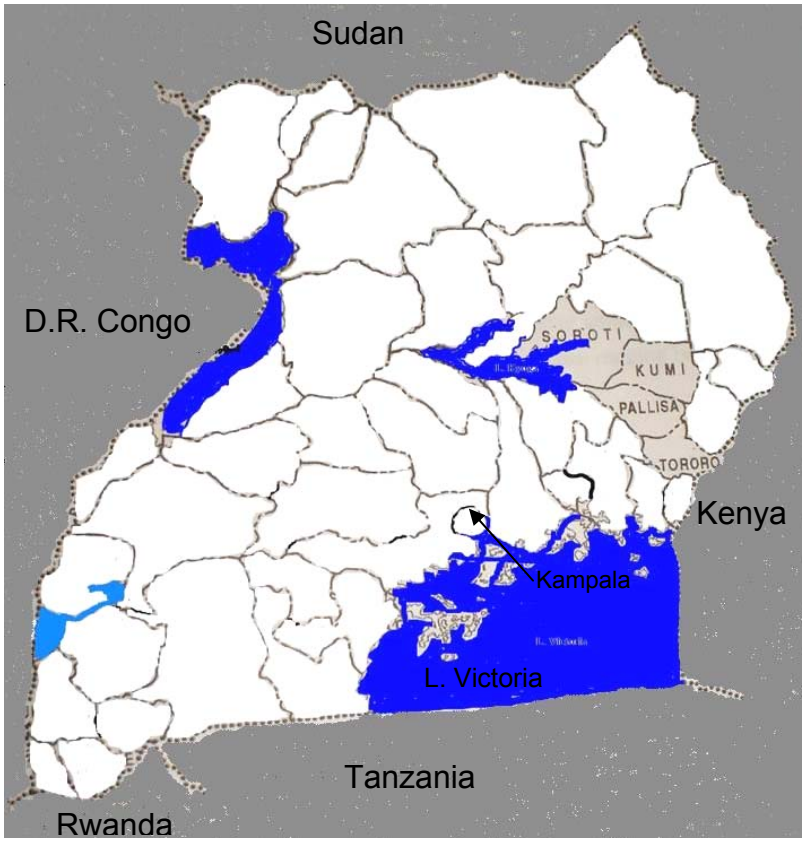

Figure 6: Map of Uganda showing the areas surveyed in cowpea growing districts of Soroti, Pallisa, Kumi and Tororo in Uganda during 2004 and 2005

3.2.3 Laboratory testing of leaf samples for viruses by Double Antibody Sandwich ELISA (DAS-ELISA)

One hundred and eight leaf samples collected from four cowpea growing districts between October and November 2004 were subjected to DAS-ELISA tests by using five antisera kits specific to CABMV, CPCMV, CPMMV, CPMV and CPSMV. During the second surveys conducted in 2005, 112 leaf samples collected in the same districts were also subjected to DAS-ELISA tests using six antisera kits specific to CABMV, CPCMV, CPMMV, CPMV and CPSMV. Leaf samples exhibiting virus symptoms were collected from different districts and placed separately in small plastic polythene bags and stored at -20°C before being subjected to DAS-ELISA to test for specific viruses. The antisera used for serological testing were provided by Dr. S. Max from Deutsche Sammlung von Milkroorganismen und Zellkulturen (DSMZ) in Germany.

Following the procedures described by Huguenot *et al*. (1993) and Shoyinka *et al*. (1997), the ELISA kits were used to test for CABMV, CPCMV, CPMMV, CPMV, CPSMV and CMV. Based on the manufacturer's instructions and the quantity of IgG provided, the microplate wells were coated with 100µl per well of virus specific IgG diluted at 1:1000 for CPMMV, CPMV, CPSMV and CMV, and 200µl per well diluted at 1:500 for CABMV and CPCMV in 0.01M sodium carbonate buffer (Na_2Co_3 and $NaHCo_3$, NaN_3 at pH 9.6) and incubated for 2-4 hr at 37°C. A cork borer that cuts disks of approximately 12 mm in diameter was used to cut leaf disks from the leaf base, middle and top sections of the leaf. The disk samples were ground and diluted at 1:10 (w/v) in 0.01M phosphate saline buffer, PBS (NaCl, KH_2PO_4, Na_2HPO_4 and KCl, NaN_3 at pH 7.4) containing 0.5 ml Tween 20 (PBS-T) and 2% polyvinylpyrrolidone (PVP), was incubated overnight at 4°C covered with adhesive film. The positive and negative controls together with blank/buffer were each loaded in the duplicate wells. The immunoglobulin-alkaline phosphatase (IgG-AP) was diluted in PBS-T-PVP-egg albumin. The dilutions of IgG-AP varied with the type of virus and were as follows: IgG-AP was diluted at 1:1000 for CPSMV and CMV; 1:500 for CABMV, CPCMV, CPMMV and CPMV in conjugate buffer (PBST, 2% PVP containing 0.2% egg albumin (Sigma A-5253)). For dilution at 1:1000, 100µl were added to all wells, while for dilution at 1:500, 200µl were added to all wells and incubated for 4hr at 37°C covered with adhesive film. The 200 µl aliquots of freshly prepared substrate [25 mg p-nitrophenyl phosphate, Pnpp (Sigma 104-105)], dissolved in 25 ml of substrate buffer (diethanolamine, distilled water, NaN_3 at pH 9.8) was added to all wells containing the bound IgG-AP and allowed to hydrolyse for 30-60 min at room temperature in order to obtain clear reactions of the yellow colour development. After adding the subtrate buffer

to each well of the ELISA microplates, they were incubated at room temperature for 90 min to obtain clear reactions and the absorbencies were measured at 405nm (A_{405}) after every 30 min. Readings indicating twice the values of the negative controls were considered positive.

3.3 Results

3.3.1 Incidence and severity of virus-like symptoms on cowpea crops in four districts in Uganda surveyed during 2004

There were highly significant ($P \leq 0.001$) differences in the levels of viral symptoms on cowpea crops among the districts surveyed. The incidence and severity of virus symptoms on cowpea crops varied significantly ($P \leq 0.01$) amongst the surveyed fields (Table 9). Similarly, a highly significant interaction ($P \leq 0.001$) was also observed between farmers' fields and the districts.

During 2004, virus symptoms were encountered in the four districts: Soroti, Kumi, Tororo and Pallisa, but with varied incidence of virus symptoms (Table 10). On average, the districts of Pallisa and Tororo had the highest incidence of viral symptoms, although there were slight variations among the surveyed fields (Table 10). This was followed by Soroti district, while Kumi district had the lowest incidence of virus symptoms. There was low incidence of virus symptoms observed in Kumi for the first, second and third fields, but with a slightly higher incidence in the fourth field. It is Interesting to note that the observation made between Pallisa and Tororo districts showed that there was a slightly similar trend of symptom appearance for the virus. For instance, Pallisa district attained an incidence of 82.7, 98.1, 98.5 and 100%, while Tororo attained incidence of 100, 94.3, 91.7 and 75.4% for the first, second, third and fourth fields, respectively (Table 10). The incidence of virus symptoms observed in cowpea fields in Kumi district were 3.5, 19.7 and 44.6% for the first, second and third field, respectively, indicating that it had the least virus symptoms compared to the other districts surveyed.

A significantly high disease severity was registered in Soroti and Pallisa in all the fields. The two districts of Kumi and Tororo had relatively low disease severity in all fields surveyed. There was high severity in virus symptoms observed in the fields with respect to the fourth field in Kumi and first field in Tororo compared to the rest of the fields surveyed. Although Tororo district registered higher disease incidence in all of the fields,

there was a low disease severity observed compared to Soroti and Pallisa districts (Table 11). A lower disease severity trend of 7.5, 11.3 and 11.3% for first, second and third fields were observed in Kumi district compared to the other districts surveyed. The interesting thing to note from this study was that when the assessment of viral disease infection progressed from one field to the next in each district, there was either a gradual decrease (Soroti and Tororo) or increase (Kumi and Pallisa) in the disease levels. The overall observations showed that Pallisa district had a high severity of viral symptoms of 30.6% and lowest in Kumi with 15.0%.

Table 9: Mean square for incidence and severity of virus diseases in the fields[1] of cowpea assessed in the four districts[2] during 2004[3]

		Mean square	
Source	DF	Virus incidence	Virus severity
Replication	3	71.8	378.5
District (D)	3	9721.2***	1135.8***
Field (F)	3	368.0**	148.3**
D x F	9	3602.4***	621.9***
Residual	45	88.3	35.2

** and *** denotes significant at P≤0.01 and highly significant at P≤0.001, respectively

[1]Fields = first, second, third and fourth; [2]Districts = Soroti, Kumi, Tororo and Pallisa, [3]Year during the second season when survey was conducted in 2004 between October and November, and this season is largely characterised by major cultivation of cowpea

Table 10: Mean incidences (%) of observed viral symptoms in the surveyed cowpea fields in the districts of Uganda during 2004

	Mean incidences (%) of viral symptoms				
	Fields surveyed				
District	First	Second	Third	Fourth	Overall mean
Soroti	93.3	95.0	90.8	28.2	76.8
Kumi	3.5	19.7	44.6	94.1	40.5
Tororo	100.0	94.3	91.7	75.4	90.4
Pallisa	82.7	98.1	98.5	100.0	94.4
LSD(0.05)		13.4			
CV%		12.4			

Table 11: Mean severity (%) of observed viral symptoms in the surveyed cowpea fields in four districts of Uganda during 2004

	Mean severity (%) of viral symptoms				
	Fields surveyed				
District	First	Second	Third	Fourth	Overall mean
Soroti	51.3	38.8	20.0	11.3	30.3
Kumi	7.5	11.3	11.3	30.0	15.0
Tororo	23.8	20.0	12.5	11.3	16.9
Pallisa	23.8	30.0	38.8	30.0	30.6
LSD(0.05)		8.4			
CV%		25.6			

3.3.2 Incidence and severity of virus symptoms on cowpea crops in four districts in Uganda surveyed during 2005

The analysis of results for the surveys conducted during 2005 showed highly significant ($P \leq 0.001$) differences in incidence and severity of virus symptoms amongst the surveyed fields (Table 12). There were highly significant ($P \leq 0.001$) differences in incidence and

severity of viral symptoms amongst the surveyed districts. Furthermore, a highly significant interaction ($P \leq 0.001$) among farmers' fields and the districts was observed, indicating great variability of viral symptoms among districts, fields within a district and fields across districts.

The results of the surveys in 2005 showed a high disease incidence in all of the districts, but with variations in disease levels among the fields within the districts. For instance, all of the fields in Kumi district had a consistently high incidence in 2005 compared to Tororo, Pallisa and Soroti (Table 13). Overall, there was a high disease incidence of 85.4% in Kumi district, followed by Soroti with 75.3%, with the lowest incidence recorded in the districts of Tororo and Pallisa. In spite of the higher disease incidence observed in the fields, there was a moderate disease incidence in the districts in 2005 compared to the 2004 surveys.

Similarly, a slightly lower disease severity was observed in all of the fields within the districts during 2005. Significantly low disease severity was registered in Tororo district. The disease severity for the districts of Soroti, Kumi and Pallisa did not differ significantly, but there was a higher disease level in Soroti compared to the former districts (Table 14). Tororo district registered a very low disease severity of 4.7%, Pallisa with 11.8%, Kumi with 13.8% and Soroti with 14.5%, meaning that there was a lower disease severity in 2005 compared to the 2004 survey.

Table 12: Mean square for incidence and severity of cowpea viral symptoms in fields[1] of cowpea from four districts[2] in Uganda during 2005[3]

		Mean square	
Source	DF	Incidence	Severity
Replication	3	185.0	102.5
District (D)	3	5126.3***	560.**3***
Field (F)	6	3919.0***	157.9***
F x D	18	2845.0***	229.6***
Residual	81	235.0	32.7

*** denotes highly significant at $P \leq 0.001$

[1]Fields = first, second, third, fourth, fifth, sixth and seventh; [2]Districts = Soroti, Kumi, Tororo and Pallisa

[3]Year during the second season when survey was conducted in 2005 between October and November, and the season is characterised by major cultivation of cowpea

Table 13: Mean incidence (%) of observed viral symptoms in cowpea fields in four districts of Uganda during 2005

District	Mean incidence (%) of viral symptoms							
	Fields surveyed							
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Overall mean
Soroti	89.7	76.5	86.6	29.4	88.3	96.0	60.7	75.3
Kumi	90.2	89.7	84.0	96.0	59.3	100.0	78.3	85.4
Tororo	67.9	82.2	81.3	66.5	51.8	34.9	6.5	55.9
Pallisa	94.4	20.8	92.2	88.2	97.2	16.0	15.6	60.6
LSD(0.05)			21.6					
CV%			22.1					

Table 14: Mean severity (%) of observed viral symptoms in cowpea fields in four districts of Uganda during 2005

District	Mean severity (%) of viral symptoms							
	Fields surveyed							
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Overall mean
Soroti	12.5	8.8	15.0	6.3	22.5	22.5	13.8	14.5
Kumi	7.5	13.8	7.5	20.0	6.3	35.0	6.3	13.8
Tororo	7.5	5.0	5.0	6.3	4.3	3.5	1.3	4.7
Pallisa	18.8	8.8	20.0	4.3	17.5	3.0	2.0	11.8
LSD(0.05)				8.0				
CV%				51.2				

3.3.3 Serological detection by DAS-ELISA

3.3.3.1 Virus detection in leaf samples collected in 2004 and 2005

The CPMMV, CABMV and CPSMV were detected in the samples collected from the four districts. The results of the study indicated that CPSMV was common in all four districts surveyed. Thus, a total of 24 (22.2%) symptomatic samples reacted positively with CPSMV antibodies, making CPSMV the most prevalent virus in the districts during the 2004 season (Table 15). This was followed by CPMMV with a total of 7 (6.5%) symptomatic samples occurring only in three districts, with the exception of Tororo district. CABMV was detected in 4 (3.7%) diseased plant samples obtained from Pallisa and Tororo districts, and it was the least frequent virus among the viruses detected in 2004. There was no reaction for CPCMV and CPMV in the samples tested.

An additional kit specific to cucumber mosaic virus (CMV) was included during 2005 surveys and this was basically to confirm whether the symptomatic leaf samples, which did not test positive for any of the antisera used in 2004, were actually free of or infected with CMV. The results showed a positive reaction for cucumber mosaic virus when some samples were tested with the antisera. Based on the results, CPMMV, CABMV, CPSMV and CMV tested positive in the samples. Thus, a total of 81 (72.3%) symptomatic samples reacted positively to CPMMV antibodies, making CPMMV the most prevalent virus in the districts during 2005 (Table 16). This was followed by CABMV with a total of 41 (36.6%) symptomatic samples occurring in all the districts. CPSMV was detected in 39 (34.8%) diseased plant samples obtained from all four districts and 32 (28.6%) symptomatic leaf samples reacted positively to CMV antibodies.

Table 15: Prevalence of five virus types tested serologically in symptomatic samples collected from four districts of Uganda in 2004

District	Samples tested	Virus serological detection by DAS-ELISA				
		CABMV	CPMMV	CPSMV	CPCMV	CPMV
Soroti	32	-	1 (3.1)	11 (34.4)	-	-
Kumi	32	-	4 (12.5)	1 (3.1)	-	-
Pallisa	22	2 (9.1)*	2 (9.1)	7 (31.8)	-	-
Tororo	22	2 (9.1)	-	5 (22.7)	-	-
Total	108	4	7	24	0	0

* Figures in parentheses are percentage incidence; – Indicate no virus was detected in the samples in any of the districts

Table 16: Prevalence of six virus types tested serologically in symptomatic samples collected from four districts of Uganda in 2005

District	Samples tested	Virus serological detection by DAS-ELISA					
		CABMV	CPMMV	CPSMV	CMV	CPCMV	CPMV
Soroti	28	15(53.6)*	22(78.6)	16(57.1)	12(42.9)	–	–
Kumi	28	6(21.4)	25(89.3)	9(32.1)	6(21.4)	–	–
Tororo	28	4(14.3)	24(85.7)	4(14.3)	4(14.3)	–	–
Pallisa	28	16(57.1)	10(35.7)	10(35.7)	10(35.7)	–	–
Total	112	41	81	39	32	0	0

* Figures in parentheses are percentage incidence; − Indicate no virus was detected in the samples in any of the districts

3.3.3.2 Single and multiple virus infections occurring in 2004 and 2005

In the 2004 surveys, 21.3% of symptomatic samples were infected with a single virus, whereas 7.4% were infected with two viruses. The most common virus in a single infection was that of CPSMV (13%), followed by CPMMV (6.5%) and the least common was CABMV (1.9%). However, CPSMV occurred in mixed infections with other viruses, namely CPSMV and CPMMV (5.6%), and CPSMV and CABMV with 1.9% (Figure 7).

In 2005, a total of 13.4% of symptomatic samples detected were infected with a single virus, while 10.7% of the samples were infected with two or more viruses. The results of the 2005 surveys indicated that CPMMV was detected as the most common virus in a single infection (11.6%) and the least common was that of CABMV (1.8%). Two or more viruses were detected interacting in the sample. For instance, four viruses CPMMV, CMV, CABMV and CPSMV interacted together in 7.1% in the samples, three viruses CPMMV, CMV and CPSMV interacted together in 0.9% in the samples, two viruses CPSMV and CMV interacted together in 0.9% in the samples, and two viruses CPMMV and CSPMV interacted together in 1.8% in the samples (Figure 8).

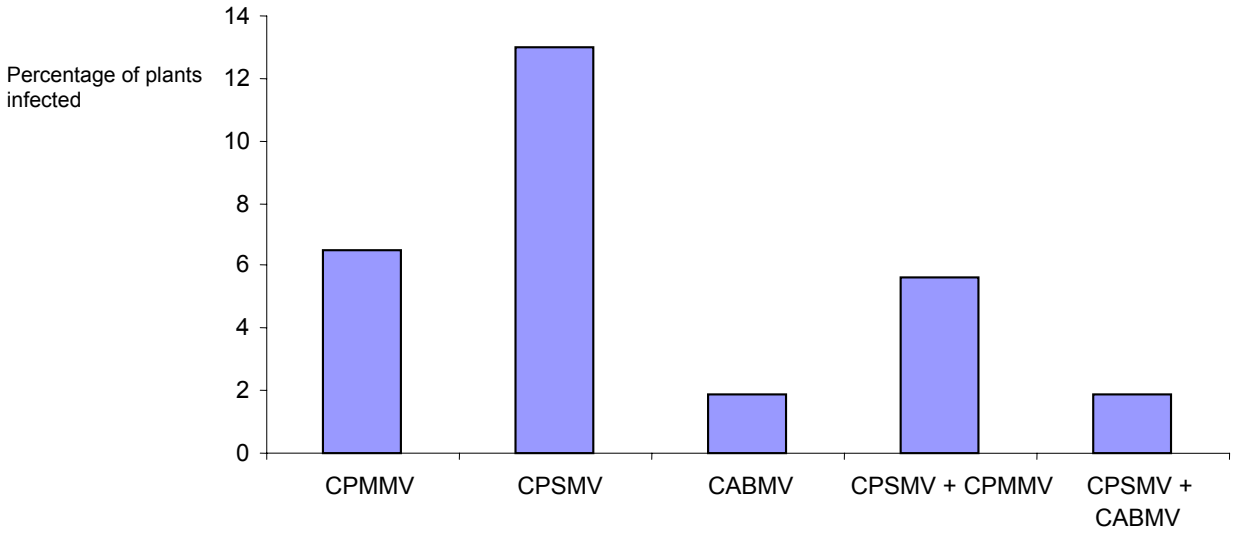

Figure 7: Occurrence of single and multiple virus infections in symptomatic cowpea plants in 2004

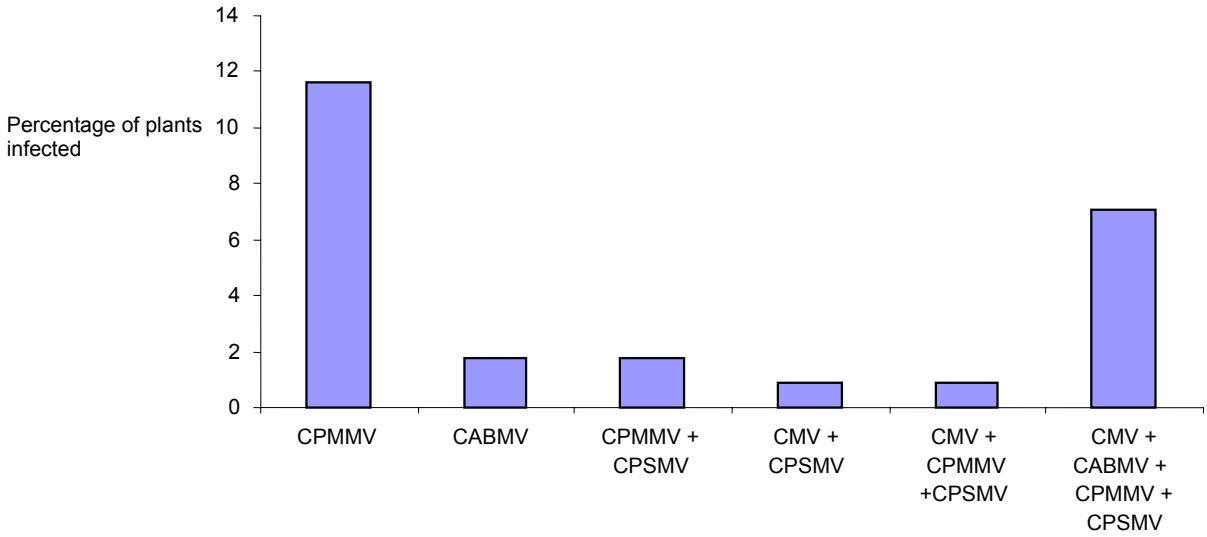

Figure 8: Occurrence of single and multiple virus infections in symptomatic cowpea plants in 2005

3.4 Discussion and conclusion

This study established the occurrence and identity of four viruses that are important in cowpea growing districts in eastern Uganda. The widespread distribution of virus symptoms on cowpea in the growing districts of Uganda reported herein and the severe levels of infection in the commonly planted cowpea, suggest that the viruses are economically important diseases of cowpea. The results obtained in this study showed that there was a substantial occurrence of viruses during 2004 and 2005. During the 2004 surveys, considerable variations of incidence and severity of virus symptoms among the districts were observed. The cowpea fields in Soroti, Tororo and Pallisa districts registered a higher incidence of virus symptoms than in Kumi, but the highest incidence was observed in Pallisa. Although the incidence of virus symptoms appeared generally high in Tororo district, the incidence of virus symptoms observed was relatively low compared to Soroti and Pallisa. The virus disease severity observed in Kumi district was consistently low in all of the fields surveyed.

In the 2005 surveys, all of the districts visited exhibited virus symptoms, with a higher incidence and severity in Kumi and Soroti districts compared to Tororo and Pallisa

districts. A similar trend of virus symptoms was observed in all fields in each district surveyed, with the exception of a few fields investigated in Tororo and Pallisa districts that registered low virus symptoms. In spite of the high virus incidence observed in the cowpea fields, there was generally a low severity in all of the districts surveyed. There was a lower virus severity registered in Tororo district than in Soroti.

The results obtained during the two years of surveys showed that viruses are widely distributed across the agro-ecological zones in the four districts. Virus incidence and severity was higher in fields surveyed in 2004 than in 2005. In 2004, the overall incidence was 75.5% and 23.2% for severity, while in 2005 the incidence was 69.3% and 11.2% for severity. However, the extent and source of infection varied greatly in the 2 y. Vacke (1983) indicated that favourable climatic conditions can prolong vector migration, enhance vector population and consequently, increase their potential to transmit wheat dwarf virus in wheat stands. Similarly, Bukvayová *et al*. (2006) has also attributed the epidemiology of vector-transmissible viruses to be related to weather conditions.

The virus severities observed in 2005 were very low, suggesting that there was probably an uneven distribution of virus vectors and consequently, low inoculum source to cause high virus incidence in fields of cowpea. Edema *et al*. (1997) and Shoyinka *et al*. (1997) attributed virus variability to changes in weather conditions within seasons and farming systems in the different environments. Perennial and weed hosts have also been shown to be important in the ecology of several viruses (Duffus, 1971; Thresh, 1974). The large populations of the virus vectors are usually found on the weeds, particularly during the second growing season, which may account for the greater population of aphids (Atiri *et al*., 1986).

On the cowpea samples exhibiting virus symptoms collected from 60 locations during 2004 and 2005 in the districts of Soroti, Kumi, Pallisa and Tororo, 228 positive samples were detected. Four virus types were identified, namely CABMV, CPSMV, CPMMV and CMV, suggesting their existence in the major cowpea growing regions in Uganda. The CPMMV was the most common virus while CPSMV was the second most prevalent virus identified in all surveyed cowpea growing districts. The results suggest that CABMV was the third most common virus identified in the samples collected from the four districts in 2004. The antisera for identifying CPCMV and CPMV did not react with the samples, suggesting these viruses may not be present in Uganda.

The results of the study showed that plant samples had a high prevalence of single virus infection compared to multiple virus infection. In single virus infected plants, CPSMV and CPMMV were the most common in 2004 and 2005, respectively, while CABMV was the least common in both years. In multiple infected plants, a combination of CPSMV + CPMMV was very common, while a combination of CPSMV + CABMV was the least common in 2004. In 2005, a combination of CMV + CABMV + CPMMV + CPSMV in the infected plants was the most common, while CMV + CPSMV and CMV + CPMMV + CPSMV were the least observed in the samples. These differences in the levels of occurrence of a particular virus being common in one year and not in the other year, may be explained on the basis of inoculum level, age of the plant, climatic conditions and cultivar type (Wisler *et al*., 1998). Studies have shown that the presence of viruses in a mixture may result in synergism or antagonism effects within the infected plants. For instance, viruses acting in synergistic manner enhance their infection rate, thus leading to the development of complexes of diseases (Vance *et al*., 1995; Fondong *et al*., 2000; Pita *et al*., 2001). Sakai *et al*., (1983) reported that some viruses may be antagonised when in a mixture with other viruses and their rate of infection may be affected compared to single virus infection. The higher infection of plants by CPSMV in the samples compared to CPMMV and CABMV could suggest its relative persistence under adverse environmental conditions over other viruses. However, there was no association between CPMMV and CABMV alone in the cowpea samples.

The study identified CABMV, CPMMV, CPSMV and CMV as the most important viruses affecting cowpea in the cultivated districts of Uganda. Since CPMMV and CPSMV were the most common viruses detected, this provides an opportunity for future breeding work for resistance in Uganda. The study showed that several viruses occur, and often in mixture, indicating how important viruses are in cowpea. The study also showed that there were variations in occurrence of virus infections in the two seasons surveyed in the districts. In a previous study, Edema *et al*. (1997) indicated that CABMV was the most common virus in the cowpea growing regions of Uganda. The study revealed the occurrence of CABMV in the four districts surveyed during 2004 and 2005. Therefore, it may not be valuable to select for CABMV resistance without taking the other viruses into account.

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CHAPTER FOUR

EVALUATION OF COWPEA GENOTYPES FOR RESISTANCE TO COWPEA APHID-BORNE MOSAIC VIRUS INFECTION IN UGANDA

Abstract

Fifty four improved cowpea genotypes including one local check were screened for resistance to CABMV during the first season of 2004 at Serere Agricultural and Animal Production Research Institute (SAARI) in Uganda. Twenty seven genotypes that showed resistance were selected for use in a cowpea improvement programme. Further screening was conducted in the second season of 2004 using the 27 genotypes. The genotypes were planted in single rows between the rows of the susceptible cultivar, *Ebelat*, at an interval of 10 d. This was to provide high pressure of aphid vector (*Aphis craccivora* Koch) and CABMV inoculum. In addition, the test genotypes were artificially inoculated with a CABMV extract on fully expanded primary leaves of fourteen day-old seedlings. The CABMV incidence and severity was assessed. Disease severity was assessed on a 0-60% visual estimation scale where 0 = with no symptoms and 60 = with severe symptoms. Serological analysis was conducted using DAS-ELISA. A specific DAS-ELISA kit to detect CABMV was used. In order to detect other viruses that attack cowpea, four additional kits were used to test for the presence of CPCMV, CPMMV, CPMV and CPSMV. The general findings from the DAS-ELISA tests revealed that 12 genotypes were positive for CABMV, four genotypes for CPCMV, 10 genotypes for CPSMV and 14 genotypes for CPMMV. These results clearly provide an indication that multiple virus infections are common among samples from field-grown cowpea. There were significant differences ($P \leq 0.001$) among the cowpea genotypes for CABMV incidence and severity. This indicated that there was genetic variability for resistance to CABMV among the test genotypes. Symptoms observed on cowpea plants included leaf mosaic, leaf chlorosis, leaf deformation and stunted plants. In the first season, the lowest and highest final incidence was 23.0 and 100.0%; severity was 10.0 and 67.5%; and area under disease progress curve was 6.8 and 47.4 at 56 d after inoculation. In the second season, the lowest and highest incidence was 53.1 and 100.0%; severity was 3.8 and 37.9%; and area under disease progress curve was 2.1 and 15.5 at 45 d after inoculation. Generally, lower disease severity was observed in the second season than in the first season. The correlation between yield and AUDPC was negative (r = -0.321,

P≤0.001), suggesting a negative association between yield and virus infection. In the screening for CABMV resistant genotypes, SECOW-2W, MU-93, BROWNMIX-SEL2, IT82D-516-2, IT85F-2841, K-80, IT82D-889, FE87, KVU419, BROWNMIX-SEL1, IT90K-109, TVX337-025, FE15 and FE60 possessed genes resistant to CABMV and other viruses that were identified in this study. The available sources with combined resistance would be used in future breeding work to improve yield.

4.1 Introduction

Studies on the occurrence of cowpea diseases under different seasons and cropping systems have shown that cowpea aphid-borne mosaic virus (CABMV) is a very common disease in cowpea growing regions in Uganda (Edema *et al*., 1997). The virus is transmitted by aphids (*Aphis craccivora* Koch) in a non-persistent manner (Atiri *et al*., 1984). The nature and severity of symptoms induced by CABMV varies with host cultivars, virus isolate and time of infection (Konate and Neya, 1996). The disease symptoms on susceptible cowpea plants show vein clearing, leaf blistering, leaf mosaic, interveinal chlorosis, stunted plants and leaf deformation (Konate and Neya, 1996). The resultant infection leads to a reduction in plant growth and consequently, in yield (Kaiser and Mossahebi, 1975; Fischer and Lockhart, 1976; Fraser, 1992; Shoyinka *et al*., 1997).

There are various methods that are widely applied for control of virus diseases. Despite considerable research, there are no chemicals that provide satisfactory control of virus diseases (Fraser, 1992). Commonly, farmers spray insecticide to prevent virus vectors from reaching the crop, but this is uneconomical. It also poses health hazards if not used judiciously by cowpea growers (Isubikalu *et al*., 2000). Studies by Isubikalu *et al*. (1999) indicated that increased use of pesticides increases the development of insect-resistance as well as affecting other beneficial insects in the ecosystem. Therefore, the use of host plant resistance remains the most effective, economical long-term control method to combat cowpea virus diseases.

The identification of resistance to viruses is therefore an important component of the genetic improvement of cowpea. At the International Institute of Tropical Agriculture (IITA), sources of resistance to CABMV have been developed (Singh *et al*., 2003). Such resistance could be incorporated into the susceptible local cowpea cultivars in order to enhance the production of cowpea. It is imperative that for resistant genotypes to be identified, rigorous screening needs to be carried out to effectively select for possible

future use in cowpea improvement. Screening for resistant genotypes is the first step when the aim is to identify resistance for breeding purposes. The objective of this study was to explore the use of artificial virus inoculation and spreader rows simultaneously under field conditions to screen for resistance to CABMV.

4.2 Materials and methods

4.2.1 Study area and site characteristics

The study was conducted at Serere Agricultural and Animal Production Research Institute (SAARI) located in the north-east of Soroti district in eastern Uganda. The average rainfall is 102.8 mm mo^{-1} and the average monthly maximum temperature is 30.4°C and minimum temperature is 18.0°C. The soil type is sandy loam and the vegetation is predominantly grassland.

4.2.2 Cowpea genotypes evaluated

A total of 54 cowpea genotypes, collected from four countries, were screened for resistance to CABMV under field conditions. The pedigree, characteristics and sources of the cowpea genotypes evaluated are presented (Table 17). Forty two genotypes were obtained from South Africa, seven from Uganda including one local check, four from Kenya and one from the International Institute for Tropical Agriculture (IITA).

4.2.3 Virus inoculum source and maintenance

To propagate the CABMV, live viruliferous aphids, especially in the wingless stage, were collected from a previously infested field of cowpea at SAARI and transferred within less than a minute onto healthy young potted seedlings of cowpea in an insect-proof cage made of shade net of 5 x 5 x 2.5 m (Figure 9). The healthy young growing cowpea seedlings were assessed visually and assumed to be uninfected by viruses before the plants were infested with aphids. This was to enhance transmission of the virus onto the healthy seedlings. The aphids were allowed to feed on the plants for a period of 2 wk for proper transmission of virus and aphids were continuously transferred and maintained on new growing cowpea seedlings in pots in an insect-proof cage. Symptom development

on the leaves was observed. To confirm that the symptomatic plants were the result of CABMV infection, the symptomatic leaves were detached and tested for CABMV with DAS-ELISA at Makerere University, Kampala, Uganda. The CABMV-infected plants served as an inoculation source for testing cowpea materials in the field. It was important that a regular transfer of live viruliferous aphids was maintained on young growing seedlings of cowpea within the insect-proof cage. This was consistently carried out for the entire period of the research study. Similarly, testing of the symptomatic leaf samples for the presence of the virus was occasionally carried out to verify the presence of the virus during the time of inoculation.

Table 17: Pedigree, characteristic and sources of cowpea genotypes evaluated

Pedigree	Growth characteristics	Maturity period in days	Origin
122BLUE	Erect	83	South Africa
CP24/FE53	Erect	78	,,
8017	Erect	78	,,
FE26	Spreader	70	,,
FE126	Spreader	78	,,
FE104	Spreader	70	,,
B359	Spreader	78	,,
3-4-11	Erect	78	,,
FE17	Erect	70	,,
FE42	Erect	78	,,
1-8-5	Erect	78	,,
FE33	Erect	78	,,
FE15	Spreader	70	,,
FE38	Erect	70	,,
FE25	Erect	70	,,
BLUEMIX	Erect	70	,,
FE28	Erect	78	,,
FE34	Erect	78	,,
FE68	Spreader	78	,,
BROWNMIX-SEL1	Erect	78	,,
CHINO E1	Spreader	78	,,
FE12	Spreader	78	,,
FE84	Spreader	78	,,
FE87	Erect	70	,,
FE69	Erect	70	,,
FE67	Erect	78	,,
CHINOMI	Erect	78	,,
FE60	Erect	70	,,
1-2-1	Erect	83	,,
BROWNMIX-SEL2	Erect	78	,,
FE125	Spreader	70	,,
FE95	Spreader	70	,,
FE96	Erect	70	,,
122RED	Spreader	78	,,
FE83	Erect	78	,,
UCR194	Erect	64	,,
FE20	Erect	70	,,
1-12-1	Erect	83	,,
FE86	Spreader	83	,,
3-411	Erect	83	,,

Table 17: Continued

Pedigree	Growth characteristics	Maturity period in days	Origin
CB5	Erect	70	,,
BECH WHITE	Erect	70	,,
KVU27-1	Spreader	78	Kenya
M66	Spreader	70	,,
K-80	Spreader	70	,,
KVU419	Erect	70	,,
IT85F-2841	Erect	78	Uganda
Blackcowpea (local check)	Spreader	70	,,
IT82D-516-2	Spreader	78	,,
SECOW-2W	Spreader	70	,,
IT90K-109	Spreader	70	,,
MU-93	Spreader	70	,,
TVX337-025	Spreader	70	,,
IT82D-889	Erect	70	Nigeria

Figure 9: Aphids reared on cowpea seedlings for CABMV transmission in an insect proof cage

4.2.4 Field establishment of cowpea genotypes

The study was carried out in two seasons at SAARI in 2004. During the first season, 54 genotypes were evaluated to screen and select for resistance to CABMV. The design was a randomised complete block design with two replications. The replicates were separated by 2 m alleys with 1 m between plots and blocks. There were nine blocks each containing six plots within a replication. An individual genotype was planted at a spacing of 900 mm between rows and 400 mm within rows in a plot size of 4 x 3.6 m (Figure 10).

Out of the total of 54 cowpea genotypes established during the first season of 2004, 27 genotypes were discarded as a result of severe infection by CABMV, while the 27 genotypes with low infection levels were retained for further evaluation in the second season of 2004. Three replications were established using a similar design, spacing and plot size as arranged during the first season. In this case, there were nine blocks each containing three plots within a replication. The screening and selection of the genotypes for further evaluation was done using the results of visual assessment and ELISA tests (section 4.2.7). This was to enable selection of genotypes with good resistance to CABMV and for possible future use in the breeding work.

Yield was determined for each cowpea genotype at the end of the maturity period by threshing and weighing the dried seeds. The two central rows of each plot were considered for yield, while disregarding 0.5 m around the plot edges to minimise border effects. Seed weight, measured in g m^{-2}, was converted to kg ha^{-1}.

4.2.5 Inoculation

Two infection methods were employed, namely, spreader row-plants and artificial inoculation. The first method was done by planting the individual genotypes in rows in each plot surrounded by a susceptible cultivar *Ebelat* as shown by red arrows (Figure 10). The susceptible cultivar was planted 10 d earlier to provide high pressure of aphids (*Aphis Craccivora* Koch) and CABMV inoculum (Figure 10). In addition, the second method was carried out on the test genotypes by artificial inoculation of fully expanded primary leaves of fourteen day-old seedlings with the virus extract. The extract was prepared by detaching and grinding the symptomatic leaves obtained from the insect-proof cage in a 0.01M phosphate buffer. The aphids were allowed to feed on the plants

for a period of 2 wk for proper transmission of the virus in an insect-proof cage (Figure 9). The symptomatic leaf extract was used to inoculate the test genotypes in the field following carborundum powder (abrasive agent) application to the leaves to be inoculated. The carborundum powder was used to induce wounds on the plants to enhance virus penetration into the plant cells. The two infection methods provided an even distribution of disease pressure in the trial.

Figure 10: Screening cowpea genotypes to CABMV resistance. Red arrow shows susceptible spreader row (Ebelat)

4.2.6 Data assessment for cowpea aphid-borne mosaic virus symptoms on cowpea genotypes

Plants were monitored for virus symptom development at intervals of one week and this was continued up to physiological maturity. The response of cowpea genotypes to CABMV inoculation was assessed as disease incidence and severity. Disease incidence and severity in a plot were determined using the method described in Chapter 3, Section 3.2.2.

Five data sets of severity assessments were used to calculate the area under disease progress curve (AUDPC) for each cowpea genotype. Thus, AUDPC was calculated as described by Anilkumar *et al*. (1994).

$$AUDPC = \sum_{i=1}^{n-1} [(X_i + X_{i+1})/2](t_{i+1}-t_i)$$

Where n = the total number of observations

X_i = disease severity in percentages at the i^{th} observation

t = time in days after virus inoculation at i^{th} observation

$t_{i+1}-t_i$ = interval between two consecutive observations

4.2.7 Double Antibody Sandwich Enzyme-linked immunosorbent assay (DAS-ELISA)

The presence of CABMV, CPCMV, CPSMV, CPMMV and CPMV was detected using DAS-ELISA as described in Chapter 3, Section 3.2.3. The ELISA kit for CMV was not available and the presence of this virus was not tested.

4.2.8 Data analysis

The data on disease incidence, disease severity, AUDPC and yield were analysed using the Genstat computer package and means were compared with the Least Significant Difference (LSD). Yield data was analysed using REML in GenStat computer package, where genotypes were considered fixed effects and blocks within replications were considered random. Phenotypic correlations between AUDPC and yield were determined

using Pearson's Correlation procedure to determine whether there is causal relationship between the two.

4.3 Results

4.3.1 Reactions of cowpea genotype to CABMV virus infection

Based on field observations, the CABMV induced disease symptoms among the cowpea genotypes. The reaction of the genotypes observed during the two seasons of 2004 consisted of symptomless plants, leaf deformation, leaf mosaic, stunted plants and chlorotic plants (Figure 11). There were significant ($P \leq 0.001$) differences among genotypes for incidence and severity for CABMV at all stages assessed in the first season of 2004 (Table 18). There were also significant ($P \leq 0.001$) differences among genotypes for AUDPC assessed in the first season of 2004 (Table 18). The significant differences observed may suggest a possibility of great variability of resistance among the cowpea genotypes to CABMV infection.

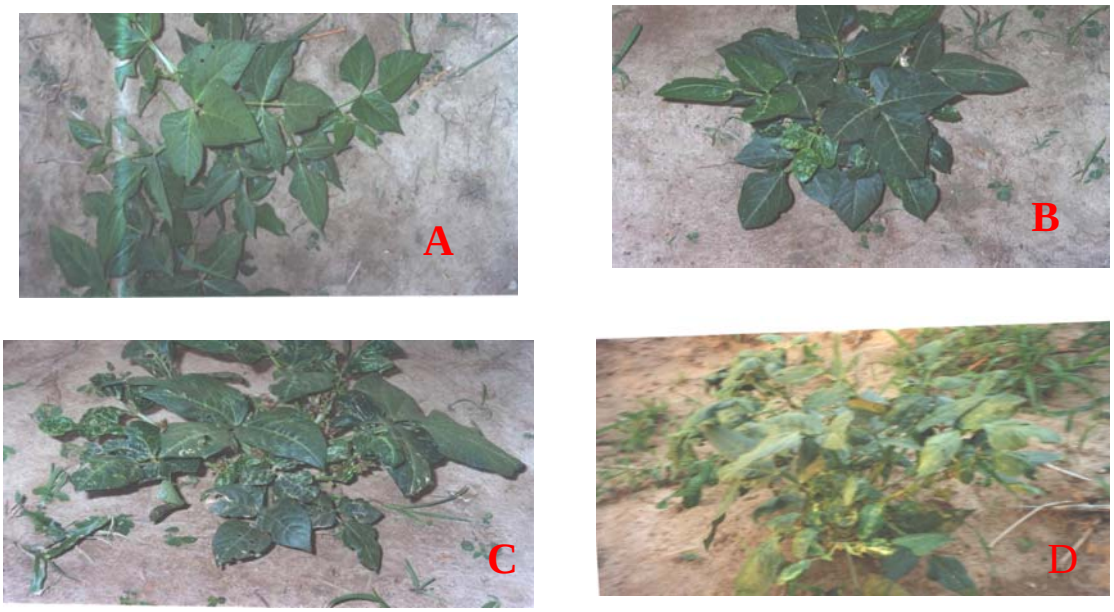

Figure 11: Symptoms of CABMV disease observed on cowpea genotypes. A, Leaf of healthy cowpea. B, Infected plant showing mild leaf mosaic. C, Severe mosaic accompanied with leaf deformation and stunted plant. D, Leaf mosaic, leaf deformation and severe chlorotic plant

4.3.2 Response of 54 cowpea genotypes to CABMV infection in first season of 2004

The results showed that symptoms developed within 14 d after inoculation on 54 cowpea genotypes. There were appreciable differences in the levels of infection with CABMV among the cowpea genotypes. In some instances, there was delayed expression of symptoms by up to 1-2 wk after inoculation (BROWNMIX-SEL1, BROWNMIX-SEL2, FE86, FE34 and FE67), but a noticeable appearance of mild to severe symptoms was later observed (Table 18). There was development of mild symptoms on some cowpea genotypes that exhibited initially mild severity, which then progressed and stabilised towards the end of the growing cycle of the plants (Table 18).

At 7 d after inoculation (DAI), most cowpea genotypes developed virus symptoms with varying levels of symptom expression. For instance, the initial mean disease incidence at 7 DAI ranged from 0.0 to 47.5%, while the final mean disease incidence at 56 DAI ranged from 23.0 to 100.0% (Table 18). Only 10 cowpea genotypes had a mean disease incidence equivalent to or below 60%, while 17 genotypes had a mean incidence of 100.0% at 56 DAI (Table 18).

There was a high incidence of the disease with most genotypes reaching more than 80% virus mean incidence (Table 18). The mean disease severity at the initial stage of assessment for virus symptom development began at 7 DAI and ranged from 0.0 to 25.0%, while the final severity at 56 DAI ranged from 10.0 to 67.5%. Thirty six cowpea genotypes had a mean disease severity of 40.0% or more at 56 DAI, while 18 genotypes had less than 40.0%. The cowpea genotypes BROWNMIX-SEL1 and BROWNMIX-SEL2 had the lowest disease severities compared to 1-12-1 and 3-411 at 56 DAI (Table 18).

The overall values for AUDPC calculated from five data sets ranged from 6.8 to 47.4 for cowpea genotypes assessed during the entire period of 56 DAI (Table 18). Thirty five cowpea genotypes had AUDPC values of greater than 25.0. Generally, lower AUDPC values were observed for BROWNMIX-SEL1 and BROWNMIX-SEL2 compared to 3-411 and FE83. The genotype BROWNMIX-SEL1 showed a range in disease level of 0.0 to 23.0% for incidence, 0.0 to 10.0% for severity at 7 DAI and 56 DAI, respectively. On the other hand, Blackcowpea, used as a local check, had high mean disease levels of 12.5 at 7 DAI to 88.0% 56 DAI for incidence, 10.0 at 7 DAI to 40.0% at 56 DAI for severity. The genotype BROWNMIX-SEL1 had a mean AUDPC of 6.8, indicating that it was the least infected by CABMV, while genotype 3-411 with a mean AUDPC of 47.4 was the

most infected (Table 18). The local check, Blackcowpea had a mean AUDPC of 28.9, indicating that it was better than genotype 3-411, but worse than BROWNMIX-SEL1. This suggested that the local cowpea cultivar (Blackcowpea) was more susceptible to CABMV disease than the cowpea genotype (BROWNMIX-SEL1); probably the latter has a resistance mechanism to slow down or withstand virus replication, mulplication and movement in the host plant cells.

The AUDPC at the end of the trial in the first season of 2004 was used to categorise each of the 54 cowpea genotypes evaluated as resistant (0.0-15.0%), moderately resistant (15.0-27.0%), moderately susceptible (27.0-35.0%) and very susceptible (>35.0%). Based on the results of the AUDPC of the grouping of reaction types, five cowpea genotypes (BROWNMIX-SEL1, BROWNMIX-SEL2, SECOW-2W, FE87 and MU-93) were considered resistant, 19 moderately resistant, 17 moderately susceptible and 13 were very susceptible (Table 18).

Table 18: Mean incidence (%), severity (%), and AUDPC, of 54 cowpea genotypes evaluated after planting in the field inoculated with cowpea aphid-borne mosaic virus during the first season of 2004

	Mean incidence (%)					Mean severity (%)					*AUDPC
	Days after inoculation (DAI)					Days after inoculation (DAI)					
Genotype	**7**	**14**	**28**	**42**	**56**	**7**	**14**	**28**	**42**	**56**	
KVU27-1	15.0	30.0	35.0	54.0	63.5	5.0	10.0	20.0	30.0	30.0	21.1
M66	25.0	35.0	47.5	72.5	80.0	10.0	20.0	30.0	30.0	40.0	27.9
1-2-1	22.5	50.0	62.5	93.8	100.0	10.0	15.0	30.0	50.0	50.0	34.0
FE126	27.5	35.0	50.0	78.0	92.0	15.0	25.0	30.0	30.0	40.0	29.3
3-4-11	35.0	50.0	67.5	100.0	100.0	15.0	30.0	40.0	50.0	50.0	40.4
8017	33.8	50	62.5	97.0	100.0	10.0	20.0	47.5	50.0	50.0	40.0
FE53	27.5	50.0	65.0	94.5	100.0	15.0	25.0	30.0	40.0	50.0	33.6
122RED	7.5	50.0	60.0	90.0	100.0	5.0	15.0	30.0	50.0	50.0	33.5
FE84	6.9	25.0	33.8	46.5	54.8	5.0	7.5	20.0	20.0	30.0	17.7
1-8-5	47.5	50.0	72.5	100.0	100.0	25.0	30.0	50.0	50.0	50.0	44.4
FE33	26.3	40.0	47.5	74.5	88.0	10.0	15.0	30.0	40.0	40.0	29.7
FE125	32.5	38.8	58.8	91.2	100.0	25.0	30.0	30.0	37.5	40.0	33.2
Blackcowpea (check)	12.5	37.5	47.5	74.5	88.0	10.0	25.0	30.0	30.0	40.0	28.9
1-12-1	37.5	66.2	90.0	96.5	100.0	20.0	25.0	50.0	50.0	67.5	45.0
CHINOE1	32.5	50.0	63.8	95.8	100.0	7.5	15.0	50.0	50.0	50.0	39.3
FE83	43.8	50.0	72.5	100.0	100.0	25.0	37.5	50.0	50.0	50.0	45.6
FE28	35.0	55.0	72.5	100.0	100.0	15.0	30.0	40.0	50.0	60.0	41.8
122BLUE	27.5	50.0	61.3	93.8	100.0	7.5	15.0	40.0	50.0	50.0	36.6
3-411	43.8	58.8	76.3	100.0	100.0	15.0	37.5	50.0	50.0	67.5	47.4
B359	27.5	35.0	50.0	78.0	92.0	15.0	25.0	30.0	30.0	40.0	29.3

Table 18: Continued

	Mean incidence (%)					Mean severity (%)					*AUDPC
	Days after inoculation (DAI)					Days after inoculation (DAI)					
Genotype	**7**	**14**	**28**	**42**	**56**	**7**	**14**	**28**	**42**	**56**	
FE68	35.0	50.0	72.5	100.0	100.0	25.0	40.0	30.0	50.0	50.0	41.1
BLUEMIX	22.5	40.0	46.3	77.8	86.0	7.5	15.0	30.0	40.0	40.0	29.5
FE20	25.0	40.0	52.5	80.5	89.5	10.0	20.0	30.0	30.0	50.0	29.3
FE26	25.0	45.0	60.0	92.5	100.0	10.0	30.0	30.0	40.0	50.0	34.7
FE104	27.5	45.0	57.5	88.5	96.0	15.0	25.0	30.0	40.0	50.0	33.2
CB5	32.5	50.0	61.3	95.8	100.0	10.0	25.0	40.0	50.0	50.0	38.8
FE96	30.0	42.5	52.5	82.5	92.5	10.0	30.0	30.0	30.0	50.0	31.4
FE42	7.5	20.0	38.8	48.0	57.0	5.0	15.0	20.0	20.0	30.0	19.3
CHINOMI	38.8	43.8	55.0	88.0	94.5	10.0	37.5	40.0	40.0	40.0	37.4
BROWNMIX-SEL1	0.0	7.5	13.8	16.8	23.0	0.0	5.0	10.0	10.0	10.0	6.8
1T82D-889	22.5	35.0	46.3	70.8	82.8	7.5	15.0	30.0	30.0	40.0	26.6
BROWNMIX-SEL2	0.0	5.0	17.5	25.5	32.0	0.0	2.5	7.5	10.0	20.0	8.4
FE60	10.0	15.0	33.8	53.3	66.3	5.0	5.0	20.0	30.0	30.0	20.0
UCR194	7.5	12.5	20.0	35.0	42.5	5.0	5.0	15.0	25.0	25.0	16.5
IT85F-2841	15.0	35.0	45.0	69.5	81.8	5.0	17.5	30.0	30.0	40.0	27.0
FE86	0.0	15.0	41.3	50.3	69.0	0.0	5.0	30.0	30.0	40.0	23.9
FE84	17.5	25.0	57.5	82.0	100.0	5.0	7.5	20.0	20.0	30.0	37.2
FE67	0.0	35.0	37.5	55.5	64.5	0.0	10.0	10.0	30.0	40.0	19.3
KVU419	15.0	25.0	35.0	52.0	60.5	10.0	10.0	15.0	20.0	30.0	17.2
IT82D-516-2	22.5	37.5	45.0	69.0	81.0	15.0	20.0	25.0	30.0	30.0	25.4
FE87	10.0	17.5	22.5	34.0	39.8	5.0	7.5	10.0	15.0	20.0	12.0
K-80	33.8	40.0	46.3	69.8	80.5	20.0	20.0	20.0	20.0	37.5	22.5
FE15	10.0	35.0	45.0	75.0	87.5	5.0	20.0	30.0	30.0	40.0	28.9

Table 18: Continued

	Mean incidence (%)					Mean severity (%)					*AUDPC
	Days after inoculation (DAI)					Days after inoculation (DAI)					
Genotype	**7**	**14**	**28**	**42**	**56**	**7**	**14**	**28**	**42**	**56**	
FE69	22.5	37.5	43.8	68.3	80.5	7.5	20.0	25.0	30.0	40.0	26.3
SECOW-2W	15.0	15.0	25.0	37.0	53.0	5.0	5.0	10.0	10.0	30.0	11.5
FE95	10.0	35.0	38.8	49.0	58.5	5.0	20.0	20.0	30.0	40.0	20.4
BECHWHITE	15.0	35.0	40.0	61.0	71.5	5.0	10.0	20.0	30.0	40.0	22.5
FE38	15.0	30.0	36.3	54.8	64.0	7.5	10.0	15.0	30.0	30.0	19.8
IT90K-109	17.5	25.0	37.5	55.5	64.5	10.0	10.0	20.0	20.0	30.0	18.6
MU-93	15.0	15.0	28.8	44.3	56.0	5.0	5.0	10.0	20.0	30.0	14.3
FE17	25.0	32.5	42.5	66.5	78.5	10.0	20.0	30.0	30.0	30.0	26.5
TVX337-025	27.5	32.5	42.5	67.5	80.0	10..0	25.0	30.0	30.0	30.0	27.5
FE25	15.0	20.0	43.8	60.3	88.0	5.0	5.0	30.0	30.0	40.0	24.3
FE 34	0.0	40.0	45.0	69.5	81.8	0.0	7.5	35.0	40.0	40.0	28.8
Mean	21.3	35.9	48.6	71.8	80.8	9.6	17.9	29.0	33.8	40.3	28.2
Significance of F	***	***	***	***	***	**	***	***	***	*	***
LSD (0.05)	21.6	21.7	14.6	21.9	23.5	12.2	16.5	18.9	19.4	24.5	14.1
CV%	50.6	30.1	15.0	15.2	14.5	63.3	46.0	32.5	28.6	30.3	24.9

*, ** and *** are significant at P≤0.05, P≤0.01 and P≤0.001 probability level, respectively

*AUDPC was calculated from five data sets of disease severity assessments

4.3.3 Yield and yield components of 54 cowpea genotypes evaluated during the first season of 2004

The mean yield and yield components were significantly ($P \leq 0.01$) different among the cowpea genotypes (Table 19). There was generally a very low mean grain yield obtained from most cowpea genotypes, with 23 genotypes attaining less than 100 kg ha^{-1} (Table 19). The mean yield for cowpea genotypes ranged from 7.9 to 277 kg ha^{-1}. The genotype FE69 attained the highest mean grain yield of 277.0 kg ha^{-1} and 1-12-1 was the lowest with 7.9 kg ha^{-1}. The genotypes MU-93, BECHWHITE, KVU27-1, TVX337-025, FE126, FE38, IT82D-889 and K-80 had intermediate mean grain yields greater than 200 kg ha^{-1} (Table 19).

Cowpea genotype SECOW-2W had the highest number of pods per plant with a mean of 33.0 (Table 19). The genotype FE125, categorised as susceptible, attained the longest pod length with a mean of 199 mm suggesting that although susceptible to the virus, it can still tolerate and perform relatively well in spite of infection. The shortest pod length was attained by FE26 genotype with a mean of 105 mm. The genotype FE125 had the highest number of seeds per pod with a mean of 17.0 and CHINOMI had the least number of seeds per pod with a mean of 4.0.

Table 19: Yield components and yield for 54 cowpea genotypes evaluated during the first season of 2004

Genotypes	Yield component			
	Number of pods/ plant	Pod length (mm)	Number of seeds/pod	Yield (kg ha^{-1})
KVU27-1	11.0	186	15.0	234.7
M66	15.0	170	15.0	155.8
1-2-1	10.0	139	9.0	38.4
FE126	15.0	164	12.0	212.9
3-4-11	10.0	149	9.0	68.6
8017	11.0	119	7.0	36.8
FE53	8.0	146	10.0	80.1
122RED	7.0	170	12.0	66.0
FE84	6.0	185	9.0	42.3
1-8-5	6.0	122	6.0	21.8
FE33	8.0	144	9.0	42.9
FE125	12.0	199	17.0	140.4
Blackcowpea (check)	6.0	138	14.0	123.7
1-12-1	6.0	117	5.0	7.9
CHINOE1	12.0	165	8.0	55.1
FE83	14.0	172	12.0	160.3
FE28	11.0	195	12.0	64.7
122BLUE	5.0	123	11.0	26.4
3-411	7.0	142	8.0	17.3
B359	13.0	190	13.0	132.0
FE68	7.0	170	13.0	112.1
BLUEMIX	12.0	148	12.0	76.9
FE20	16.0	136	9.0	131.0
FE26	16.0	105	9.0	19.5
FE104	15.0	181	10.0	114.0
CB5	12.0	165	9.0	52.6
FE96	22.0	152	11.0	70.8
FE42	16.0	180	16.0	107.7
CHINOMI	7.0	128	4.0	23.8
BROWNMIX-SEL1	6.0	178	13.0	139.3
1T82D-889	8.0	179	14.0	201.2
BROWNMIX-SEL2	6.0	154	11.0	28.8
FE60	13.0	164	13.0	169.5
UCR194	19.0	149	11.0	33.5
IT85F-2841	11.0	166	13.0	102.2
FE86	7.0	145	9.0	21.7
FE84	6.0	185	9.0	112.6

Table 19: Continued

Genotypes	Yield component			
	Number of pods/ plant	Pod length (mm)	Number of seeds/pod	Yield (kg ha^{-1})
FE67	6.0	144	13.0	78.7
KVU419	19.0	155	12.0	151.3
IT82D-516-2	14.0	162	14.0	63.3
FE87	10.0	190	13.0	152.4
K-80	12.0	190	16.0	200.8
FE15	8.0	152	11.0	142.0
FE69	26.0	180	15.0	277.0
SECOW-2W	33.0	166	14.0	194.9
FE95	19.0	157	10.0	136.4
BECHWHITE	17.0	177	13.0	241.4
FE38	17.0	165	12.0	208.7
IT90K-109	17.0	149	14.0	157.5
MU-93	23.0	157	13.0	242.4
FE17	12.0	193	12.0	173.0
TVX337-025	17.0	166	14.0	215.0
FE25	13.0	172	11.0	122.0
FE 34	7.0	187	13.0	80.0
Mean	12.1	161	11.5	112.6
Significance of F	***	***	***	**
LSD (0.05)	9.9	36.0	4.3	141.6
CV%	40.8	11.1	18.7	62.7

** and *** are significant at P≤0.01 and P≤0.001 probability level, respectively

4.3.4 Phenotypic correlation of AUDPC for CABMV, yields and yield components of cowpea

The phenotypic correlations between yield and yield components, and yield and AUDPC were significant (P≤0.05) (Table 20). Similarly, the correlations between pod numbers per plant and pod length, and number of seeds per pod were also significant and positive. Correlation between pod length and seeds per pod was significant and positive (Table 20). Correlations between AUDPC with pod length, and number of seeds per pod were significant and negative. The pod number per plant and seeds per pod (r = 0.506, P≤0.001) and pod length (r = 0.481, P≤ 0.001) were positively correlated. The positive correlations of number of seeds per pod, pod length and pod numbers per plant are indicative of the major factors contributing to increases in yields of cowpea. In contrast,

the correlation between yield and AUDPC was significantly negative (r = -0.321, P≤0.001) indicating that yield can be negatively affected by the disease.

Table 20: Phenotypic correlation matrix for yield and yield components associated with AUDPC of CABMV infection in the field during first season of 2004

	Pods per plant	Pod length	Seeds per pod	AUDPC
Pod length	0.209*			
Seeds per pod	0.266**	0.694***		
AUDPC	-0.247**	-0.284**	-0.379***	
Yield	0.506***	0.481***	0.506***	-0.321***

*, ** and *** significant at P≤0.05, P≤0.01 and P≤0.001, respectively

4.3.5 Detection of CABMV and other cowpea viruses in cowpea leaf samples using DAS-ELISA

The results of the ELISA tests revealed that of the 54 cowpea genotypes screened, 18 genotypes tested positive to CABMV, while none was positive to CPMV, CPMMV, CPMV and CPSMV (Table 21). Despite the visual observations of virus symptoms in the field, there was no detection of any of the viruses by DAS-ELISA tests, suggesting that probably there was too low a virus concentration in the plants to be detected. A large number of genotypes showed virus symptoms, but no detections were observed on M66, 8017, 122RED, 1-8-5, FE33, FE125, 1-12-1, CHINOE1, FE28, 3-411, B359, FE68, BLUEMIX, FE104, CB5, FE96, FE42, IT82D-889, FE60, FE86, FE67, KVU419, K-80, FE15, FE69, SECOW-2W, FE95, BECHWHITE, FE38, IT90K-109, MU-93, FE17, TVX337-025 and FE34. The test of leaf samples of a particular genotype showed a range of reactions to the CABMV antiserum. However, some plants exhibited symptoms that did not test positive to any of the above viruses.

4.3.6 Criteria used for selection of cowpea genotypes with good resistance to CABMV for further evaluation

The assessment, which was based on final severity of disease and AUDPC, enabled the selection of genotypes with resistance to CABMV. In addition, ELISA tests also permitted the selection of some cowpea genotypes that reacted negatively to the antisera. The genotypes that had AUDPC values ranging from 0.0-15.0 and 15.0-27.0 were considered resistant and moderately resistant, respectively, in spite of the traces of weak yellow colouration shown by the ELISA tests in some cases. The two criteria (visual and DAS-ELISA assessment) were useful for the identification of resistant genotypes. The 27 cowpea genotypes were selected for further evaluation in the field during the second season of 2004. This enabled selection of the most resistant genotypes to CABMV for use in the development of virus disease resistant cultivars.

Table 21: Reactions of leaf samples of the 54 cowpea genotypes in DAS-ELISA test to CABMV, CPCMV, CPMMV, CPMV and CPSMV in first season of 2004

Genotype	**Reaction response of viruses**				
	CABMV	CPCMV	CPMMV	CPMV	CPSMV
KVU27-1	+	-	-	-	-
M66	-	-	-	-	-
1-2-1	+++	-	-	-	-
FE126	+++	-	-	-	-
3-4-11	+++	-	-	-	-
8017	-	-	-	-	-
FE53	+++	-	-	-	-
122RED	-	-	-	-	-
FE84	+++	-	-	-	-
1-8-5	-	-	-	-	-
FE33	-	-	-	-	-
FE125	-	-	-	-	-
Blackcowpea (check)	+++	-	-	-	-
1-12-1	-	-	-	-	-
CHINOE1	-	-	-	-	-
FE83	+++	-	-	-	-
FE28	--	-	-	-	-
122BLUE	+++	-	-	-	-
3-411	-	-	-	-	-
B359	-	-	-	-	-

Table 21: Continued

Genotype	Reaction response of viruses				
	CABMV	CPCMV	CPMMV	CPMV	CPSMV
FE68	-	-	-	-	-
BLUEMIX	-	-	-	-	-
FE20	+++	-	-	-	-
FE26	-	-	-	-	-
FE104	-	-	-	-	-
CB5	-	-	-	-	-
FE96	-	-	-	-	-
FE42	-	-	-	-	-
CHINOMI	+++	-	-	-	-
BROWNMIX-SEL1	+	-	-	-	-
1T82D-889	-	-	-	-	-
BROWNMIX-SEL2	+	-	-	-	-
FE60	-	-	-	-	-
UCR194	+	-	-	-	-
IT85F-2841	+	-	-	-	-
FE86	-	-	-	-	-
FE84	+	-	-	-	-
FE67	-	-	-	-	-
KVU419	-	-	-	-	-
IT82D-516-2	+	-	-	-	-
FE87	+	-	-	-	-
K-80	-	-	-	-	-
FE15	-	-	-	-	-
FE69	-	-	-	-	-
SECOW-2W	-	-	-	-	-
FE95	-	-	-	-	-
BECHWHITE	-	-	-	-	-
FE38	-	-	-	-	-
IT90K-109	-	-	-	-	-
MU-93	-	-	-	-	-
FE17	-	-	-	-	-
TVX337-025	-	-	-	-	-
FE25	+	-	-	-	-
FE 34	-	-	-	-	-

- no reaction observed with DAS-ELISA test
+ Positive reaction, but with very weak yellow coloration with ELISA test after elapse of 90 minutes from the time a substrate was added,

+++ positive reaction with very strong yellow coloration

4.3.7 Response of the selected 27 cowpea genotypes to CABMV infection in second season of 2004

There were significant ($P \leq 0.001$) differences among the 27 genotypes for incidence, severity and AUDPC of CABMV (Table 22). The results revealed that the cowpea genotypes were susceptible to CABMV, but some exhibited milder symptoms than others. The reactions exhibited by the cowpea genotypes included stunted plants, mosaic leaves, leaf mottling, leaf deformation and mild leaf chlorosis. The results showed that virus symptoms developed within 7 DAI and the symptoms progressed rapidly on the individual plants up to maturity periods, especially at 45 d after inoculation (Table 22). For instance, the initial mean disease incidence ranged from 17.4 to 72.6% at 7 DAI and final mean disease incidence ranged from 53.1 to 100.0% at 45 DAI (Table 22). Four cowpea genotypes, namely: IT85F-2841, BROWNMIX-SEL2, SECOW-2W and MU-93, had a mean disease incidence of less than 70.0%. However, there were moderate levels of mean disease severity among the genotypes tested. The initial mean disease severity at 7 DAI ranged from 1.2 to 6.8% and the final mean disease severity at 45 DAI ranged from 3.8 to 37.9%. The genotypes; BROWNMIX-SEL2, IT85F-2841, IT82D-516-2 K-80, SECOW-2W and MU-93 had the lowest mean disease severity less than 10.0% at 45 DAI. The values of AUDPC computed from five data sets ranged from 2.1 to 15.5. The genotypes; MU-93, SECOW-2W, IT82D-516-2, IT85F-2841 and BROWNMIX-SEL2 had the lowest values of AUDPC, suggesting a resistance mechanism among them. On the other hand, however, the genotype UCR194 showed a final incidence of 100%, severity of 37.9% and AUDPC of 15.5, yet it showed no reaction to any virus antiserum. This may suggest that the plant cells are very sensitive and can react very rapidly following an attack by a virus(es) even when the concentration of the virus may be very low in the plant's tissues. Generally, there was an appreciable increase in the disease mean incidence of most genotypes in spite of moderate levels of mean disease severity and AUDPC among the tested genotypes in the second season of 2004.

Since the main focus of this study was to screen for and select the genotypes with good levels of resistance, resistance types were categorised based on the cowpea genotype reactions to CABMV infection. The genotypes were grouped on percentage scores of <10% (resistant), 10-20% (moderately resistant), 20-30% (susceptible) and >30% (very susceptible). Based on this grouping of visual assessment, cowpea genotypes; SECOW-2W, MU-93, BROWNMIX-SEL2, IT82D-516-2, K-80, KVU27-1 and IT85F-2841 were considered resistant. Cowpea genotypes; FE86, FE67, IT82-889, FE87, FE69, FE38, FE84, KVU419, BROWNMIX-SEL1, IT90K-109, TVX337-025, FE15 and FE60 were

considered moderately resistant. The cowpea genotypes; BECH WHITE, FE17, Fe34, FE25 and FE42 were considered susceptible, while genotypes UCR194 and FE95 were very susceptible (Table 22). Analysis of the grouping of the genotypes showed that the distribution was in favour of resistance (7 resistant; 13 moderately resistant; 5 susceptible; 2 highly susceptible genotypes).

Table 22: Mean incidences (%), severities (%) of cowpea aphid-borne mosaic virus, and AUDPC, of 27 cowpea genotypes selected for further evaluation during the second season of 2004

	Mean incidence (%)					Mean severity (%)					AUDPC
	Days after inoculation (DAI)					Days after inoculation (DAI)					
Genotype	7	14	21	38	45	7	14	21	38	45	
FE42	45.7	61.1	66.6	83.0	86.7	3.6	5.3	6.8	11.2	21.9	6.6
KVU27-1	50.0	65.0	68.1	77.5	84.3	4.5	6.3	6.7	7.5	10.0	6.9
BROWNMIX-SEL1	23.3	54.2	64.0	93.3	95.8	2.3	4.2	5.3	8.7	14.6	6.6
1T82D-889	38.2	57.5	61.1	72.4	87.1	3.3	4.8	6.0	9.5	17.0	7.4
BROWNMIX-SEL2	24.5	35.9	40.5	54.0	63.0	1.2	2.0	2.6	4.4	7.9	3.4
FE60	38.3	60.0	64.2	77.0	92.5	3.0	4.3	5.3	8.2	14.7	6.5
UCR194	48.4	70.9	76.1	91.5	100.0	6.1	10.8	12.6	18.0	37.9	15.5
IT85F-2841	17.4	29.2	30.8	35.8	53.1	1.7	2.3	2.5	3.0	5.3	2.8
FE86	49.2	87.5	88.5	91.7	91.7	4.5	6.4	8.0	12.8	17.0	9.5
FE84	57.4	72.5	75.3	83.8	95.2	4.3	6.1	7.1	9.8	19.5	8.5
FE67	61.3	81.1	84.2	93.3	100.0	6.1	8.3	9.0	11.2	16.2	9.8
KVU419	54.7	71.9	75.2	85.0	96.7	3.7	5.1	6.0	8.7	16.0	7.3
IT82D-516-2	44.2	45.8	51.9	70.0	80.6	3.5	3.8	4.2	5.4	6.5	4.6
FE87	38.2	57.1	60.5	70.3	75.9	5.4	4.9	5.3	7.2	12.4	6.4
K-80	52.6	75.0	75.8	78.3	86.7	5.0	6.6	6.8	7.5	8.4	7.0
FE15	72.6	86.7	89.2	96.7	100.0	6.8	8.4	8.9	10.4	15.6	9.6
FE69	59.7	87.5	88.5	91.7	100.0	5.4	7.6	8.6	11.6	15.4	9.5
SECOW-2W	21.7	36.7	38.9	45.6	67.5	1.9	2.7	3.1	4.3	5.7	3.4
FE95	52.5	75.8	80.8	95.8	100.0	4.8	7.9	9.4	14.0	31.2	11.9
BECHWHITE	59.9	84.4	87.5	96.7	100.0	6.0	8.5	9.7	13.3	25.6	11.5

Table 22: Continued

	Mean incidence (%)					Mean severity (%)					AUDPC
	Days after inoculation (DAI)					Days after inoculation (DAI)					
Genotype	**7**	**14**	**21**	**38**	**45**	**7**	**14**	**21**	**38**	**45**	
FE38	60.6	78.2	81.9	93.0	100.0	4.1	7.2	8.5	12.2	14.8	9.4
IT90K-109	61.3	75.0	78.8	90.3	90.3	5.1	6.2	7.0	9.5	14.1	8.0
MU-93	23.3	23.3	27.4	40.0	53.3	1.5	1.5	1.8	2.7	3.8	2.1
FE17	61.1	85.6	86.8	90.5	90.5	6.0	7.9	9.1	12.4	25.3	11.0
TVX337-025	35.8	49.2	57.1	80.8	80.8	3.3	5.4	6.1	8.2	13.9	7.0
FE25	53.3	88.9	90.3	94.4	100.0	4.9	7.9	9.2	13.0	24.7	11.0
FE34	66.7	91.7	93.8	100.0	100.0	7.7	10.0	11.3	15.0	25.7	13.0
Mean	47.1	66.2	69.8	80.5	87.8	4.3	6.0	6.9	9.6	16.3	8.0
Significance of F	***	***	***	***	***	*	***	***	***	***	***
LSD(0.05)	26.1	27.8	25.0	23.0	21.8	3.6	4.1	4.2	6.1	12.0	4.6
CV%	33.8	25.6	21.9	17.4	15.1	50.8	41.3	36.7	39.0	44.9	34.9

*, ** and *** are significant at P≤0.05, P≤0.01 and P≤0.001 probability level, respectively

4.3.8 Grain yield and yield components of cowpea genotypes

The yield data analysed in REML showed significant differences (P<0.001) among the cowpea genotypes (Table 23). There was a relatively low mean yield achieved from most cowpea genotypes, with BROWNMIX-SEL1, IT82D-889, BROWNMIX-SEL2, FE86, FE95, BECHWHIT and FE17 attaining the lowest mean yield (39.9, 85.9, 23.5, 75.8, 75.7, 73.9, 90.1 and 99.1 Kg ha^{-1}, respectively). The mean yield ranged from 39.9 to 294.0 kg ha^{-1}. The genotypes FE42, K-80, FE15 and FE38 attained mean yields greater than 200.0 kg ha^{-1} (Table 23).

There were significant (P<0.001) differences in yield components among the genotypes (Table 23). The cowpea genotype FE42 had the highest number of pods per plant with a mean of 50.0 and BROWNMIX-SEL2 with the least mean of 6.0 (Table 23). The genotype FE17 attained the longest pod length with a mean of 247 mm, while FE86 had the shortest pod length of 116 mm. The genotype KVU27-1 had the highest number of seeds per pod with a mean of 15.0 and the least was FE86 with a mean of 7.0.

Table 23: Yield and yield components of 27 cowpea genotypes evaluated during the second season of 2004

Genotypes	Yield component			
	Number of pods/ plant	Pod length (mm)	Seeds/pod	Mean yield (Kgha^{-1})
KVU27-1	21.0	177	15.0	142.3
FE84	12.0	161	8.0	117.2
FE42	50.0	141	13.0	232.5
BROWNMIX-SEL1	8.0	176	11.0	39.9
1T82D-889	15.0	178	12.0	85.9
BROWNMIX-SEL2	6.0	116	8.0	23.5
FE60	15.0	153	13.0	125.2
UCR194	16.0	155	9.0	192.0
IT85F-2841	15.0	152	11.0	119.2
FE86	11.0	116	7.0	75.8
FE67	15.0	152	12.0	178.0
KVU419	15.0	152	13.0	147.3
IT82D-516-2	13.0	148	12.0	126.2
FE87	13.0	240	11.0	75.7
K-80	37.0	198	14.0	294.0
FE15	24.0	158	14.0	246.5
FE69	21.0	213	13.0	149.1
SECOW-2W	25.0	145	14.0	148.8
FE95	13.0	126	10.0	73.9
BECHWHITE	23.0	161	12.0	90.1
FE38	42.0	152	12.0	227.5
IT90K-109	21.0	142	14.0	166.8
MU-93	23.0	145	13.0	139.1
FE17	18.0	247	13.0	99.1
TVX337-025	16.0	143	13.0	122.4
FE25	19.0	163	11.0	110.9
FE 34	24.0	153	8.0	104.9
Mean	19.7	162	11.6	133.6
Significance of F	***	**	***	***
LSD (0.05)	16.5	5.8	3.6	126.8
CV%	51.1	22.2	19.2	48.0

** and *** are significant at P≤0.01 and P≤0.001 probability level, respectively

4.3.9 Phenotypic correlation of AUDPC, yield and yield components of cowpea

The phenotypic correlations for all possible comparisons among the four traits studied are presented (Table 24). The number of seeds per pod exhibited significant ($P \leq 0.001$) positive correlation (r =0.495) with yield, followed by number of pods per plant. This suggested that number of seeds per pod and number of pods per plant are indicative of the major factors that contribute to increases in yield of cowpea. The AUDPC exhibited significant ($P \leq 0.05$) positive correlation (r = 0.231) with yield of cowpea. The AUDPC was not significantly correlated to any of the yield components (Table 24).

Table 24: Correlation matrix for yield and yield components associated with AUDPC of CABMV infection in the field during the second season of 2004

	Number of Pods plant^{-1}	Pod length	Number of Seeds pod^{-1}	AUDPC
Pod length	-0.003			
Number of seeds per pod	0.324**	0.295**		
AUDPC	0.195	0.078	-0.003	
Yield	0.473***	0.073	0.495***	0.231*

*, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively

4.3.10 Detection of CABMV and other viruses in 27 cowpea genotypes by serological test

Five viruses CABMV, CPCMV, CPSMV, CPMMV and CPMV were detected among the genotypes by ELISA tests. Of the 27 genotypes tested, 12 genotypes tested positive for CABMV, while 15 genotypes showed a negative reaction to the CABMV antiserum (Table 25). In the reaction of the 27 genotypes to other antisera, four genotypes showed a positive reaction to the CPCMV antiserum, 10 genotypes showed a positive reaction to the CPSMV antiserum, 15 genotypes showed a positive reaction to CPMMV and seven genotypes reacted positively to the CPMV antiserum (Table 25). The results of the ELISA tests indicated that the genotypes UCR194, IT82D-889, BECHWHITE, FE87, FE95, FE34, KVU419 and FE25 did not test positive to any of the five antisera (Table 25). Two genotypes, FE86 and BROWNMIX-SEL2, only reacted positively to the antiserum of CABMV, while MU-93 reacted positively only to the CPMMV antiserum (Table 25). On the other hand, some genotypes reacted positively to more than one antiserum. For instance, four genotypes reacted positively to two antisera, six genotypes

reacted positively to three antisera, two genotypes reacted positively to four antisera and one genotype reacted positively to all the five antisera (Table 25).

Table 25: Reaction of leaf samples of cowpea genotypes in DAS-ELISA test to CABMV, CPCMV, CPSMV, CPMMV and CPMV

	Reaction of cowpea genotypes to viruses				
Genotype	**CABMV**	**CPCMV**	**CPSMV**	**CPMMV**	**CPMV**
FE86	++	-	-	-	-
FE67	+	+	-	+	-
UCR194	-	-	-	-	-
IT82D-889	-	-	-	-	-
BECHWHITE	-	-	-	-	-
FE87	-	-	-	-	-
FE69	+	-	+++	++	++
SECOW-2W	+	++	-	-	++
FE17	++	++	++	+	++
FE95	-	-	-	-	-
MU-93	-	-	-	+	-
FE34	-	-	-	-	-
FE38	+	-	+++	++	-
BROWNMIX-SEL2	+	-	-	-	-
FE84	+	-	-	+	-
KVU419	-	-	-	-	-
FE25	-	-	-	-	-
BROWNMIX-SEL1	-	-	++	+	-
Fe42	+++	-	-	++	+++
IT90K-109	-	-	+	++	-
TVX337-025	-	-	++	++	-
IT82D-516-2	+	-	+	+	+
FE15	-	+	-	-	-
FE60	-	-	+	+	-
K-80	-	-	++	++	-
KVU27-1	+	-	++	++	+
IT85F-2841	+	-	-	++	+

The symbols; **−** denote no reaction, **+** very weak positive yellow coloration, **++** moderate positive yellow coloration and **+++** strong positive yellow coloration with the DAS-ELISA tests

4.4 Discussion and conclusion

The 54 cowpea genotypes screened at SAARI for resistance to CABMV during the first season of 2004 had a consistently high virus incidence and severity. Notably, the 27 selected genotypes had a consistently low virus severity, but a high virus incidence in the second season. There was a significant and negative correlation between AUDPC and yield during the first season, but this was positive during the second season of 2004. The negative correlation indicated that yield was affected by CABMV infection during the first season. The relationships between yields and number of pods per plant, and number of seeds per pod provided strong positive correlations, indicating that they are the major contributors to increases in yield, rather than the pod length.

In two seasonal trials, conducted at SAARI to screen and select for the genotypes for CAMBV resistance, varying levels of symptoms developed within weeks after inoculation. In several cases, there was a range of symptoms observed that consisted of leaf deformation, leaf mosaic, stunted plants, leaf mottling and minor interveinal chlorosis on some genotypes. Fischer and Lockhart (1976) observed that cowpea plots infected with a strain of CABMV appear to have similar symptoms. These symptoms, caused by CABMV, have also been reported by Pietersen (1995), Bashir and Hampton (1996b) and Thottappilly and Rossel (1997).

The infection by CABMV gradually developed and increased from 7 to 14 DAI. Umaharan *et al*. (1997a) indicated that resistance to symptom development cannot be determined by assessments made over a period shorter than 3 wk after inoculation. Furthermore, Umaharan *et al*. (1997a) indicated that such a delay is not difficult to comprehend, since the presence of one to two alleles that confer resistance may be expected to slow the virus multiplication rate, thus delaying the expression of symptoms. To some extent, the virus infection increased for most cowpea genotypes, especially at seedling stage and then steadily when cowpea genotypes were nearing the maturity period between 42 to 56 DAI. The symptom development suggests that virus infection may be influenced by a number of factors such as changes in plant nutrients, physiological age of the plant, defence mechanisms in response to attack and other environmental factors (Hewings *et al*., 1990; De Koeijer and van der Werf, 1995; Gaunt, 1995; Bachand and Castello, 1998). Van Loon (1983) reported that disease progression in a plant, with respect to virus disease, occurs only when new leaves develop, since symptoms cannot be expressed in already expanded leaves. Seasonal effects

associated with temperature and relative humidity have been reported to have an effect on the disease development in the plant (Schuerger and Hammer, 1995). In the second season of 2004, with the 27 genotypes evaluated, the progress of virus infection developed slowly in most cowpea genotypes. Thresh (1974) observed that shortly after anthesis, plants tend to become more resistant to infection and the number of healthy plants available for new infections also decreases as the season progresses. Johansen *et al*., (1994) suggested that symptom expression may be dependant upon the virus strain, host cultivar and environmental conditions. This was in agreement with the findings of Gumedzoe *et al*. (1998), who pointed out that sometimes the expression of symptoms depended on the age of the plant, architecture of the plant and environmental factors, suggesting the existence of genetic variations within the host plants.

The cowpea genotypes evaluated in the field under artificial inoculation for two seasons in 2004 developed symptoms of CABMV infection. The levels of infection varied significantly among the genotypes, which suggested that there was genetic variability for resistance to CABMV. Umaharan *et al*. (1997b) reported that differences in host reaction among virus isolates are evident in the comparison with the reaction of some germplasm lines to cowpea severe mosaic virus. The expression of virus symptoms observed in the first season showed that some genotypes, such as BROWNMIX-SEL2, BROWNMIX-SEL1, FE67 and FE34, exhibited variable levels of resistance to the virus. The study also showed that higher virus incidences were observed on most cowpea genotypes during the first season than in the second season of 2004. Since a large number of genotypes obtained from diverse agro-ecological zones were involved in the screening trial during the first season and whose resistance was not well known, it was possible that such differences in infection could have occurred. It was established that most genotypes had high virus incidences of up to 100% in the first season compared to the second season. For instance, 17 genotypes registered a virus incidence of up to 100.0% in the first season while the same percentage of incidence was observed in only eight genotypes in the second season. The results also showed that in the first season, there was a virus severity of 67.5% and AUDPC of 47.4 at 56 DAI. In contrast, the second season had a virus severity of 37.9% and AUDPC of 15.5 at 45 DAI, indicating that the second season had a lower severity than the first season. The high mean incidence during the first season was due to the fact that there were more susceptible genotypes compared to those used in the second season. Studies by Grumet *et al*. (2000) indicated that the genetic background or environmental factors may also influence the apparent relative effectiveness of the resistant genes of the plant, resulting in a lot of genotypes becoming susceptible to a virus attack. The low mean severity observed in the second season

demonstrated that the 27 genotypes selected exhibited some kind of tolerance or resistance to the virus in spite of symptom occurrences.

The results of the study revealed that CABMV was identified in the cowpea genotypes evaluated in the two seasonal trials conducted in 2004. Of the 54 cowpea genotypes evaluated in the first season of 2004, only 18 genotypes tested positive for CABMV, while none was positive for CPCMV, CPMMV, CPMV and CPSMV. However, some plants exhibited symptoms that did not test positive for any of the above viruses. Bachand and Castello (1998) pointed out that if the virus concentration in the seedlings does not exceed 5-25ng g^{-1}, then an ELISA test would assess the plant samples as negative. Based on that reasoning, it may be true that the virus concentration in the inoculated genotypes was too low to be detected by the ELISA technique used. Therefore, it is at this point that a more robust method of detecting viruses is required, especially with the use of polymerase chain reaction (PCR), to amplify the virus presence. Nevertheless, when the trial was repeated in the second season with the selected 27 genotypes, four other viruses were identified in addition to CABMV. Out of the 27 genotypes tested, eight genotypes did not react to any of the five virus antisera used, an indication of a low rate of virus proliferation, which may itself be a result of genetic resistance to CABMV and other viruses, namely CPMV, CPSMV, CPCMV and CPMMV. The ELISA tests also showed that some genotypes reacted positively to more than one virus antisera. Twelve genotypes reacted positively for CABMV, four genotypes for CPCMV, 10 genotypes for CPSMV and 14 genotypes for CPMMV.

The research results showed that two infection methods, namely susceptible spreader rows and artificial inoculation, were successfully used in this study. Genotypes with some levels of resistance to CABMV were identified and these can provide sources of resistance for future breeding work. Although this study did not identify genotypes with immunity, several lines with good levels of resistance were identified. Infection in field with other viruses is difficult to avoid, especially if viruses have similar symptoms. Based on the findings from field assessments and ELISA tests, the genotypes with less than 10.0% disease severity that reacted negatively or very weakly to the ELISA tests were categorised as resistant. Under this classification the genotypes SECOW-2W, MU-93, BROWNMIX-SEL2, IT82D-516-2, IT85F-2841 and K-80 were partially resistant. In addition, the range between 10-20% of disease severity was categorised as moderately resistant as long as the ELISA tests showed a negative reaction. This grouping included the genotypes IT82D-889, FE87, KVU419, BROWNMIX-SEL1, IT90K-109, TVX337-025, FE15 and FE60. These genotypes could be used as sources of resistance to CABMV. It

is anticipated that the genotypes selected will provide a broad spectrum of resistance or tolerance not only to CABMV, but also to a number of other viruses that attack cowpea. In this way, over-dependence on chemical use will be reduced, with many farmers acquiring improved varieties for them to increase yield and subsequently household income.

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CHAPTER FIVE

INHERITANCE OF RESISTANCE TO COWPEA APHID-BORNE MOSAIC VIRUS IN COWPEA

Abstract

Cowpea is an important source of protein for resource poor farmers in Uganda, but its production is constrained by several factors, particularly cowpea aphid-borne mosaic virus (CABMV). The objective of this study was to determine the nature of inheritance governing resistance to CABMV. Segregating cowpea populations were evaluated for resistance to CABMV under field conditions, using two infection methods. The first method was by spreader row-plants in which the susceptible cultivar, Ebelat, was planted around each plot planted with cowpea crosses. The second method was by inoculating the crosses artificially with CABMV inoculum. The cowpea genotypes IT82D-889, IT85F-2841, IT82D-516-2, MU-93 and SECOW-2W, were selected from the previous evaluation after rigorous screening under field conditions for resistance to CABMV. Hybridisation of the resistant male parent MU-93, moderate resistant male parents IT82D-889, IT85F-2841, IT82D-516-2 and SECOW-2W, and susceptible female parents Ebelat, Ecirikukwai and Blackcowpea were carried out in a North Carolina mating design II scheme. The resultant F_1, F_2 and BC_1F_1 populations, together with their parents, were evaluated in the field to assess their reaction to CABMV and also to study the inheritance of resistance to CABMV. The GCA and SCA effects were significant, indicating that both additive and non-additive genetic factors are important. However, GCA may be more important than SCA in determining the expression of inheritance for resistance in cowpea to CABMV infection. The proportions (%) of the sum of squares for crosses attributable to GCA and SCA for CABMV severity were 51.4% for GCA females (GCA_f), 8.4% for GCA males (GCA_m) and 40.2% for the SCA effects (SCA). The results showed that resistance to CABMV was conditioned by more than one recessive gene in cowpea in eight populations, but resistance was conditioned by a single recessive gene in seven populations. The observation of transgressive segregation in same populations provided evidence of additive gene action and suggests quantitative inheritance, which involves many genes. Consequently, heritability ranged from moderate (48%) to high (87%). The GCA variance was slightly higher than SCA variance, suggesting that additive effects were more important than non-additive effects. The significance of GCA

variance suggests that resistance could be improved by recurrent selection to accummulate the additive genes for resistance.

5.1 Introduction

Cowpea is one of the food legume crops that provides an important source of protein for people in many countries of the world (Bashir *et al*., 2002). Viral diseases are considered an important constraint on yield in all agro-ecological zones, wherever cowpea is grown (Emechebe and Lagoke, 2000; Bashir *et al*., 2002). Among the seed-borne viruses, CABMV is considered economically important, as it causes crop losses of up to 87% under field conditions (Kaiser and Mossahebi, 1975; Mali and Thottappilly, 1986; Bashir and Hampton, 1996b; Shoyinka *et al*., 1997). The virus is transmitted non-persistently by several aphid species, but *Aphis craccivora* Koch is the major aphid vector (Atiri *et al*., 1984). It is a pathogen of many crops, including common beans (*Phaseolus vulgaris* L.) Fabaceae and has a wide host range (Behncken and Maleevsky, 1977).

In the management of virus diseases, the use of host plant resistance is the most economical. Heritable forms of resistance have been found in certain cultivars or landraces (Fraser, 1992). Byoung-Cheorl *et al*. (2005) noted that the use of resistant varieties is cost-effective for farmers, but considerable time and cost may be involved in developing varieties with appropriate levels of resistance. Although several measures have been examined for control of the virus, host plant resistance is viewed as the most economical, practical and environmentally-friendly approach to control CABMV disease of cowpea (Bashir and Hampton, 1996b). Sources of resistance to CABMV have been identified and are being used in cowpea improvement (Van-Boxtel *et al*., 2000). Breeding for resistance has become an increasingly common practice in developing methods for the control of viral diseases (Arshad *et al*., 1998).

The CABMV, a member of the genus potyvirus belonging to the family potyviridae, is one of the plant viruses that causes the most widespread disease in cowpea in the world (Rybicki and Pietersen, 1999). Like other potyviruses, CABMV is characterised by common features such as filamentous particles, size of caspid protein and sedimentation of nucleic acid (Bock, 1973; Taiwo *et al*., 1982; Rybicki and Pietersen, 1999). The intriguing characteristics of potyviral diseases are the appearance of mosaic, vein clearing, mottling, deformation and stunted plants, which are characteristics of CABMV.

The genetic code of CABMV in the nucleic acid gives the appropriate instructions to the host cell to replicate viral nucleic acid. In the virus, there is rearrangement of equivalent genes between the genomes of different particle types, and this increases the variability resulting directly from mutations (Russell, 1978). The potential genetic variability of the virus is of great importance to the plant breeder, because of the danger that new resistance-breaking virus strains may arise. In reinforcing the control mechanism in the host plant against the virus, host resistance can occur by interruption of the virus life cycle at one or more of several stages. For instance, Siegel (1979) identified such steps upon which resistance could act: 1) entry into the cell, 2) uncoating of the nucleic acid, 3) translation of viral proteins and 4) replication of the viral nucleic acid. At the molecular level, evidence has supported resistance mechanisms involving alterations in the function of virus-encoded protease, movement or replicase proteins (Jayaram *et al*., 1992). In vitro studies suggested that leaves of cowpea cultivar (Arlington) contain a protease inhibitor that can inhibit proteolytic processing of a virus-encoded poly-protein (Sanderson *et al*., 1985).

The available literature shows that inheritance of resistance to potyviruses in crops is governed by both dominant and recessive genes (Gilbert-Albertini *et al*., 1995). For instance, resistance to watermelon mosaic virus (WMV) is governed by a dominant gene (Cohen *et al*., 1971). In papaya ringspot virus (PRV), zucchini yellow fleck virus (ZYFV) and celery mosaic virus (CeMV), resistance has been reported to be governed by a recessive gene (Wang *et al*., 1984; Gilbert-Albertini, 1995; D'Antonio *et al*., 2001). In leguminous crops like peas, bean leaf roll virus (BLRV) is conferred by a major recessive gene (Baggett and Hampton, 1991). Similarly, in common bean, resistance to bean yellow mosaic virus strain (BYMV-S) has been reported to be conditioned by a single recessive gene (Park and Tu, 1991). However, resistance to CABMV and blackeye cowpea mosaic viruses (BICMV) in *Phaseolus Vulgaris* (L.) Fabaceae has been reported to be conferred independently by single dominant factors that appear to be closely linked (Provvidenti *et al*., 1983).

Previous reports indicate that several sources of genetic resistance to viruses in cowpea have been identified (Bashir and Hampton, 1996b; Muhammand *et al*., 1996; Umaharan *et al*., 1997b). The concerted efforts by the IITA research team have transferred resistant genes into popular cowpea landraces to boost production for cowpea growers in West and Central Africa (IITA, 1998). In Uganda, the major cowpea growing areas are in the eastern and northern regions, where the crop is grown for food security and also to generate cash income. Despite the strong interest among farmers to cultivate cowpea for

commercial use, production is often hampered by the epidemics of CABMV. The CABMV disease is thus a major hindrance to cowpea production in these regions, sometimes causing up to 100% yield losses in fields grown with susceptible cowpea cultivars. Indeed, CABMV has been reported to be common in the cowpea growing regions of Uganda (Edema *et al*., 1997), and is a threat to cowpea production. There have been no efforts to improve resistance to CABMV in the local susceptible cowpea in Uganda. This work focused on the development of resistant cultivars.

Arshad *et al*. (1998) described resistance to BICMV as governed by a single recessive gene pair in cowpea lines. In the case of CABMV, it has been reported that resistance in cowpea is governed by a single dominant or recessive gene (Taiwo *et al*., 1981; Fisher and Kyle, 1994, 1996). Patel *et al*. (1982) reported resistance to CABMV to be expressed by minor or modifier genes. The modifier genes have been reported to possess small quantitative effects on the levels of expression of another gene. Therefore, knowledge of genetic inheritance is needed when developing cowpea materials resistant to CABMV, as this enables breeders to develop an appropriate breeding strategy. Therefore, the study aimed to determine the nature of inheritance governing resistance to CABMV in cowpea in Uganda.

5.2 Materials and methods

5.2.1 Hybridisation

Among the large collections of cowpea genotypes that were screened and selected in 2004, only five genotypes that showed some low levels of CABMV infection were selected and used for hybridisation with the susceptible cultivars. They were used as males for crossing with susceptible cultivars as females. The selection of the genotypes was based on the assumption that those cultivars with fewer symptoms when infected with CABMV and low or no traces of virus titres with ELISA tests were categorised as resistant (Chapter 4, Sections 4.3.5, 4.3.6 and 4.3.10). Based on that assumption, the selected resistant male genotypes were IT82D-889, IT85F-2841, IT82D-516-2, MU-93 and SECOW-2W. The susceptible female parents were Ebelat, Ecirikukwai and Blackcowpea, and these are the common cowpea cultivars which are widely grown by farmers in Uganda, but are highly susceptible to CABMV. The male and female parents were planted separately in pots in a greenhouse and watered whenever necessary. In order to synchronise the flowering period of both parents, the planting was done at an

interval of 5 d for either of the parents. In this study, the North Carolina II mating design scheme was used because there were two sets of lines (males and females) and it was convenient in generating crosses between resistant and susceptible genotypes. The resistant set was used as males, while the susceptible set was used as females. The crosses were made and the F_1 progenies produced by mating the male parents to each of the female parents in a scheme in June 2005. Part of the seeds of the F_1 progenies produced was retained for field evaluation, while some F_1 seeds were planted and allowed to self produce F_2 progenies and the other for backcrosses in the greenhouse at Serere Agricultural and Animal Production Research Institute (SAARI).

5.2.2 Field evaluation of parental, F_1, F_2 and BC_1F_1 populations

As the seeds of each population did not mature at the same time, planting was carried out at different times of the year, but at the same location (SAARI). Therefore, the individual seeds of the parental and F_1 populations were planted in October 2005 and the seedling stages evaluated for CABMV resistance in a randomised complete block design with three replications at SAARI. The F_2 and BC_1F_1, planted in April 2006, were evaluated for CABMV resistance in a randomised complete block design with three replications. The F_2 and parents, BC_1F_1 and parents were planted in separate experiments with a plot size of 4 m long and 3.6 m wide, with plants spaced 900 mm between rows and 400 mm within rows.

5.2.3 Virus inoculum preparation and the inoculation techniques

Virus inoculum was generated by growing adequate plants of the susceptible cultivar, Ebelat, in pots, as indicated in Chapter 4, Section 4.2.3. These were then infested with live viruliferous aphids onto plants in order to aid the transmission of CABMV into the plant cells. Two methods of inoculating the test genotypes were used. In the first method, the test genotypes were planted in rows in each plot 10 d after spreader rows of Ebelat had been planted in order to generate adequate inoculum in the spreader rows. In the second method, the test genotypes were artificially inoculated as described in Chapter 4, Section 4.2.5.

5.2.4 Data collection

Following inoculation of the crosses with CABMV inoculum, test plants were observed for symptom development at 1 wk intervals starting from 7 DAI and this continued up to physiological maturity. Data were collected in the manner described in Chapter 3 (Section 3.2.2). These were used to calculate the AUDPC as described in Chapter 4 (Section 4.2.6). The percentage severity was rated according to the rating scale presented in Chapter 3 (Table 8).

5.2.5 Evaluation of progeny for resistance by DAS-ELISA antisera

The presence of CABMV, CPCMV, CPSMV, CPMMV, CPMV and CMV was detected as described in Chapter 3 (Section 3.2.3). The CMV antibodies were included in this study, because previous studies showed that some samples were not detected, despite exhibiting virus symptoms. The six ELISA kits were provided by Dr. S. Max at DSMZ Plant Virus Laboratory in Germany. The procedures followed for virus detection with ELISA kits in the laboratory were those provided by the manufacturer and they have already been described in Chapter 3 (Section 3.2.3). A cross was considered susceptible if the sample exhibited moderate or strong intensity of the yellow colouration as denoted by the signs ++ and +++, respectively.

5.2.6 Evaluation of CABMV resistance in the presence of other viruses

The survey of cowpea viruses (Chapter 3) has shown that several viruses will occur on cowpeas at the same time. It will therefore be possible that viruses other than the CABMV will eventually play a role in the field trial. In order to ensure that CABMV is the dominant virus, a high pressure of the disease was employed by the two inoculation methods as described in Chapter 4 (Sections 4.2.3 and 4.2.5). It is accepted that a small degree of confounding of the results could have taken place.

5.2.7 Data analyses

The parental and F_1 severity mean data were analysed using the procedure in the Genstat computer package. The analyses of the variance of component of genotypes were further partitioned into variations due to parents, crosses and one degree of freedom orthogonal contrast (parent versus cross). The crosses were also further partitioned into females, males and their parental interactions (female x male). Using the method described by Dabholkar (1999), the female and male main effects were used to estimate general combining ability (GCA) due to male and female contributions, whereas the female x male interaction provided estimates of specific combining ability (SCA). The statistical model underlying the analysis was:

$$\mathbf{Y_{ijk} = \mu + m_i + f_j + (m \times f)_{ij} + \varepsilon_{ijk}}$$

Where; Y_{ijk} is the kth observation on ith male x jth female progeny

μ is the general mean

m_i is the GCA effect of ith male

f_j is the GCA effect of jth female

$(m \times f)_{ij}$ is the interaction effect or SCA effect

ε_{ijk} is the error associated with each observation.

The combining ability estimates were calculated based on the methods described by Singh and Chaudhary (1985), and Huff and Wu (1992) as follows:

$$\textbf{GCA} = \frac{\text{marginal sum of a parent}}{\text{number of crosses involved}} - \frac{\text{grand total of all parents}}{\text{total number of crosses}}$$

Independent GCA effects were calculated for male and female parents using the same formula.

Predicted value of a cross = GCA of female parent **+** GCA of male source **+** grand mean of all crosses

GCA was regarded as significantly different from zero using a t-test, $t = \frac{GCA - 0}{SE}$ at 44 degree of freedom (error)

SCA = observed value of a cross **–** predicted value of a cross.

SCA was regarded as significantly different from zero using a t-test, $t = \frac{SCA - 0}{SE}$ at 44 degree of freedom error)

The relative importance of GCA and SCA was determined by calculating the percentage of the sum of squares for the crosses attributable to GCA and SCA effects (Hallauer and Miranda, 1988; Kang, 1994; Menkir and Ayodele, 2005). Observed and expected phenotypic segregation ratios of resistant to susceptible crosses were tested by using chi-square (X^2) for goodness of fit, assuming a monogenic model for inheritance of resistance.

$$\mathbf{X^2 = \sum \frac{(Observed\ value - Expected\ value)^2}{(Expected\ value)}}$$

Narrow sense heritabilities were estimated by regressing F_2 on F_1 progenies (Vogel *et al*., 1980; Casler, 1982), and F_1 progenies on mid-parents (Falconer and Mackay, 1996) and F_2 progenies on mid-parents (Cross *et al*., 2000).

The regression statistical model was: **Y = bx + c**

Where: Y is the relationship between F_2 and Mid-parent populations

b is regression value from the graph which estimates heritability

X is the intercept

C is the constant

5.3 Results

5.3.1 Reaction of cowpea crosses and their parents to CABMV infection

The results showed that highly significant ($P \leq 0.001$) differences for severity and AUDPC were observed for all of the genotypes (Table 26), indicating that there is variability in reaction to CABMV infection. The characteristic symptom development of CABMV was initially observed at 21 DAI on the parents and crosses when they had attained 36 days from the time of germination. The observed symptoms were leaf mosaic, mild stunted plants, leaf mottling and appearance of chlorotic patches. None of the cowpea crosses studied showed complete resistance to the virus disease, but varying levels of partial resistance to CABMV was observed among the populations. These were categorised in the range of 0-20% based on a visual assessment of the final severity (Table 26). In this case, the F_1 populations that exhibited with partial resistance included Ecirikukwai x IT82D-516-2, Ecirikukwai x MU-93, Ecirikukwai x SECOW-2W, Blackcowpea x IT82D-

889, Blackcowpea x IT85F-2841and Blackcowpea x MU-93. The F_2 populations included Ebelat x SECOW-2W, Ecirikukwai x IT85F-2841, Ecirikukwai x IT82D-516-2, Ecirikukwai x MU-93, Ecirikukwai x SECOW-2W, Blackcowpea x IT82D-516-2 and Blackcowpea x MU-93. The backcross populations included Ecirikukwai x IT85F-2841, Ecirikukwai x IT82D-516-2, Ecirikukwai x MU-93 and Ecirikukwai x SECOW-2W.

The results of the study showed that the parents MU-93, IT82D-516-2 and IT85F-2841 displayed a low level of disease severity and AUDPC values, suggesting a partial resistance in these parents. The mean severity for the parents ranged from 2.3-38.7%, and AUDPC ranged from 1.7-26.5 over all the seasons (Table 26). The parent MU-93 showed a resistance reaction of about 5.0% for the final severity and 2.0 for AUDPC, while parents IT82D-516-2 and IT85F-2841 showed moderate resistance reactions of about 20.0% for final severity and 13.0 for AUDPC. The parents Ebelat, Ecirikukwai and Blackcowpea were highly susceptible to CABMV infection, with mean disease severity levels of about 25.0% and 15.0 for AUDPC.

When the F_1 progenies were assessed, most of them exhibited a higher level of susceptibility than their parents. A few of them showed moderate reactions to CABMV infection, suggesting that non-additive gene effects were present. The five progenies, Ecirikukwai x IT82D-516-2, Ecirikukwai x MU-93, Ecirikukwai x SECOW-2W, Blackcowpea x IT82D-889 and Blackcowpea x IT85F-2841 all exhibited moderate resistance, falling into a category grouping of 10-20% based on the final severity assessment. This suggests the significance of additive gene action in these populations, since they were crosses between resistant and susceptible parents. In contrast, the progenies Ebelat x IT85F-2841 (35.0%, 22.3), Ebelat x IT82D-516-2 (26.0%, 16.5), Ebelat x MU-93 (33.7%, 20.2), Ebelat x SECOW-2W (26.3%, 16.5) and Blackcowpea x SECOW-2W (33.3%, 23.5) significantly expressed higher final severity and AUDPC values, respectively than their parents (Table 26). The existence of susceptibility in these progenies to CABMV infection suggests that susceptibility was dominant over resistance. The resistance exhibited in the progeny Ecirikukwai x IT82D-516-2 suggests that there was complete dominance for resistance because the F_1 population had resistance which was similar to the better parent. The resistance exhibited in the progenies Ecirikukwai x SECOW-2W and Blackcowpea x IT85F-2841 suggests that there was partial dominance for resistance. The resistance in the progeny Blackcowpea x IT82D-889 was due to over dominance for resistance because the F_1 population was more resistant than the better parent. The final severity (41.5%) and AUDPC (24.8) values for the cross Ebelat x

IT82D-889 were not significantly different from their parents Ebelat and IT82D-889 at P = 0.05 despite high values exhibited by the cross (Table 26).

The F_2 progenies segregated into varying levels of resistance to CABMV infection, indicating that the involvement could have been in the control of parents. Based on disease severity estimates, six segregates were categorised for disease resistance in the ranges of 0-20%, while 9 segregates were susceptible with a disease severity greater than 20 (Table 26). The segregates of Ebelat x IT82D-889 and Ebelat x IT85F-2841 were highly susceptible to CABMV infection. Most BC_1F_1 crosses were highly susceptible, and only four crosses were classified as resistant, while 11 crosses were susceptible. The results further showed that the crosses Ebelat x IT82D-516-2, Ebelat x IT82D-889, Ebelat x SECOW-2W, Ebelat x MU-93 and Ebelat x IT85F-2841 were also highly susceptible to CABMV infection.

5.3.2 Evaluation of F_2 and BC_1F_1 populations to CABMV disease by DAS-ELISA test

The F_1 crosses were not tested with the ELISA kits because during that time, ELISA kits had not been secured. The results presented under this section for the analysis with ELISA tests are only for F_2 and BC_1F_1 crosses. Two ELISA kits, namely CPMV and CPCMV, did not detect the target viruses in the samples of the tested crosses. In the F_2 crosses, CABMV was not detected in Ebelat x IT82D-889, Ecirikukwai x IT82D-516-2 and Blackcowpea x IT82D-889. The virus CPMV was also not detected in Ecirikukwai x IT82D-889, Ebelat x IT82D-889 and Ecirikukwai x IT82D-516-2. The CPSMV was not detected in 9 crosses (Table 27). The intensity of the colour observed with characteristics of yellow colouration of the three antisera with samples of other crosses was moderate. However, CPMMV was detected in all of the crosses and a strong yellow colouration was observed.

In contrast, all of the BC_1F_1 crosses showed a positive reaction with CABMV and CPMMV antisera, with CPMMV showing a strong yellow colouration. This indicated that the concentration of virus titres of CPMMV in the leaf samples was probably very high compared to CABMV. The CMV antiserum showed a negative reaction with only two crosses of BC_1F_1, namely Blackcowpea x MU-93 and Ecirikukwai x IT82D-516-2. The CPSMV was detected only in one cross (Ebelat x IT85F-2841). The results showed that the three crosses were free of CABMV (Table 27). Interestingly, the crosses Ecirikukwai

x IT82D-516-2 and Ebelat x IT82D-889 were also free of other viruses such as CMV and CPSMV. The CPMMV was the only virus which was detected in these two crosses (Table 27). However, all the BC_1F_1 crosses showed positive reactions with CABMV and CPMMV antisera, with the exception of Ecirikukwai x IT82D-516-2 and Blackcowpea x MU-93, which did not react with the CMV and CPSMV antisera.

Table 26: Mean severity and AUDPC* of cowpea crosses and parents planted in a field inoculated with CABMV at SAARI in Uganda

Genotypes	F_1 and parents in 2005		F_2 and parents in 2006		BC_1F_1 and parents in 2006	
	Final severity	AUDPC	Final severity	AUDPC	Final severity	AUDPC
Parents						
Ebelat	38.7	26.5	27.9	18.9	29.4	21.6
Ecirikukwai	29.6	17.0	25.7	17.0	27.2	19.5
Blackcowpea	28.9	20.4	27.5	18.9	26.3	18.7
IT82D-889	33.0	21.2	23.3	13.4	20.0	12.7
IT85F-2841	21.5	12.2	11.2	7.4	10.6	8.2
IT82D-516-2	15.7	11.2	11.0	8.6	8.0	8.5
MU-93	4.7	5.5	4.0	1.7	2.3	2.0
SECOW-2W	20.0	13.4	33.7	21.2	31.2	21.8
Crosses						
Ebelat **x** IT82D-889	41.5	24.8	39.2	21.5	34.3	24.3
Ebelat **x** IT85F-2841	35.0	22.3	31.3	18.0	29.2	19.7
Ebelat **x** IT82D-516-2	26.0	16.5	21.7	11.9	36.7	23.1
Ebelat **x** MU-93	33.7	20.2	22.2	14.5	29.1	18.8
Ebelat **x** SECOW-2W	26.3	16.5	18.2	12.6	32.5	22.3
Ecirikukwai **x** IT82D-889	25.3	16.5	23.1	15.6	24.7	18.0
Ecirikukwai **x** IT85F-2841	24.3	16.3	13.1	12.5	15.7	13.3
Ecirikukwai **x** IT82D-516-2	11.4	10.8	18.6	13.2	18.8	15.4
Ecirikukwai **x** MU-93	12.2	10.3	17.3	11.8	18.3	12.8
Ecirikukwai **x** SECOW-2W	15.5	13.5	16.8	11.5	17.4	10.2
Blackcowpea **x** IT82D-889	15.4	16.8	25.8	15.9	27.2	17.8
Blackcowpea **x** IT85F-2841	17.8	15.3	20.9	15.0	24.3	17.7
Blackcowpea **x** IT82D-516-2	24.0	17.5	17.7	15.1	23.1	16.7
Blackcowpea **x** MU-93	20.8	14.5	20.8	10.8	26.2	18.8
Blackcowpea **x** SECOW-2W	33.3	23.5	24.3	14.5	25.8	18.3
Mean	24.1	16.6	21.5	14.0	23.4	16.5
Significance of F	***	***	***	***	***	***
LSD (0.05)	9.8	5.7	10.2	5.9	8.8	4.6
CV%	24.7	20.7	28.9	25.6	22.8	17.0

*** denotes highly significant at $P \leq 0.001$

*AUDPC (area under disease progress curve) was calculated from five data sets of disease severity assessments

Table 27: Reaction of F_2 and BC_1F_1 crosses to six antisera of CABMV, CMV, CPMMV, CPSMV, CPMV and CPCMV

Populations	Reaction response of antisera					
	CABMV	CMV	CPMMV	CPSMV	CPMV	CPCMV
F_2						
Ecirikukwai x IT85F-2841	++	++	+++	++	-	-
Ecirikukwai x SECOW-2W	++	++	+++	++	-	-
Ecirikukwai x IT82D-889	++	++	+++	-	-	-
Ecirikukwai x MU-93	++	++	+++	++	-	-
Ecirikukwai x IT82D-516-2	-	-	+++	-	-	-
Ebelat x SECOW-2W	++	++	+++	-	-	-
Ebelat x IT82D-516-2	++	++	+++	-	-	-
Ebelat x MU-93	++	++	+++	++	-	-
Ebelat x IT85F-2841	++	++	+++	++	-	-
Ebelat x IT82D-889	-	-	+++	-	-	-
Blackcowpea x x MU-93	++	++	+++	-	-	-
Blackcowpea x IT82D-889	-	++	+++	++	-	-
Blackcowpea x IT85F-2841	++	++	+++	-	-	-
Blackcowpea x SECOW-2W	++	++	+++	-	-	-
Blackcowpea x IT82D-516-2	++	++	+++	-	-	-
BC_1F_1						
Ecirikukwai x IT85F-2841	++	++	+++	-	-	-
Ecirikukwai x SECOW-2W	++	++	+++	-	-	-
Ecirikukwai x IT82D-889	++	++	+++	-	-	-
Ecirikukwai x MU-93	++	++	+++	-	-	-
Ecirikukwai x IT82D-516-2	++	-	+++	-	-	-
Ebelat x SECOW-2W	++	++	+++	-	-	-
Ebelat x IT82D-516-2	++	++	+++	-	-	-
Ebelat x MU-93) x Ebelat	++	++	+++	-	-	-
Ebelat x IT85F-2841	++	++	+++	++	-	-
Ebelat x IT82D-889	++	++	+++	-	-	-
Blackcowpea x MU-93	++	-	+++	-	-	-
Blackcowpea x IT82D-889	++	++	+++	-	-	-
Blackcowpea x IT85F-2841	++	++	+++	-	-	-
Blackcowpea x SECOW-2W	++	++	+++	-	-	-
Blackcowpea x IT82D-516-2	++	++	+++	-	-	-

CABMV (cowpea aphid-borne mosaic virus), CMV (cucumber mosaic virus), CPMMV (cowpea mild mottle virus), CPSMV (cowpea severe mosaic virus), CPMV (cowpea mosaic virus) and CPCMV (cowpea chlorotic mosaic virus)

5.3.3 Relationship within the parents and F_1 crosses for resistance to CABMV infection

With respect to disease severity, there were significant ($P \leq 0.001$) differences among the crosses for disease severity (Table 28). The parents and female x male interactions were highly significant ($P \leq 0.001$), suggesting great variability of resistance among the parents. The significant GCA mean squares due to the female parents and the interactions (SCA) indicated that both additive types of gene action due to female parents, as well as non-additive types of gene action, respectively, are important for determining severity of CABMV infection. Additionally, the magnitude of GCA mean squares was significant ($P \leq 0.001$) and higher than that of SCA (Table 28). The high magnitude of GCA in comparison to SCA is an indication of the greater contribution of additive gene effects over the non-additive gene effects in the inheritance of resistance to CABMV infection. The proportions (%) of the sum of squares for crosses attributable to GCA and SCA for CABMV severity were 51.4% for GCA due to females, 8.4% for GCA due to males and 40.2% for the SCA due to female x male interactions. This indicated that GCA may be more important than SCA in determining the inheritance of resistance to CABMV infection. However, the GCA mean squares due to male parents was not significant at P = 0.05 (Table 28), indicating the additive variance could be entirely attributed to the female parents. This indicated that the female parents contributed more than male lines towards resistance.

Strong negative values of GCA effects of the parents show contribution of GCA towards resistance for CABMV disease. The positive significant values indicate contributions to susceptibility among the parents. The expression of resistance, which is reflected in negative values, is due to high gene frequency for resistance, while the positivevalues are due to low gene frequency for CABMV resistance. The female parent Ecirikukwai expressed a higher negative GCA effect for virus resistance (-6.4), while Ebelat had positive GCA and Blackcowpea had non-significant GCA effects. This indicates susceptibility levels varied among the female parents, and Ecirikukwai was the least susceptible (Table 29). The resistance levels varied among the male parents, where IT82D-516-2 was the most resistant as indicated by significant negative GCA effects (-3.7). The male genotype IT82D-889 had the least level of resistance as indicated by significant positive GCA effects (3.2). The remaining male parents IT85F-2841, MU-93 and SECOW-2W had non-significant GCA effects, indicating that they had similar levels of resistance. This probably explains why the GCA mean squares for males was not significant at P = 0.05 (Table 28). Selection from the progenies resulting from the

hybridisation of the parents with negative GCA effects would be expected to produce progenies with greater resistance than the parents. The parental strains with negative GCA effects were regarded as desirable combiners for resistance, while those with positive GCA effects were undesirable combiners for resistance to CABMV disease (Table 29).

Three superior crosses were observed, with negative SCA effects (Table 30). The cross Blackcowpea x IT82D-889 was the best specific combiner among the crosses, while Blackcowpea x SECOW-2W was positive and the poorest specific combiner. The cross involving susceptible general combiner (Ebelat) and intermediate resistant combiner (SECOW-2W), had negative SCA effects, indicating a general relationship between resistance of a parent and its SCA (Table 30). This further reflects the heritability of the resistance in the next generations, probably due to the significance of non-additive gene effects in the crosses. The results also showed that resistance in these crosses was moderately higher than would be expected from the mean resistance of their respective parents.

Table 28: Analysis of variance for CABMV assessment of three females, five males and F1 progenies evaluated at SAARI in June 2005

Source of variation	DF		Sum of square	Mean square
				Final severity
Replication	2		485.6	242.8
Genotypes	22		5797.1	263.5***
Parents (P)	7		2454.5	350.7***
G.C.A/Females (F)		2	1716.8	858.4***
G.C.A/Males (M)		4	280.3	70.1**ns**
SCA/F* M		8	1345.1	168.1**
Crosses (C)	14		3342.2	238.7***
P versus C	1		0.4	0.4**ns**
Error	44		1560.0	35.5
Proportions of the sums of squares for crosses	GCA_f		0.514***	
	GCA_m		0.084ns	
	SCA_{fxm}		0.420**	

** and *** significant level at P≤0.01 and P≤0.001, respectively.

ns = not significant

Table 29: Estimates of general combining ability (GCA) effects for severity and AUDPC of CABMV infection on eight cowpea parents

Parents	GCA effects	
	Final Severity	**AUDPC**
Female parents		
Ebelat	8.3***	3.0**
Ecirikukwai	-6.4***	-3.5***
Blackcowpea	-1.9	0.5
SE	**1.5**	**0.9**
Male parents		
IT82D-889	3.2*	2.4**
IT85F-2841	1.5	0.9
IT82D-516-2	-3.7**	-2.1*
MU-93	-1.9	-2.0*
SECOW-2W	0.9	0.8
SE	**1.9**	**1.2**

** and *** indicates significance at P = 0.01 and 0.001, respectively

Table 30: Estimates of specific combining ability (SCA) effects for final severity and AUDPC of CABMV infection on F1 crosses

Crosses	SCA effects	
	Final severity	**AUDPC**
Ebelat x IT82D-889	5.8	2.4
Ebelat x IT85F-2841	1.0	1.3
Ebelat x IT82D-516-2	-2.8	-1.5
Ebelat x MU-93	3.1	2.2
Ebelat x SECOW-2W	-7.1**	-4.4**
Ecirikukwai x IT82D-889	4.3	0.7
Ecirikukwai x IT85F-2841	5.1	1.9
Ecirikukwai x IT82D-516-2	-2.7	-0.6
Ecirikukwai x MU-93	-3.6	-1.2
Ecirikukwai x SECOW-2W	-3.1	-0.8
Blackcowpea x IT82D-889	-10.1***	-3.1
Blackcowpea x IT85F-2841	-6.1	-3.2
Blackcowpea x IT82D-516-2	5.5	2.1
Blackcowpea x MU-93	0.5	-1.0
Blackcowpea x SECOW-2W	10.2***	5.2**
SE	**3.4**	**2.0**

** and *** indicates significance at P = 0.01 and 0.001, respectively

5.3.4 Evaluation of monogenic inheritance model for resistance to CABMV in F_2 and backcross populations

The frequency distribution of segregating F_2 crosses was not normal but displayed skewed distributions (Figures 13, 14 and 15), indicating that there was segregation among the crosses. Large numbers of susceptible plants within the individual cross were observed with IT82D-889 x Ecirikukwai, MU-93 x Ecirikukwai, IT85F-2841 x Ebelat, IT82D-516-2 x Ebelat, MU-93 x Ebelat, IT82D-889 x Ebelat, IT82D-889 x Blackcowpea, SECOW-2W x Blackcowpea and MU-93 x Blackcowpea. Nevertheless, a few plants within the crosses were observed with moderate resistance to CABMV infection (Figures 13, 14 and 15). This suggests that there is more than one gene controlling resistance to CABMV in the individual parents.

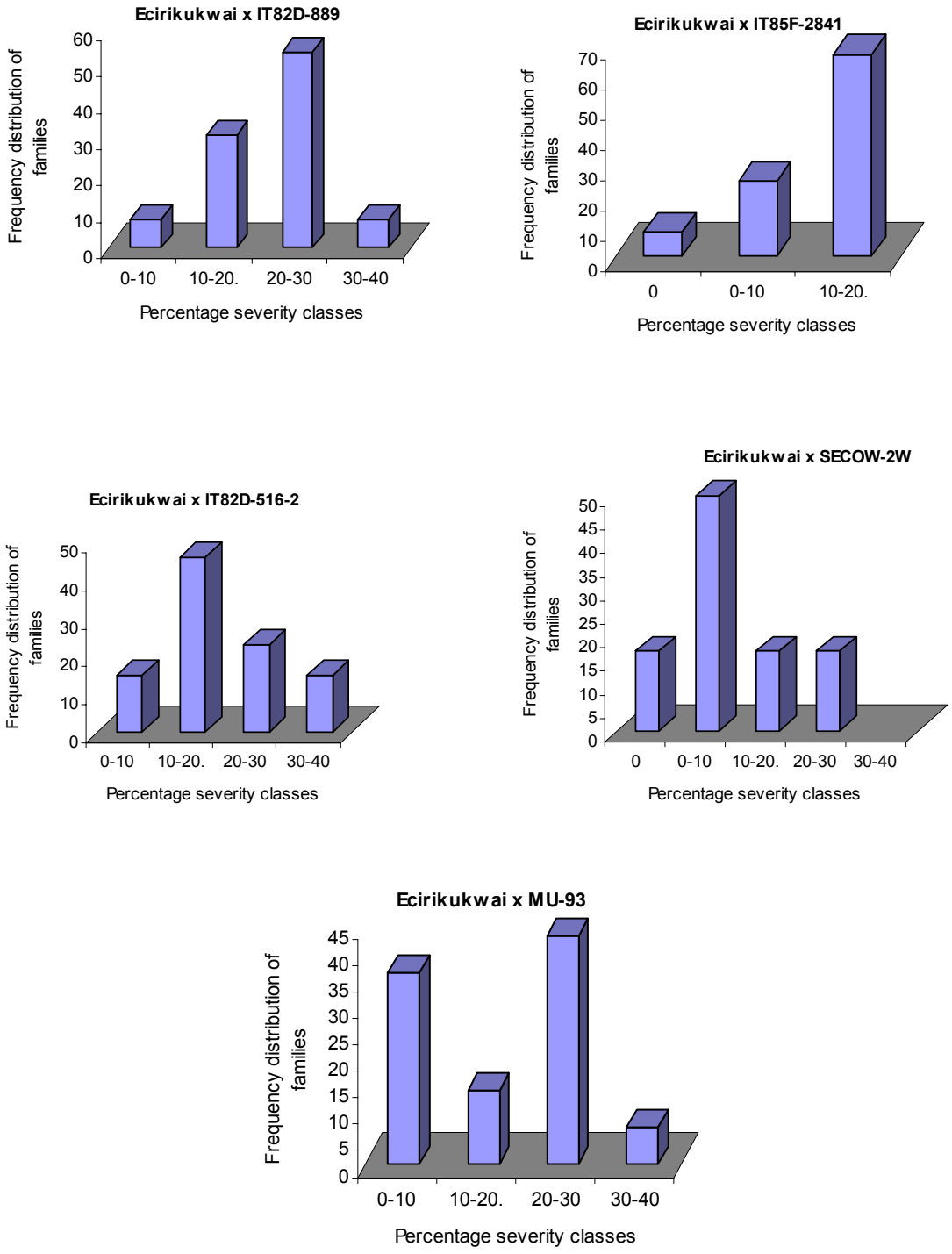

Figure 12: Frequency distribution for percentage severity of F2 crosses involving the susceptible cultivar Ecirikukwai with the resistant ones MU-93, SECOW-2W, IT85F-2841, IT82D-516-2 and IT82D-889

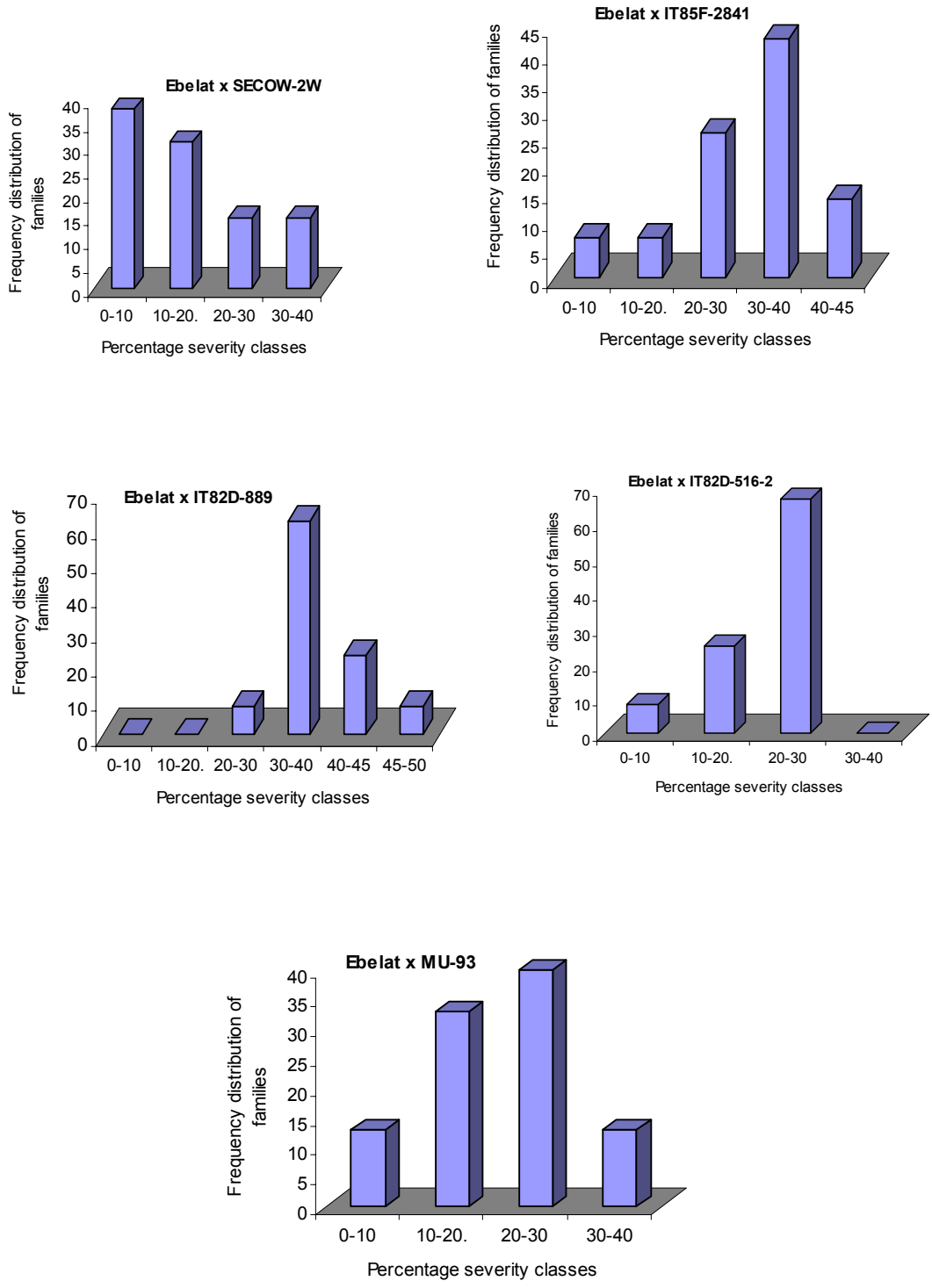

Figure 13: Frequency distribution for percentage severity of F2 crosses involving the susceptible cultivar Ebelat with the resistant ones MU-93, SECOW-2W, IT85F-2841, IT82D-516-2 and IT82D-889

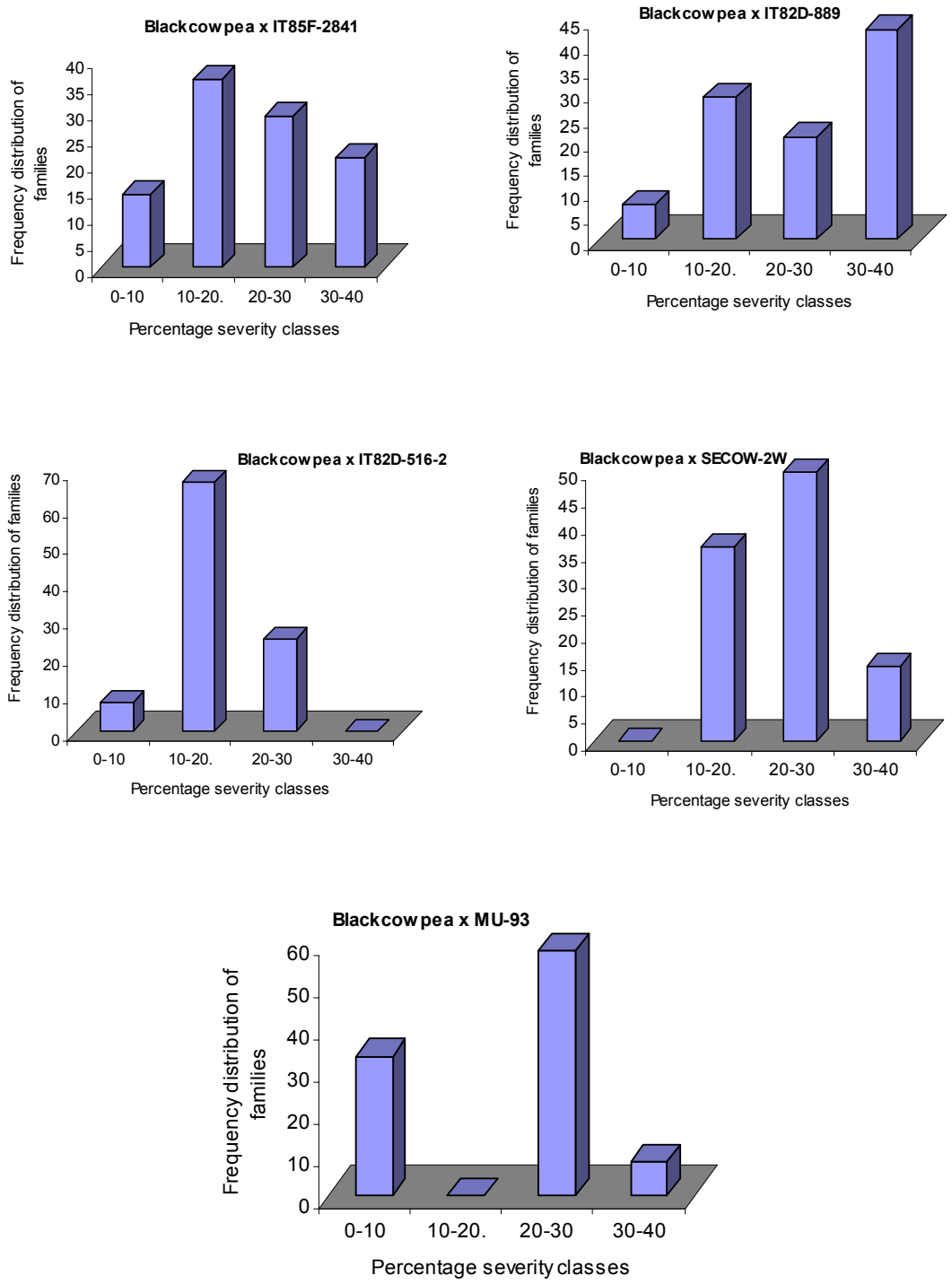

Figure 14: Frequency distribution for percentage severity of F2 crosses involving the susceptible cultivar Blackcowpea with the resistant ones MU-93, SECOW-2W, IT85F-2841, IT82D-516-2 and IT82D-889

The virus symptoms observed on the progenies consisted of chlorosis, mottling and mosaic, with mosaic symptoms being prominent on F_2 and backcross plants. The majority of the plants in backcrosses were very susceptible to CABMV infection. When the F_2 populations were analysed with a critical chi-square (X^2) value of 3.84 at P = 0.05 with one degree of freedom, some progenies showed a good fit to chi-square in a segregation ratio of 1 resistant: 3 susceptible, while other crosses did not, suggesting that more than one recessive gene is involved in the inheritance of resistance to CABMV (Table 31). Other backcross progenies segregated in ratios of 1 resistant: 1 susceptible, as identified by the chi-square analysis (Table 32). Thus, the chi-square test seemed to show that resistance to CABMV in segregating F_2 and backcross populations was conditioned by more than one recessive gene, with minor gene at a different locus.

Table 31: Phenotypic ratios of resistant (R) : susceptible (S) F_2 populations when fitted on 1:3 genetic model

Cross	Phenotype	Observed	Expected	X^2
Blackcowpea x IT82D-889	R (0-10%)	3	9.75	6.23**
	S (>10%)	36	29.25	
Blackcowpea x MU-93	R (0-10%)	11	8.25	1.22
	S (>10%)	22	24.75	
Ecirikukwai x IT85F-2841	R (0-10%)	11	8.25	1.22
	S (>10%)	22	24.75	
Ecirikukwai x SECOW-2W	R (0-10%)	6	7.00	0.19
	S (>10%)	22	21.00	
Ebelat x SECOW-2W	R (0-10%)	11	9.00	0.59
	S (>10%)	25	27.00	
Blackcowpea x IT85F-2841	R (0-10%)	6	9.75	1.92
	S (>10%)	33	29.25	
Ecirikukwai x MU-93	R (0-10%)	14	10.50	1.56
	S (>10%)	28	31.50	
Ecirikukwai x IT82D-889	R (0-10%)	3	9.25	5.63*
	S (>10%)	34	27.75	
Ebelat x IT82D-516-2	R (0-10%)	3	8.50	4.75*
	S (>10%)	31	25.50	
Ebelat x IT82D-889	R (0-10%)	3	9.75	6.23**
	S (>10%)	36	29.25	
Ebelat x IT85F-2841	R (0-10%)	3	9.00	5.33*
	S (>10%)	33	27.00	
Ebelat x MU-93	R (0-10%)	6	11.25	3.27
	S (>10%)	39	33.75	
Ecirikukwai x IT82D-516-2	R (0-10%)	6	9.25	1.52
	S (>10%)	31	27.75	
Blackcowpea x IT82D-516-2	R (0-10%)	3	8.50	4.75*
	S (>10%)	31	25.50	
Blackcowpea x SECOW-2W	R (0-10%)	3	9.75	6.23**
	S (>10%)	36	29.25	

Critical chi-square (X^2) value for one degree of freedom at $P \leq 0.05$ = **3.84**

Table 32: Phenotypic ratios of resistant (R) : susceptible (S) BC_1F_1 populations when fitted on 1:1 genetic model

Cross	Phenotype	Observed	Expected	(X^2)
Blackcowpea x IT82D-889	R (0-10%) S (>10%)	8 25	16.5 16.5	8.76**
Blackcowpea x MU-93	R (0-10%) S (>10%)	0 39	--	--
Ecirikukwai x IT85F-2841	R (0-10%) S (>10%)	39 0	--	--
Ecirikukwai x SECOW-2W	R (0-10%) S (>10%)	16 18	17.0 17.0	0.12
Ebelat x SECOW-2W	R (0-10%) S (>10%)%	11 22	16.5 16.5	3.67
Blackcowpea x IT85F-2841	R (0-10%) S (>10%)	11 19	15.0 15.0	2.13
Ecirikukwai x MU-93	R (0-10%) S (>10%)	33 0	--	--
Ecirikukwai x IT82D-889	R (0-10%) S (>10%)	14 27	20.5 20.5	4.12*
Ebelat x IT82D-516-2	R (0-10%) S (>10%)	0 36	--	--
Ebelat x IT82D-889	R (0-10%) S (>10%)	0 42	--	--
Ebelat x IT85F-2841	R (0-10%) S (>10%)	0 34	--	--
Ebelat x MU-93	R (0-10%) S (>10%)	0 33	--	--
Ecirikukwai x IT82D-516-2	R (0-10%) S (>10%)	22 14	18.0 18.0	1.78
Blackcowpea x IT82D-516-2	R (0-10%) S (>10%)	36 0	--	--
Blackcowpea x SECOW-2W	R (0-10%) S (>10%)	8 30	19.0 19.0	12.74***

Critical chi-square (X^2) value for one degree of freedom at $P \leq 0.05$ = **3.84**

5.3.5 Heritability estimates for CABMV resistance in cowpea populations

The narrow sense heritability estimates for CABMV resistance, using three different methods for area under disease progress curve and final disease severity, are summarised in Table 33. The regression of F_1 on mid-parents provided heritability estimates of 0.87 $\pm$ 0.26 and 0.84 $\pm$ 0.41 for AUDPC and final disease severity, respectively (Figures 16 and 17). However, regression of F_2 on F_1 progenies resulted in heritability estimates of 0.49 $\pm$ 0.13 and 0.48 $\pm$ 0.15 for AUDPC and final disease severity, respectively (Figures 18 and 19). The regression of F_2 progenies on mid-parents provided heritability estimates of 0.63 $\pm$ 0.16 and 0.79 $\pm$ 0.23 (Figures 20 and 21). The above results have shown that the high regression estimates obtained from the respective methods provided an indication that resistance to CABMV infection among the parental populations was highly heritable. Considering the fact that heritability estimates obtained by regressing F_2 on F_1 progenies was low, it shows that an effect on a character due to genetic effects and environmental variability can affect the heritability and thus, lowers its value.

Table 33: Summary of heritability estimates by regressing F1 on Mid-parents, F2 on F1 progenies, and F2 on Mid-parents for CABMV infection

Components observed	Method	Heritability (B)	Standard error (SE)	Regression R^2 value	Mean	Disease range
Area under disease progress curve	F_1/Mid-parents	0.87	0.26	0.47	17.0	10.3 - 24.8
	F_2/F_1 progenies	0.49	0.13	0.54	15.7	10.3 - 24.8
	F_2/Mid-parents	0.63	0.16	0.55	15.7	10.8 - 23.9
Disease severity	F_1/Mid-parents	0.84	0.41	0.25	24.9	11.4 - 41.5
	F_2/F_1 progenies	0.48	0.15	0.45	23.1	11.4 - 41.9
	F_2/Mid-parents	0.79	0.23	0.42	23.9	13.1 - 39.2

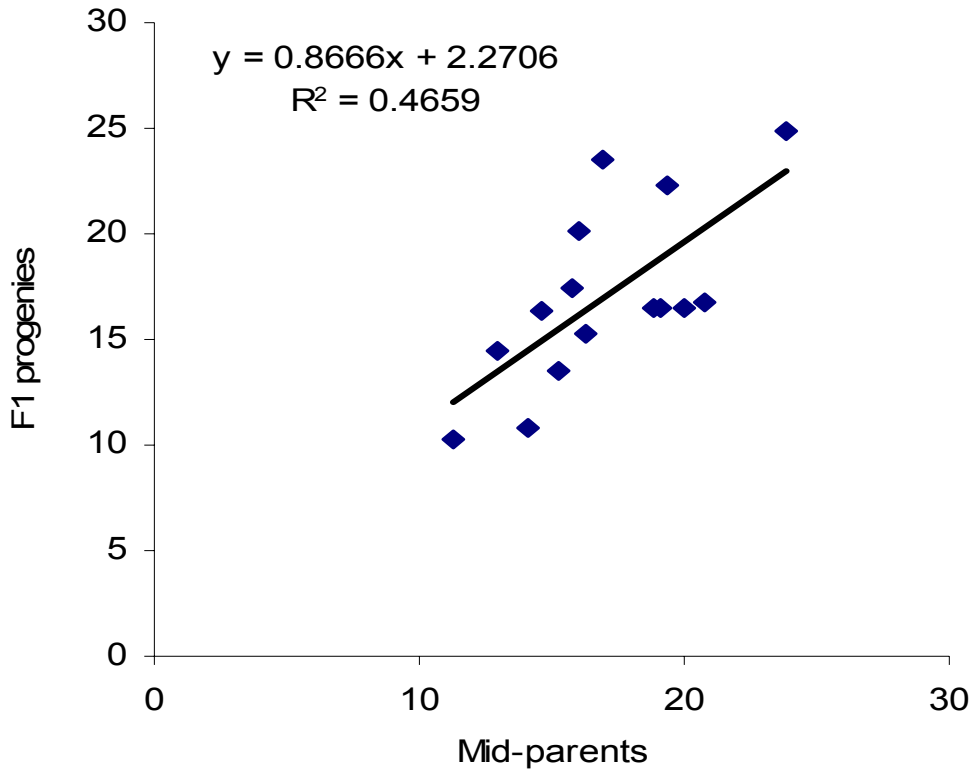

Figure 15: Regression of F_1 progenies on Mid-parents using AUDPC of CABMV infection

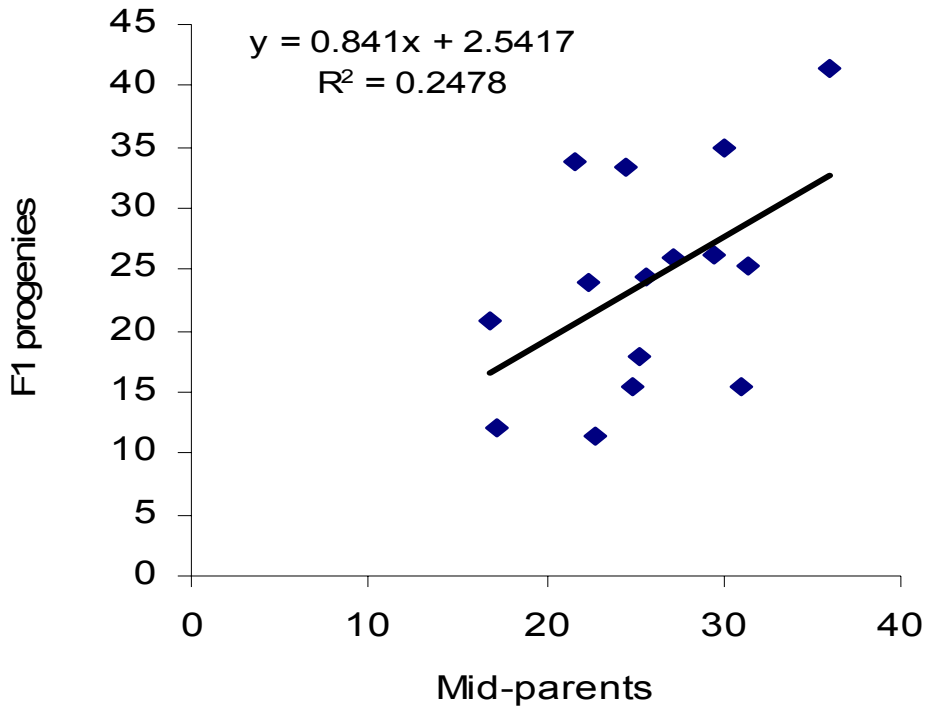

Figure 16: Regression of F_1 progenies on Mid-parents using final disease severity of CABMV infection

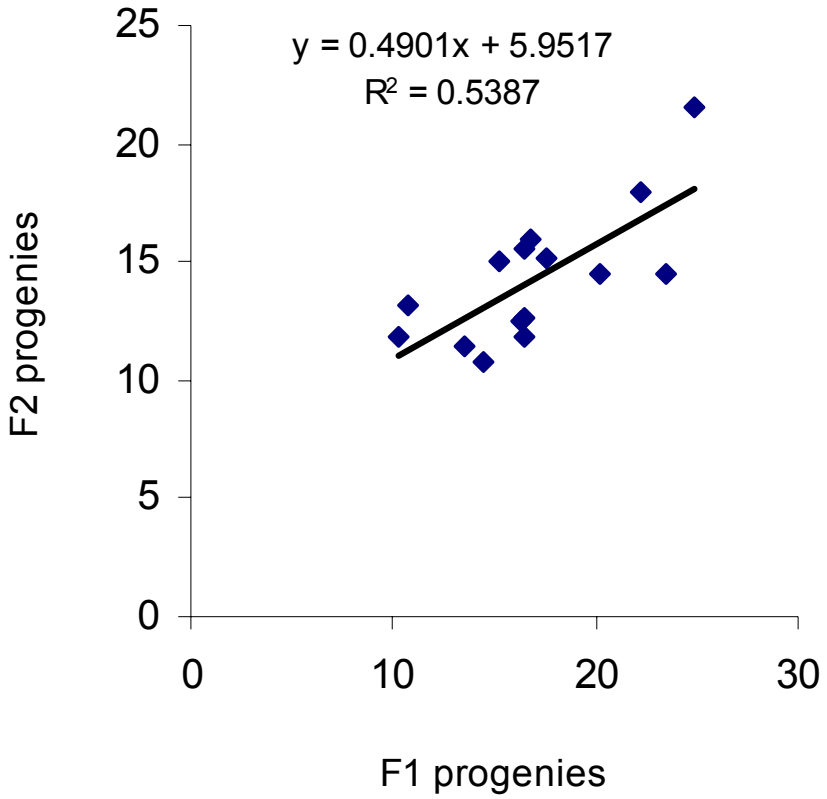

Figure 17: Regression of F_2 on F_1 progenies using AUDPC of CABMV infection

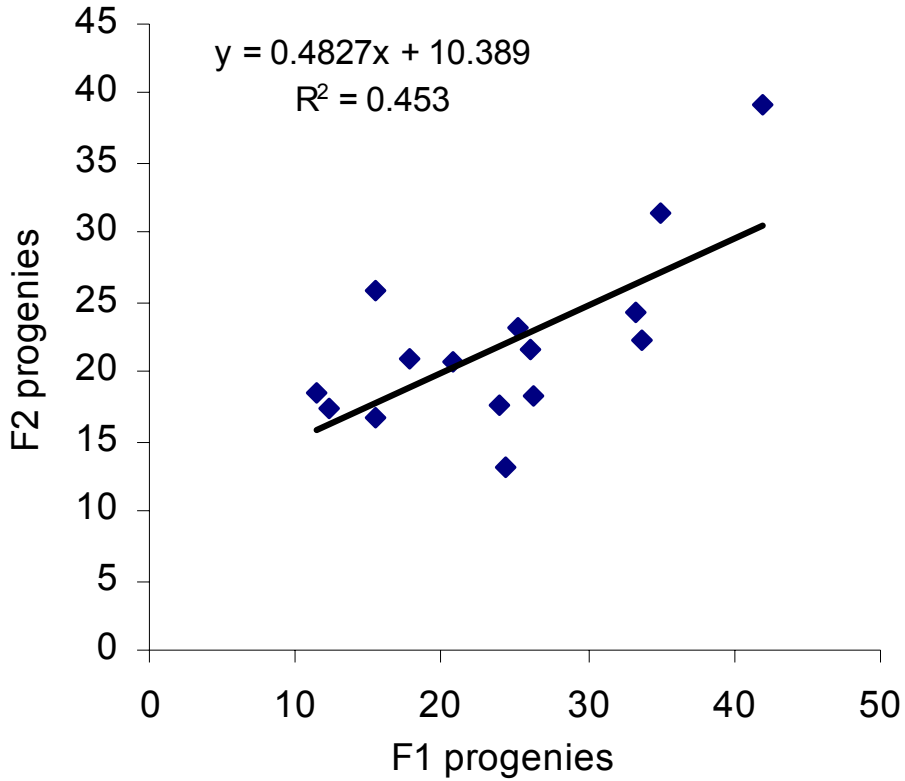

Figure 18: Regression of F_2 on F_1 progenies using final severity of CABMV infection

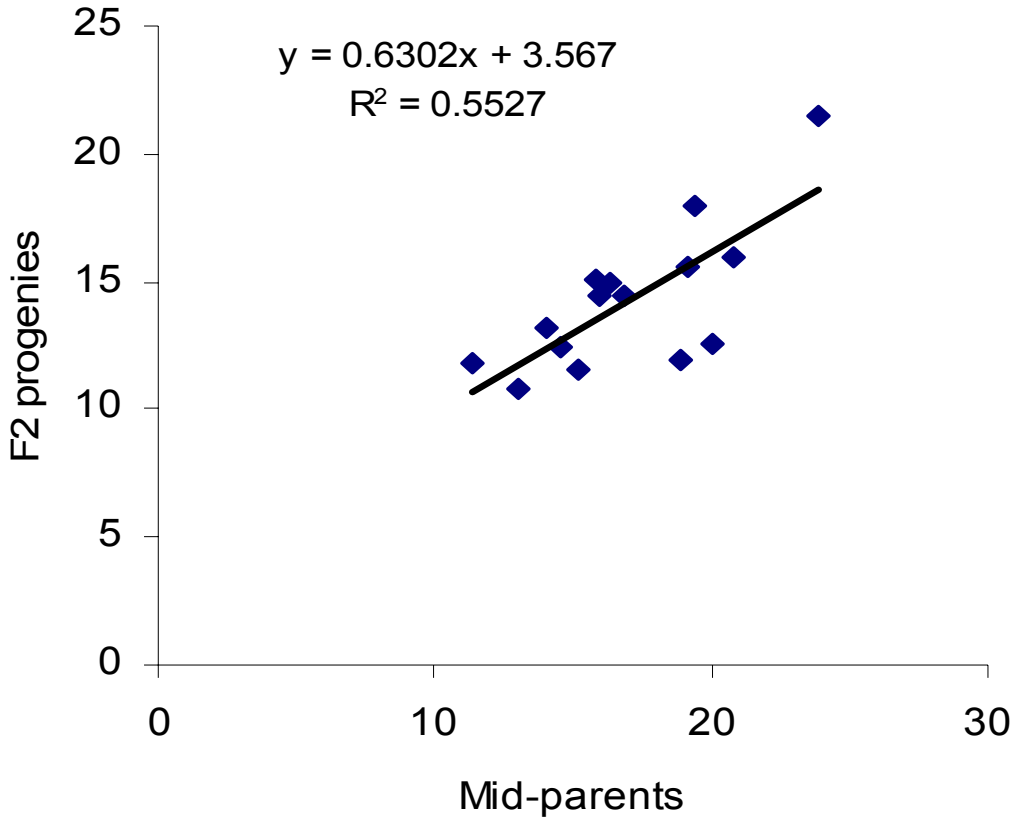

Figure 19: Regression of F_2 on Mid-parents using AUDPC of CABMV infection

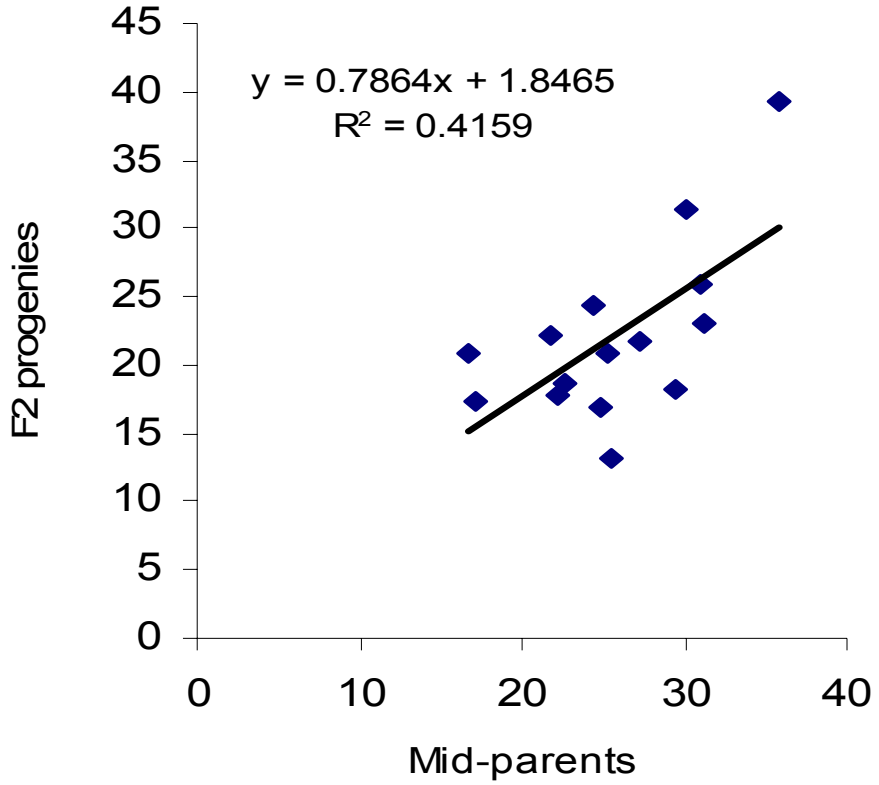

Figure 20: Regression of F_2 on Mid-parents using final disease severity of CABMV infection

5.4 Discussion and conclusion

5.4.1 Response of cowpea crosses and parents to CABMV infection

There was a range of expression of symptoms among the progenies that included chlorosis, mosaic and mottling, with mosaic symptoms being pronounced. Park and Tu (1991) indicated that light intensity has a great effect on the expression of symptom development of the viruses and therefore varying symptom development may occur. Furthermore, Provvidenti and Schroeder (1973) noted that susceptibility of beans to bean yellow mosaic virus Strain (BYMV-S) may be affected by changes in the environmental effects. The cowpea parents and their crosses succumbed to CABMV infection, but with varied levels of symptom development. For instance, Ebelat was highly affected, while MU-93, IT82D-516-2 and IT85F-2841 exhibited moderate symptom development.

In the F_1 populations, six crosses had their mean value less than the overall mean, indicating that they were moderately resistant to CABMV infection. Based on disease severity, some deviations in F_1 crosses were noted, e.g., Ecirikukwai x IT82D-516-2, Ecirikukwai x SECOW-2W, Blackcowpea x IT82D-889 and Blackcowpea x IT85F-2841, all of which exhibited more resistance than their parents. The observation that some F_1 crosses were more resistant than their parents, suggests the involvement of overdominance gene action for resistance. In the case of other F_1 crosses, the observation that they were more susceptible than their parents, suggests overdominance gene action for susceptibility. This suggests that there was both positive and negative overdominance gene action. In this study, it was noted that when the F_1 crosses were selfed, the F_2 populations varied in resistance to CABMV. This was expected because of segregation. For instance, in the F_2 populations, eight crosses had mean disease severity values lower than the overall mean values. However, the cross Blackcowpea x IT82D-889, which showed moderate resistance to CABMV in the F_1 generation, became susceptible in F_2 generation, suggesting that most of the segregants were susceptible and some were even worse than their parents. In contrast, Ebelat x SECOW-2W, which expressed susceptibility in the F_1 cross, showed moderate resistance in the F_2 generation. This might be a result of transgressive segregation for resistance and suggests a quantitative mode of resistance. The observation of transgressive segregation for both resistance and

susceptibility also supports the importance of additive gene action in controlling resistance/susceptibility.

5.4.2 Detection of CABMV and other viruses in the F_2 and backcross populations by DAS-ELISA

The results of the ELISA analyses showed that the F_2 crosses Ecirikukwai x IT82D-516-2, Ebelat x IT82D-889 and Blackcowpea x IT82D-889 did not react with the antisera of CABMV, indicating the absence of the virus in the crosses, although symptoms were observed. The absence of detectable CABMV indicated that these crosses possessed some levels of resistance, rather than simply an asymptomatic infection. Grube *et al*. (2000) revealed that, even if the plant is inoculated and there are inhibiting factors within the plant cells, the replication or cell-to-cell movement of the virus could be slowed dramatically within the inoculated leaf. Previous reports have indicated that several mechanisms of virus resistance can interfere with viral multiplication (Nono-Womdim *et al*., 1993). It was also observed that other viruses like CMV, CPSMV, CPMV and CPCMV were not detected in the crosses. However, there was a strong reaction of all the F_2 crosses with CPMMV, indicating that the virus is also important in cowpea production in Uganda. All of the backcross populations reacted positively with the antisera of CABMV, indicating the susceptibility of the backcrosses to the virus. In relation to other viruses, the results of the study showed that the backcrosses Ecirikukwai x IT82D-516-2 and Blackcowpea x MU-93 reacted positive only to CABMV and CPMMV antisera.

5.4.3 Combining ability estimates for inheritance of resistance

In this study, both GCA and SCA effects were important in the inheritance of resistance to CABMV. This indicated that both additive and non-additive types of gene action were involved in the inheritance of resistance to CABMV infection in cowpea. The magnitude of GCA effect was approximately higher than the SCA effect. This suggests that early generation selection would be effective in breeding for resistance to CABMV in cowpeas. The utilisation of good general combiners such as MU-93, IT82D-516-2, SECOW-2W and IT85F-2841 in hybridisation work followed by selection in segregating populations would be beneficial in a breeding programme. This could be done by adopting progeny selection techniques for exploiting additive genetic variance to improve inbred progenies with a superior performance than the

parents (Jatasra, 1980; Hanson *et al*., 1998). Jatasra (1980) noted that even if the SCA were high, it does not necessarily result in a good performance by the hybrid as well. The proportions (%) of the sum of squares for crosses attributable to GCA and SCA for CABMV severity were 51.4% for GCA due to females, 8.4% for GCA due to males and 40.2% for the SCA. This suggested that additive gene action provided a larger contribution in the populations than the non-additive gene action.

5.4.4 Evaluation of monogenic inheritance model for resistance to CABMV in F_2 and backcross populations

The genetic basis for resistance was determined in this study in order to understand the nature of inheritance of CABMV resistance in the progenies, and also to improve CABMV resistance in the local cowpea cultivars. Genetic variability for resistance to CABMV among the F_2 populations was observed in the study. The susceptibility to CABMV in F_2 populations showed that susceptibility was dominant to resistance. In the current study, the frequency distribution of segregating F_2 crosses was not normal, but had skewed distributions which may be explained by the dominance gene action that was exhibited in some populations. Bjarko and Line (1988) reported that lack of discrete classes in the segregating populations of the crosses may result in low heritabilities, due to segregation of several genetic factors. Furthermore, Bjarko and Line (1988) noted that the lack of normal distribution may be due to the presence of dominance, epistasis or linkage between the leaf rust resistance genes. However, the segregation observed in some F_2 populations may indicate that each of the crosses appeared to possess more than one gene for resistance to CABMV.

Using the chi-square analysis in determining the inheritance of resistance, a ratio of 1 resistant: 3 susceptible was obtained with some of the F_2 progenies. This indicated that resistance to CABMV in these progenies was controlled by a single recessive gene. In the other progenies, the chi-square values were significantly larger than the critical chi-square value in the table, indicating the involvement of more than one recessive gene conditioning resistance to CABMV. The F_2 populations Blackcowpea x IT82D-889, Ecirikukwai x IT82D-889, Ebelat x IT82D-516-2, Ebelat x IT82D-889, Ebelat x IT85F-2841, Blackcowpea x IT82D-516-2 and Blackcowpea x SECOW-2W had significant chi-square values larger than critical value, indicating that there was more than one gene controlling resistance in these populations. A survey of literature indicated that quantitative resistance to CABMV has not been previously reported in

cowpeas. However, the involvement of single or few genes has been previously reported. Shukler *et al*. (1978) and Pal *et al*. (1991) reported that resistance to yellow mosaic virus in cowpea was conditioned by double recessive genes. Taiwo *et al*. (1981) reported that inheritance of resistance to CABMV was conditioned by a single recessive gene. It can be concluded that the resistance was probably controlled by a single gene in seven populations, but it was under the control of many genes in eight populations in the current study.

5.4.5 Heritability estimates for CABMV resistance in cowpea populations

The narrow sense heritability estimate was moderate when F_2 populations were regressed on mid-parents. The large values of heritability estimates for F_1 progenies on mid-parents and F_2 progenies on mid-parents indicated that selection would be expected to be effective. This was consistent with previous findings (Dudley, 1969). Cross *et al*. (2000) showed that the high values of heritability estimates are likely to be due to the control of additive genetic effects. However, Bjarko and Line (1988) indicated that progenies with low heritability estimates would probably be difficult to select for resistance, especially during early generations, but that selection could be done at a later generation, under conditions of severe disease pressure. This showed that a process of hybridisation of parents, followed by selection in segregating populations, should yield inbred progenies with better resistance than the parents. In this study, enhanced levels of resistance to CABMV in the cowpea progenies were achieved. The study also revealed that primarily additive genetic variances governed the severity and AUDPC of CABMV infection, which explains the moderate to high heritability estimates.

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CHAPTER SIX

OVERVIEW OF RESEARCH FINDINGS AND THE WAY FORWARD FOR COWPEA BREEDING IN UGANDA

Cowpea (*Vigna unguiculta* (L.) Walp.) is one of the important cash income and food security crops for most rural farmers in Uganda, and extensive cultivation of the crop is carried out in the northern and eastern regions. However, cowpea production is constrained by a number of factors, among which insect pests and diseases are important. Previous studies have indicated CABMV to be common and a threat to cowpea production in Uganda, where it causes up to 100% yield losses in field-grown susceptible cowpea cultivars. This study was conducted with the aim of improving resistance to CABMV in local cowpea varieties, to the benefit of Ugandan farmers. The following significant findings have been made in this study:

1) The PRA approach was an efficient and effective technique that enabled farmers to provide detailed information about the cowpea cropping system. Farmers prioritised cowpea constraints and listed preferred traits of varieties.

2) The CABMV was common and appears to be a threat in cowpea-producing regions. Other viruses were also encountered, such as CPSMV, CPMMV and CMV. Incidence and severity of the viruses varied from season to season.

3) Several cowpea genotypes (MU-93, SECOW-2W, IT82D-889, IT85F-2841 and IT82D-516-2) possessed good levels of resistance to CABMV, and these were used in breeding work to improve on resistance in the susceptible cowpea cultivars.

4) One or more recessive genes condition resistance to CABMV, and both additive and non-additive gene effects were important in cowpea.

Crop diversification appeared to be a major practice amongst the majority of farmers in the region. The most significant crops mentioned by farmers were cassava, groundnuts, cowpea and sweet-potatoes. The focus of the study was to obtain views on cowpea production from a cross-section of farmers. It was clear that there is an increasing demand for cowpea grain, but that the present cowpea varieties don't

always have the best traits for marketing. The produce therefore often does not fetch premium prices. During the focus group discussions, farmers suggested that research should target certain traits, such as white seed colour, determinate growth, good palatability, good yield, and resistance to diseases and insect pests. Breeders should develop varieties that keep farmers' interests in mind and any deviation from this may lead to the rejection of such cultivars.

Four viruses, namely CABMV, CPSMV, CPMMV and CMV, were found. The viruses were widely distributed, highlighting their potential to cause severe yield losses in all the cowpea growing regions in Uganda. The CABMV was common in association with other viruses (CPSMV, CPMMV and CMV) in the districts surveyed. However, the study showed that there was variability of the viruses from season to season implying that environmental factors have an influence on the occurrence of viruses. This was evident with CPSMV and CPMMV, if the 2004 and 2005 results are compared. The occurrence of new cowpea viruses and their distribution in a wide geographical area is a big challenge for breeders. In selection for CABMV resistance other viruses will need to be taken into account, as seldom will one virus occur without the presence of one or more other viruses.

Several genotypes were identified with good resistance and five were chosen to become the sources of resistance in the breeding programme, namely MU-93, IT85F-2841, SECOW-2W, IT8D-516-2 and IT82D-889. The resistance detection was based on visual observations as well as ELISA tests.

The results of the introgression revealed that the F_1 progenies from crosses Ecirikukwai x MU-93, Ecirikukwai x IT82D-516-2, Ecirikukwai x SECOW-2W and Blackcowpea x IT82D-889 were identified as moderately resistant to CABMV, while F_1 progeny from Ebelat x IT82D-889, Ebelat x IT85F-2841, Ebelat x MU-93 and Blackcowpea x SECOW-2W were susceptible. This study showed that both additive and non-additive genetic factors were important, although GCA may be more important than SCA in determining the inheritance of resistance to CABMV. The high magnitude of GCA compared to SCA is an indication of the additive gene effects rather than the non-additive gene effects for the inheritance of resistance to CABMV infection. This shows that these components are very useful in breeding programmes, as there may be a possibility of transferring important traits to the next generation for better resistance. This suggested that additive gene action provided a large contribution in the populations over the non-additive gene action.

Results indicated that a single recessive gene conditions resistance to CABMV in cowpea. On the other hand, the progenies Blackcowpea x IT82D-889, Ebelat x IT82D-889 and Blackcowpea x SECOW-2W did not fit to a chi-square value due to the large values, indicating that more than one recessive gene conditions resistance to CABMV in these combintions. The backcross progenies Ecirikukwai x SECOW-2W and Ecirikukwai x IT82D-516-2 showed a good fit in segregation ratio of 1 resistant: 1 susceptible, while Blackcowpea x SECOW-2W and Blackcowpea x IT82D-889 did not fit the chi-square value due to the large values. Therefore, the chi-square test seemed to show that resistance to CABMV in segregating F_2 and backcross populations may be conditioned by more than one recessive gene, but with minor gene acting in different locus. The results of F_1 regressed on mid-parents (0.87 and 0.84) and F_2 regressed on mid-parents (0.63 and 0.79), showed high regression estimates, indicating that resistance to CABMV among the parental populations was highly heritable. The regression of F_2 on F_1 showed low heritability estimates, implying that it would probably be difficult to select for resistance in those crosses at early generation for resistance, but a number of generations have to be conducted with high disease pressure before selection can be done.

The need for improved, disease-resistant cowpea cultivars for Uganda has been clearly established. Farmers have given their input regarding the preferred traits for new varieties and these will be important criteria in formulating selection in the segregating populations of the breeding programme. The CABMV will be a major breeding focus, while selection for resistance to other viruses cannot be ignored. Good resistance has been identified in the F_2 and backcross generations and this programme will need to be continued in order to develop the improved cowpea cultivars. It will be necessary to involve farmers in some of the later selections in order to ensure that the best genotypes are identified.