EVALUATION AND INHERITANCE OF SINGLE AND MULTIPLE RESISTANCE TO VIRAL DISEASES OF COWPEA (*Vigna unguiculata* (L.) Walp)

By

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ABSTRACT

Viral diseases usually occur as multiple infections and significantly reduce yield in cowpea. Planting resistant cowpea varieties is economical and effective in controlling viral diseases. However, information on mode of inheritance of virus resistance required for cowpea breeding programmes is limited. Thus, single and multiple resistance and inheritance patterns of resistance to viral diseases were investigated in some selected cowpea breeding lines.

Nine cowpea genotypes comprising eight improved lines and Ife brown (susceptible check) were evaluated for resistance to *Bean common mosaic virus-blackeye cowpea mosaic strain* (BCMV-BlCM), *Southern bean mosaic virus* (SBMV) and *Cucumber mosaic virus* (CMV) in Screenhouse and Field Experiments (SaFE) in IITA, Ibadan. Virus identity was confirmed by RNA sequence similarity search in GenBank databases using BLASTN. Cowpea seedlings were mechanically inoculated seven days after sowing with viruses in 8 viral treatments comprising single and mixed infections. Pots were arranged in 8 by 9 factorial experiment in a completely randomised design (r=3). Disease incidence and severity data were taken at weekly intervals for eight Weeks Post-Inoculation (WPI). Cowpea leaf samples were tested for viruses at five WPI using Enzyme Linked Immunosorbent Assay with negative results confirmed by Polymerase Chain Reactions. Yield parameters were taken while seeds from infected cowpea plants were tested for seed-transmitted viruses. In field evaluations, cowpea lines were planted (r=4) using inoculated Ife brown as spreader rows. Cowpea lines were classified into resistant/susceptible plants using data from disease severity, area under disease progress curves and virus detection test. Two resistant/tolerant and two susceptible cowpea lines were selected and crossed. Parental lines, F_1, F_2, BC_1 and BC_2 were evaluated for virus resistance. Data were analysed using chi-square, ANOVA and PPMC at p=0.05.

Virus identity revealed 92%, 95% and 98% homology to SBMV, BCMV-BlCM and CMV respectively. Disease severity in SaFE was negatively correlated with number of pods/plant (r= -0.9, -0.8), seeds/pod (r= -0.8,-0.6) and total seed weight (r= -0.6,-0.7). Higher seed transmission rates were observed for CMV (2-26%) and BCMV-BlCM (2-25%) than SBMV (0-2%). Cowpea line IT98K-1092-1 had multiple-resistance to BCMV-BlCM and SBMV and tolerance to CMV while IT97K-1042-3 showed multiple-resistance to BCMV-BlCM and SBMV. Lines IT97K1069-6 and IT04K-405-5 showed single

resistance to SBMV. However, IT99K-1060 and IT98K-503-1 were susceptible to the three viruses while other genotypes were susceptible to one or two viruses. Goodness-of-fit for 1 resistant to 3 susceptible segregation ratios (χ^2=1.28) indicated that inheritance of resistance to BCMV-BlCM is controlled by a single recessive gene pair in IT97K-1042-3. Segregation ratios 15 resistant to 1 susceptible plants (χ^2=0.30 and 1.39) suggested that duplicate dominant genes conditioned resistance to SBMV and tolerance to CMV in IT98K-1092-1. Reciprocal crosses supported the monogenic and digenic natures of inheritance and indicated absence of maternal or cytoplasmic effects.

Some cowpea lines showed single resistance to *Southern bean mosaic virus* while some had multiple-resistance to the viruses. Inheritance patterns were monogenic or digenic. The most promising line can be released as a new variety after further trials or its resistance genes introgressed into a susceptible higher yielding variety.

Keywords: Multiple-resistance, *Bean common mosaic virus*, *Southern bean mosaic virus*, *Cucumber mosaic virus*, Cytoplasmic effects.
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DEDICATION

This work is dedicated to the Almighty God for his favour and kindness and to my wife and children for their unending love.

CERTIFICATION

We certify that this work was carried out by Mr. Kayode Ezekiel OGUNSOLA of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan in collaboration with the International Institute of Tropical Agriculture (IITA), Ibadan.

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CHAPTER ONE

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most economically and nutritionally important indigenous African grain legumes. It is cultivated in the tropics and sub-tropical regions in Asia and Oceania, the Middle East, southern Europe, Africa, southern USA, and Central and Southern America (Singh *et al.*, 2002). It is an annual crop believed to have originated in Africa (Padulosi and Ng, 1997). Cowpea is well adapted to the dry savanna in the West African sub-region, where it is mostly grown by small-scale farmers in association with millet, sorghum, maize and groundnut (Boukar *et al*., 2013). The world total production of cowpea is about 4.9 million MT annually from about 10.4 million hectares of land and Africa alone accounts for over 9.7 million hectares, of which over 90% lies in West and Central Africa (FAOSTAT, 2013). Nigeria is the largest producer of cowpea grain with approximately 3.2 million ha under cultivation (FAOSTAT, 2013).

Cowpea grain is valued for its high nutritive quality and short cooking time and serves as a major source of protein in the daily diets of people of the developing tropical world. The seed protein content ranges from 23 to 32 % of seed weight, rich in lysine and tryptophan and a substantial amount of mineral and vitamins (Hall *et al.*, 2003). Cowpea is a staple food crop in Nigeria (Olakojo *et al.* 2007) where it serves as an important source of protein for the teeming population. Farmers in the dry savanna use cowpea haulms as a nutritious fodder for their livestock. The plant's ability to fix atmospheric nitrogen helps maintain soil fertility and its tolerance to drought extends its adaptation to drier areas considered marginal for most other crops (Singh *et al.*, 1997).

However, the average cowpea yield in Nigeria is low, approximately 583 kg/ha (FAO, 2013). This is due to several production constraints, mainly infestation by insect pests, parasitic weeds and diseases caused by many fungal, bacterial and viral pathogens (Jackai and Adalla, 1997). Serious insect pests of cowpea in Nigeria include aphids, thrips, pod sucking bugs, pod borers and storage weevils (*Callosobruchus spp*) (Singh *et al.*, 2003).

Plant viral diseases cause serious economic losses in crops by reducing yield and quality. Viral diseases remain a major constraint to production of cowpea and several other crops

in Nigeria (Shoyinka *et al.*, 1988; Taiwo and Shoyinka, 1988; Thottappilly and Rossel 1992). Estimated yield losses due to viral infection of cowpea are between 10% and 100% (Rachie, 1985). Natural infection of cowpea with about 15 different viruses has been recorded in different parts of the world. Of these, nine viruses were reported to infect cowpea in Nigeria (Taiwo, 2003). These are *Cowpea aphid-borne mosaic virus* (CABMV), genus *Potyvirus*, family *Potyviridae*; *Bean common mosaic virus* - blackeye cowpea mosaic strain (BCMV - BlCM), genus *Potyvirus*, family *Potyviridae*; *Cowpea mosaic virus* (CPMV), genus *Comovirus*, family *Secoviridae*; *Southern bean mosaic virus* (SBMV), genus *Sobemovirus*; *Cowpea mottle virus* (CPMoV), genus *Carmovirus*, family *Tombusviridae*; *Cucumber mosaic virus* (CMV), genus *Cucumovirus*, family *Bromoviridae*; *Cowpea mild mottle virus* (CPMMV), genus *Carlavirus*, family *Betaflexiviridae*; *Sunn-hemp mosaic virus* (SHMV) genus *Tobamovirus*, family *Virgaviridae* and *Cowpea golden mosaic virus* (CGMV), genus Begomovirus, family *Geminiviridae*) (ICTV, 2012). Some of these viruses are seed transmitted. Seed-borne cowpea viruses, after establishment in plants, are typically spread within fields by insect vectors such as aphids (e.g. *Aphis craccivora*), whitefly (*Bemisia tabacci*) and leaf beetles (e.g. *Ootheca mutabilis*) (Hampton *et al.*, 1997). Thus, seed and insect vector transmissions play important roles in the spread and epidemiology of viral diseases.

Among the seed transmitted viruses causing economic losses to cowpea are BCMV - BlCM, SBMV and CMV. These viruses were detected in the 3-year survey throughout all agro-ecological zones in Nigeria (Shoyinka, *et al.* 1997). In cowpea, 40 % yield loss by BCMV - BlCM on the field (Zettler and Evans, 1972), 59 % by SBMV (Givord, 1981) and 14 % yield loss due to CMV (Pio- Ribeiro *et al*., 1978) have been reported.

Compounding the devastating effects of viruses on cowpea is the occurrence of mixed infections. Most attention in virology research has traditionally been given to properties of individual virus species, whereas comparatively little attention has been paid to within-host interactions between viruses or between viruses and other pathogens in multiple infections (Lidsky *et al.*, 2009; Rentería-Canett *et al.*, 2011). Meanwhile, accumulating evidence for ubiquitous viral infections in the plant strongly suggests that mixed viral infections may be the rule rather than the exception in nature (DaPalma *et al.*, 2010). Surveys conducted by Shoyinka *et al.*, (1997) in Nigeria further confirmed that viruses occur in mixtures naturally, causing mixed-infections in cowpea. Though, double

infections are more prevalent, multiple infections caused by four or five viruses have been observed in cowpea (Shoyinka, *et al.*, 1997). Synergistic interaction of BCMV - BlCM and CMV resulted into cowpea stunt disease which caused significant losses in cowpea production (Anderson *et al.*, 1994; Gillaspie, 2001). This double infection of BICMV + CMV caused a yield loss of between 32 – 85 % (Kuhn, 1990). Effective management strategies are thus required to mitigate the devastations caused by the diseases.

Breeding for resistance against pests, either weeds, insects, nematodes, fungi, bacteria or viruses has a common environmental justification; alternatives are needed to pesticides. Cowpea cultivation is heavily dependent upon pesticides and every effort needs to be made to find alternatives. The use of host plant resistance is considered to be the most economical and environment friendly in the management of virus diseases (Orawu *et al*., 2013). Knowledge of the pattern of inheritance of resistance to the virus responsible for causing the diseases is essential for the success of any breeding programme. The first step in the study of resistance to a pathogenic virus is to determine whether the resistance response is heritable and if so, how many genes are involved and their mode of inheritance (Kang, *et al.* 2005). A number of genetic studies have been carried out on virus diseases in cowpea and some of these have led to the identification of resistance (R) genes (Bashir and Hampton 1996; Umaharan *et al*., 1997). According to Fraser (1992), genes for resistance to some viruses have been detected in some cowpea cultivars and landraces. Genes for resistance to a number of viruses have been incorporated into several breeding lines and varieties by the International Institute of Tropical Agriculture (IITA) (1998), as well as some national and international agricultural research institutes (Cardoso *et al.*, 1990). The genes confer good levels of resistance to viruses, thereby boosting productivity of cowpea, especially in West and Central Africa.

Cowpea lines with individual and combined resistance to several cowpea viruses have been identified at IITA (Thottappilly and Rossel, 1992). But in spite of this, viruses are still detected on commercially cultivated cowpeas in Nigeria. Hence, more resistant lines are required for the development of elite cowpea varieties with stable and durable virus resistance and resultant higher yield. Research efforts continue to identify sources for durable virus resistance genes for use in the development of improved cowpea varieties (Boukar *et al*., 2013).

The BCMV-BlCM, SBMV and CMV diseases are major threats to cowpea productivity in sub-Saharan Africa. Although, studies of viral disease resistance in cowpea have been reported, the available reports on modes of inheritance of resistance to BCMV-BlCM, SBMV and CMV diseases are insufficient and seemed to be variety dependent. For instance, Walker and Chambliss (1981) reported that single recessive gene governed inheritance of BCMV - BlCM resistance in cowpea cultivar "Worthmore" and Taiwo *et al.*, (1981) also reported the same inheritance for four cowpea lines (TVu-2740, TVu-3273, TVu-2657 and TVu-2845). In contrast, single dominant gene was reported on the same virus for cowpea cultivars "White Acre-BVR" (Quatara and Chambliss, 1991) and "Pinkeye Purple Hull BVR" (Strniste, 1987). Similar occurrence was observed for SBMV resistance. According to Hobbs *et al.*, (1987), single gene with partial dominance conferred SBMV resistance in cowpea lines "Early Pinkeye" and "PI 186465" whereas multiple genes with incomplete dominance conditioned resistance to the same virus in "Iron" cultivar. For resistance to CMV in cowpea, one dominant gene has been reported (Dezeeuw and Crum, 1963; Fery, 1980). There are however limited reports on tolerance to CMV in cowpea, though it was reported in pepper to be incompletely dominant and quantitatively inherited (Lapidot *et al.*, 1997).

Thus, determination of virus R genes and their modes of inheritance in newly developed improved breeding lines will be useful in breeding programmes. Due to the incidence of multiple virus infections, development of cowpea lines with durable multiple disease resistance to economically important viruses is required to effectively manage cowpea virus diseases (Taiwo *et al.*, 2007). Also, the interactive effects of multiple-viral infections on yield and seed transmission of single and multiple viruses in cowpea have not been adequately reported. Therefore, the objectives of this study are to:

1. Evaluate eight improved cowpea breeding lines for single and multiple resistance against three economically important viruses
2. Investigate the effects of single and mixed infections of BCMV - BlCM, SBMV and CMV on yield parameters of cowpea.
3. Carry out genetic studies to determine the mode of inheritance of resistance to BCMV - BlCM, SBMV and CMV diseases in cowpea, and
4. Determine virus seed transmission under single and mixed virus infections.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and taxonomy of cowpea

Cowpea is indigenous to Africa, with a probable centre of origin in the former Transvaal region, now Gauteng and Mpumalanga provinces, of South Africa due to the abundance of wild varieties in this region (Padulosi and Ng, 1997). Although some authors have suggested that cowpea originated in Asia, much of the published evidence suggested that it originated in Africa (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 is the primary centre of cowpea domestication. Southeast Asia appears to be a secondary centre of cowpea diversity since significant genetic variability occurs on the subcontinent (Baudoin and Marechal, 1985).

Cowpea (*V. unguiculata*) is a diploid species (2n = 2x = 22), self-pollinated and belongs to the family Fabaceae (Padulosi and Ng, 1997). It is a dicotyledonous crop in the order *Fabales*, Family *Fabaceae*, subfamily *Faboideae* (Syn. Papillionoideae), tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna*, and section Catiang (Verdcourt, 1970; Maréchal *et al.*, 1978). The genus *Vigna* is pantropical and with differing reported number of species: 184 (Philips, 1957), 170 (Faris, 1965), between 150 and 170 (Summerfield and Roberts, 1985), 150 (Verdcourt, 1970), 154 (Steele, 1976) and about 84 of which 50 species are indigenous to Africa (Marechal *et al.*, 1978). In addition to cowpea, other members include mungbean (*V. radiata*), adzuki bean (*V. angularis*), blackgram (*V. mungo*), and the bambara groundnut (*V. subterranean*). *V. unguiculata* subspecies *unguiculata* includes four cultigroups: *unguiculata*, *biflora* (or *cylindrica*), *sesquipedalis*, and *textilis* (Ng and Maréchal, 1985).

2.2 Biology and ecology of cowpea

Cowpea is a warm-season, annual, herbaceous legume with spreading growth habit and erect shoots up to 80 cm or more in height. Its leaves are glabrous and taproot is stout with laterals near soil surface. The roots have large nodules and the stems are usually procumbent, often tinged with purple. The first leaves above cotyledons are simple and

opposite, and subsequent trifoliolate leaves are alternate. The terminal leaflet is often bigger and longer than the two asymmetrical laterals. Petioles are stout, grooved, 5 to 15 cm long, leaflets ovoid-rhombic, entire or slightly lobed, and apex acute. The leaflets are usually 6.5 to 16 cm long, and 4 to 11 cm wide and the lateral leaflets are oblique. Inflorescence is axillary, within two to four flowers, crowded near tips of short peduncles 2.5–15 cm long (Duke, 1983).

According to Davis *et al.* (1991), cowpea is generally day neutral. However, short-day photoperiod sensitive types occurs (Dugje *et al.*, 2009). Flowers are borne in multiple racemes of 20 to 50 on flower stalks (peduncles) that arise from the leaf axil. Two or three pods per peduncle are common and up to four pods can also be carried on a single peduncle. Cowpea is primarily self pollinating. Its pods are smooth, 15 to 25 cm long, cylindrical and generally somewhat curved (Davis, *et al.*, 1991). Cowpea seeds, the most widely utilized part of the plant, vary in size from the very small wild types up to nearly 12 mm long. The seed coat can be either smooth or wrinkled and of various colours including white, cream, green, buff, red, brown, and black (Davis *et al.*, 1991). Plant types are often categorized as erect, semi-erect, prostrate, or climbing. Cowpea is generally strongly tap-rooted. It thrives on many kinds of soil, from highly acid to neutral but less well adapted to alkaline. Crop grows and yields at relatively low fertility levels, but often responds to phosphorus fertilization while nitrogen applications are rarely effective on well-nodulated plants. The crop can withstand considerable drought and a moderate amount of shade, but is less tolerant of water logging than soybean (Duke, 1983).

2.3 Constraints to cowpea production

The average cowpea yield in Nigeria is low, approximately 583 kg/ha, compared with its potential of over 3,000 Kg/ha (FAO, 2013). This is due to a complex of abiotic and biotic factors. The abiotic ones include poor soil fertility, drought, heat and soil acidity (Singh and Ajeigbe, 2002). The biotic factors are insect pests, parasitic plants and pathogen infections. Insect pests represent the most serious constraints to cowpea production throughout Africa. Cowpea is attacked by several insect pests. Fatokun *et al.*, (1997) reported susceptibility of large proportion of the available germplasm cowpea lines to major pests, especially to the *Maruca* pod borer and pod-sucking bugs. Those of economic importance are the aphids (*Aphis craccivora* Koch), flower thrips (*Megalurothrips sjostedti* Trybom), pod borers (*Maruca vitrata* Geyer), a complex of pods sucking bugs,

especially (*Clavigralla* spp.) and storage bruchids (*Callosobruchus* spp.), (Karungi *et al.*, 2000a, 2000b; Singh *et al.*, 2003).

Thirty five major diseases are reportedly caused by viruses, fungi, bacteria and nematodes, while 20 major insect pests have also been reported to be responsible for up to 100 % yield loss in cowpea in Africa (Emechebe and Lagoke, 2002). Pythium soft stem rot is a fungal disease caused by *Pythium aphanidermatum* which appears to be important only in warm, humid tropical condition such as those of the rain forest and the southern part of southern guinea savanna of West and Central Africa (Adandonon *et al.*, 2004). Bacterial blight, induced by *Xanthomonas axonopodis* pv. *vignicola* (Burkholder) Dye, is probably the most widespread bacterial disease of cowpea reported from all regions of the world where cowpea is cultivated (Emechebe and Florini, 1997). Kishun (1989) reported grain yield loss of 2.7 to 92.20% to bacterial blight depending on susceptibility of the variety. Viral diseases have long been associated with yield losses ranging from 10 to 100% in field grown cowpea crops (Shoyinka *et al.*, 1997), depending on the virus-host-vector relationships, as well as prevailing epidemiological factors.

2.4 Virus diseases of cowpea

Viruses are known to be a major constraint to production wherever cowpea is grown in the world. Review and research articles on cowpea frequently state that virus diseases are economically important and are major constraint to production (Thottapilly and Rosell, 1988; Mali and Thottapilly, 1986). Field studies to determine the effect of virus diseases on cowpea seed production have not been well documented. Such studies are difficult to conduct because virus-free control treatments, frequently, if not always, become infected with the viruses from treated plants located nearby (Ogundiwin, 2000). Out of more than 20 viruses infecting cowpea worldwide, nine are known to occur in Africa (Taiwo and Shoyinka, 1988) which also infect cowpea in Nigeria (Taiwo, 2003). Seven seed-borne viruses considered most damaging to cowpea include: BCMV - BlCM, CABMV, CMV, CPMV, SBMV, CPMoV and CPSMV. Two non-seedborne viruses considered important (Hampton *et al.*, 1997) are CGMV and *Cowpea chlorotic mottle virus* (CCMV, genus *Bromovirus*). Some of the cowpea viruses of economic importance in Nigeria are described below:

2.4.1 Bean common mosaic virus - blackeye cowpea mosaic strain

Blackeye cowpea mosaic virus is regarded as a distinct strain of the *Bean common mosaic virus* (ICTV, 2010). Bean common mosaic virus - blackeye cowpea mosaic strain occurs more or less worldwide and is transmitted non-persistently by several aphids' species including *Aphis craccivora* (Purcifull and Gonsalves, 1985). Its occurrence has been reported in Brazil, India, Kenya, Nigeria and other parts of the world (Mali *et al.*, 1983). The virus particles are filamentous, with modal lengths ranging from 740 to 800 nm (Lima *et al.*, 1979; Taiwo *et al.*, 1982; Murphy *et al.*, 1984). It contains single stranded RNA and induces the formation of cytoplasmic cylindrical inclusions and associated scrolls in its hosts (DPV, 2012). Symptoms usually consist of discoloration of leaves, showing mosaic, mottling, vein banding, vein chlorosis, leaf deformation and yellow spots and the affected plants may also show growth reduction (CPC, 2007; Taiwo and Shoyinka, 1988).

Diagnosis can be by purification or serology. BCMV - BlCM bears etiological and morphological resemblance to the CABMV but through the protein coat digestion, amino acid sequence analysis of the peptides and serology, studies have shown that BCMV - BlCM is distinct from CABMV and both are distinct potyviruses (Taiwo *et al.* 1982; Thottapilly *et al.* 1993; Bashir and Hampton, 1996). The virus is readily transmissible by sap inoculation. At least 36 species in 7 dicotyledonous families are susceptible, with cowpea being a major natural host. Natural infections are also reported in *Crotalaria* and *Desmodium.* The virus is seed-borne and seed transmitted, causing economic loss in cowpea. BCMV - BlCM strains reported are in symptoms and host range variants (Murphy *et al.*, 1984). A major symptom variant is an isolate which causes red, necrotic ring spots and reddish veinal necrosis on cowpea cultivar Knuckle Purple Hull.

2.4.2 *Southern bean mosaic virus*

The cowpea strain of SBMV (SBMV-CP or strain C) also called *Southern cowpea mosaic virus* often occurs in mixtures with other beetle-transmitted viruses, including CCMV and CPSMV (Hampton *et al.* 1997). SBMV is mainly found in tropical and subtropical areas. The particle of SBMV is isometric ca 28 - 30 nm diameter. It contains a single species of single-stranded, positive-sense RNA of 4194 nucleotides (DPV, 2012). SBMV gives necrotic local lesions in some cowpea cultivars. However, it spreads systemically in most cowpea cultivars causing vein clearing, mosaic and leaf distortion (CPC, 2007). SBMV is highly antigenic. The bean strain (strain B) infects common bean varieties but not cowpea

(DPV, 2012). The virus can be diagnosed serologically and bean and cowpea strains can be distinguished by the Reverse Transcription-Polymerase Chain Reaction (RT-PCR). It is transmitted by leaf beetles (Chrysomelidae) probably in a circulative manner. In North America, strains B and C are transmc itted by *Ceratoma trifurcata* and *Epilachna varivestis* while *Ootheca mutabilis* transmits strain C in Nigeria (Allen *et al.*, 1981). Strain C can be transmitted by *C. trifurcata* for up to 19 days following acquisition access feedings but beetle species differ in the length of time for which they continue to transmit virus without renewed access to source plants (DPV, 2012).

2.4.3 *Cucumber mosaic virus*

Cucumber mosaic virus is distributed worldwide. It has increasingly been reported as the causal agent in several disease epidemics of major crops throughout the world, especially in the tropics (Palukaitis *et al.*, 1992). CMV has the widest host range of any virus and is one of the most damaging viruses of temperate agricultural crops worldwide (Gallitelli, 2000). Hosts include over 1200 species in over 100 families of monocots and dicots, including many vegetables, ornamentals and woody and semi-woody plants (Zitter and Murphy, 2009). Also, CMV has been detected in leguminous and tomato plants (Zitikaite and Staniulis, 2006) and is one of the most common plant viruses of substantial agricultural importance (Van Regenmortel *et al.*, 2000).

It is transmitted by numerous species of aphid in a non-persistent manner. It is also emerging as a major virus, especially in the tropics. CMV particles contain three functional pieces of single-stranded RNA, packaged in three classes of icosahedral particles about 28 nm in diameter, all sedimenting at the same rate (DPV, 2012). The particles are isometric (Table 2.1). CMV is a single-stranded positive-sense tripartite genome RNA (Boari *et al.* 2000; Colariccio *et al.*, 2002). Most CMV strains cause systemic infections, which are sometimes symptomless. The most common symptoms include severe mosaic, mottling, chlorosis, necrosis and distortion in leaves and fruits. In beans, early infected plants may yield no or few pods because CMV causes flower abortion and abnormal development. These pods are mostly curved, mottled and reduced in size (Zitter and Murphy, 2009). Diagnostic hosts include *Chenopodium amaranticolor* and *C. quinoa* (CPC, 2007).

Table 2.1 Properties of viruses infecting cowpea in Nigeria

Virus name	Genus	Shape	Sizes (nm)	Vector	Symptoms[a]
Cowpea aphid borne mosaic	*Potyvirus*	Filamentous	750	Aphid	DGVB
Cowpea yellow mosaic	*Comovirus*	Isometirc	24	Beetle	DYMo
Southern bean mosaic	*Sobemovirus*	Isometric	28	Beetle	VoC, Mo, M
Cowpea mottle	*Carmovirus*	Isometric	30	Beetle	M, BoY
Cowpea golden mosaic	*Geminivirus*	Geminate	20 x 30	Whitefly	BoY
Cucumber mosaic	*Cucumovirus*	Isometric	28	Aphid	Mo, M, R
Cowpea mild mottle	*Carlavirus*	Flexous rod	650	Whitefly	mM
Suhn-hemp mosaic	*Tobamovirus*	Rigid rod	300	-	Mo, mM
Bean common mosaic	*Potyvirus*	Filamentous	750	Aphid	DGVB

[a]DGVB, Dark green vein-banding; DYMo, Distinct yellow mosaic; Mo, Mosaic; M, Mottle; VoC , Vein clearing; BoY, Bright yellow; R, Ringspot; mM , Mild mottle. **Source**: Taiwo, (2003)

2.4.4 *Cowpea aphid-borne mosaic virus*

The CABMV is a cosmopolitan virus of cowpea found in Europe, USA, Asia and Africa (Thottappilly and Rossel. 1992; Shoyinka *et al.*, 1997). In surveys throughout Nigeria during the past several years, CABMV has been found to occur in all ecologic zones and is considered one of the most widespread and important viral diseases of cowpea (Thottappilly and Rossel, 1985). The virus has been reported to be transmitted by several aphid species in a stylet-borne non-persistent manner, but *Aphis craccivora* is reported to be the most efficient vector (Atiri *et al.*, 1984). CABMV has flexuous filamentous particles 727 - 765 nm in length. The nature and severity of the symptoms induced by CABMV vary with host cultivars, virus strain and the time of infection (Thottappilly and Rossel, 1985). Natural infection of cowpea causes various mosaics, mottling, interveinal chlorosis and vein-banding (CPC, 2007).

2.4.5 *Cowpea mosaic virus*

CPMV is an RNA-containing virus with isometric particles. It is characterized by isomeric particles averaging 24 nm in diameter (Table 2.1), having two kinds of nucleoprotein particles that are similar in size but differ in their single-stranded RNA content (Thottappilly and Rossel, 1985). It has a limited host range and is transmitted mainly by beetles and readily by sap inoculation (DPV, 2012). Owning to its common occurrence, epidemic potential and pathogenicity, CPMV, also called *Cowpea yellow mosaic virus* (CYMV) is one of the most important cowpea viruses in Africa (Hampton *et al.* 1997). Surveys have revealed that CPMV ranked next to CABMV in importance in Nigeria (Shoyinka et al., 1997). Most locally grown cowpea varieties appear highly sensitive and susceptible. Host plants of CPMV include *Chenopodium quinoa* (quinoa), *Crotalaria juncea* (sunn hemp) and *Glycine max* (soyabean). Its RNA genome has been sequenced and defined in a classic series of investigation by van Kammen and colleagues, as reviewed by Mathews (1991).

2.4.6 *Cowpea severe mosaic virus*

CPSMV has assumed worldwide distribution via movement of infected seed lots and appeared to be more common than CPMV in the cowpea cultivars of Southern Europe and the Americas and less common in old world cowpea-growing regions (Bashir and Hampton, 1993). According to Hampton *et al.*, (1997), CPSMV comprises of at least nine serotypes and an unknown number of pathogenic variants. The virus is characterized by

isometric particles or approximately 25 nm in diameter. It has two kinds of nucleoprotein particles that are morphologically identical but contain different single-stranded RNA molecules (Thottappilly and Rossel, 1985).

2.4.7 *Cowpea mottle virus*

Cowpea mottle virus (CPMoV) was first isolated in Nigeria by Shoyinka *et al.* (1978) and has been reported from Benin, Cote d'Ivoire, Pakistan and Togo (Hampton *et al.* 1997). Extensive surveys in Nigeria revealed that the virus typically occurs in cowpea when grown in association with Bambarra groundnut. Geographically, within Nigeria, the virus almost exclusively occurs in the riverine area of middle belt, which has a southern guinea savanna climate, where most Bambara groundnut is also grown (Thottappilly and Rossel, 1985). The symptoms of CPMoV are dense clustering of branches at the top of the plants, a feature known as witches' broom syndrome. This virus also causes severe mottling, leaf distortion and stunting (Taiwo and Shoyinka, 1988). The modes of transmission of CPMoV are by seeds, vectors and mechanical. Some of the vectors that transmit the virus are the beetles, *Ootheca mutabilis* and *Paraluperodes quaterus.* Its particle is isometric and measure about 30 nm in diameter containing a single-stranded RNA, which sediments as a single component. It can be detected through transmission test, serology and molecular techniques. Gillaspie *et al.* (1999) described a sensitive RT-PCR method for detection of CPMoV.

2.5 Multiple-virus infections

Mixed viral infections usually result in a more severe disease symptom culminating in significant reductions in quantitative parameters such as plant height, weight and subsequently yield and at times causing death. Viruses in mixed infections may interact synergistically or antagonistically causing changes in the concentration of either or both viruses (Murphy and Bowen, 2006). Antagonism usually occurs when the co-infecting viruses are related, resulting in interference or cross-protection while synergism normally occurs in mixed infection of unrelated viruses, resulting in more severe disease symptoms than those produced by single infection (Walkey and Payne, 1990). Moreover, mixtures of synergistic and antagonistic interactions, creating usually unpredictable biological and epidemiological consequences, are likely to occur in plants. The mechanisms of some of these are still unknown (Syller, 2011; Murphy and Bowen, 2006).

Viral diseases usually occur in multiple infections. Multiple infection involving CABMV + SBMV and CABMV + CPMoV have been reported to cause stunting and premature death in some commercial cowpea cultivars in Nigeria (Taiwo *et al.*, 2007). This suggested synergistic interaction between CABMV and CPMoV which was further confirmed by the increased symptoms observed on the cowpea inoculated with a mixture of the two viruses. Owolabi *et al.*, (1988) also reported a 78 – 100 % reduction in the pod number of two cowpeas cultivars (Ife Brown and Nigeria B7) inoculated with BCMV - BlCM and CYMV. Some form of synergistic Potyvirus interactions has also been reported by a number of workers in soybean (Calvert and Ghabrial, 1983), in pepper (Murphy and Bowen, 2006) and in cucurbits (Wang *et al.*, 2002). Apart from cowpea, mixed infections have been reported in other commercial vegetables such as pumpkin, watermelon, pepper, Irish potatoes, tomatoes and wheat (Bowen *et al.*, 2003, Murphy and Bowen, 2006).

2.6 Intra-host virus-virus interaction

Multiple infections lead to a variety of intra-host virus–virus interactions, many of which may result in the generation of variants showing novel genetic features and thus change the genetic structure of the viral population. Hence, virus–virus interactions in plants may be of crucial significance for the understanding of viral pathogenesis and evolution and consequently for the development of efficient and stable control strategies (Syller, 2011). Related and unrelated viruses can often replicate in the same cells and may interact synergistically or antagonistically (Otsuki and Takebe, 1976), while the concentration of one or both may significantly increase. The synergistic interaction has a facilitative effect on both or at least one of the viral partners and is manifested by an increase in virus replication in the host plant (Syller, 2011). Different mechanisms have been reported for enhancement of virus under mixed infections. In a mixed infection involving *Potato virus* X (PVX) genus *Potexvirus* and *Potato virus* Y (PVY), family *Potyviridae*, genus *Potyvirus*. PVX was reportedly enhanced while PVY remained unchanged (Vance, 1991). In contrast, no marked increase in the accumulation of PVX was recorded in *Nicotiana benthamiana* plants co-infected with PVY, *Tobacco etch virus* (TEV) or *Plum pox virus* (PPV) despite the severe reaction leading to systemic necrosis of leaves and stems and finally plant death. This showed that enhancement of disease symptom is not simply due to increase in PVX accumulation in plants and it was suggested that synergy between PVX and a potyvirus is host dependent (Gonzalez-Jara *et al.*, 2004, 2005). Goodman and Ross, (1974) reported that the increase in PVX by PVY or TMV in tobacco (*Nicotiana tabacum*

L.) resulted from enhanced concentration and increased synthesis per cell and not by increase in the number of cells infected. Meanwhile, in co-infection involving CMV and *Turnip mosaic virus* (TuMV), family *Potyviridae* genus *Potyvirus*, the enhancement of CMV was largely attributed to an increased number of CMV infected cells (Ishimoto *et al.*, 1990). Baker (1987) attributed enhancement of *Potato leaf-roll virus* (PLRV) genus *Luteovirus* by PVY in tobacco to the enhanced transport of the virus. Balogun *et al.*, (2002) reported that mixed infection of tomato with TMV and PVX, which results in more disease symptoms, involves alterations in the accumulation of PVX and is influenced by virus strains and tomato cultivar where cultivar with specific resistance gene is the best hope for curtailing the viral disease.

Despite the differences in sequences and genome organization, taxonomically distinct species of plant viruses have frequently been demonstrated to exhibit complementary functions in virus cell-to-cell and long distance transport (Rao *et al.*, 1998) Complementation, a process by which function affected by mutation is provided in trans by fully competent genotypes in multiple-infected cells may result to host range extension (Fraile *et al.*, 2008). Recombination has also been reported especially with regard to virus evolution, synergistic interactions between related viruses invading the same cells (Syller, 2011). Mixed infections provide the opportunity for recombination between co-infecting viruses to give rise to new variants or species while some of these new entities might become a severe phytopathological problem (Rentería-Canett *et al.*, 2011).

Antagonism or cross-protection occurs when a previous infection with one (protecting) virus prevents or interferes with subsequent infection by a homologous virus (DaPalma *et al.*, 2010). Several mechanisms have been proposed for this phenomenon. Some of these include a prevention of the disassembly of the challenging virus by the expression of the coat protein of the protecting virus (Sherwood and Fulton, 1982) and the induction of RNA silencing by the protecting virus, presumably by sequence-specific degradation of the challenging virus RNA (Fagoaga *et al.*, 2006). Also, the coat protein may interfere with the process of replication of the challenging virus (Sarika *et al.*, 2010). Also, mutual exclusion, exhibited by mild symptoms developed by plants soon after inoculation, followed by complete recovery of the plant from which no virus could be detected, has been reported in mixed viral infections (Syller, 2011).

2.7 Insect vectors of plant virus

Insect vectors of plant viruses are found in 7 of the 32 orders of the class Insecta. Most plant viruses depend on vectors for their survival and spread. Most vectors are found in two orders Thysanoptera and Hemiptera, which are the piercing-sucking insects that transmit plant viruses either the circulative virus (CV) or the non-circulative virus (NCV). NCV are carried on the lining cuticle of vectors' stylets while CV cut the vectors gut, move internally to the salivary gland, cross the membrane to be ejected upon feeding (Raccah and Fereres, 2009). Few vector species are found in five orders of chewing insects which include Orthoptera, Dermaptera, Coleoptera, Lepidoptera and Diptera (Raccah and Fereres, 2009).

2.7.1 Mode of virus transmission by insects

The basis for assigning viruses to their modes of transmission was the duration of virus retention in the vector. Non-persistent are for short retention or less than the time the virus survives in leaf extracts and persistent for extended retention, often for live. Later on, semi-persistent viruses were identified. After this time, different terminologies were proposed for mode of transmission based on the site at which the virus is carried in the insect. The non-persistent viruses were termed stylet-borne whereas persistent were termed circulative. The circulative or internal mode of transmission means the virus crosses body barriers and enters the circulatory system of the insect and accumulates inside the salivary glands. The non-circulative or external is where the virus remains attached to the cuticle of the insect and does not cross body barrier (Raccah and Fereres, 2009). Examples of important virus vectors are aphids, foliage beetles, thrips and leaf hoppers.

2.7.2 Aphids

Aphids are by far the most important vectors, transmitting nearly 30 % of all plant virus species described to date. Several different interaction patterns have evolved between viruses and aphid vectors (Brault *et al.*, 2010). Aphids are certainly by far the most frequent and efficient vectors of plant viruses. They transmit hundreds of plant pathogens, mostly viruses and cause large economic losses. They have adopted a complex life cycle with alternating asexual and sexual phases and show remarkable phenotypic plasticity. Three transmission modes were defined, the non-persistent mode with viruses acquired within seconds and retained for only a few minutes by their vectors; the semi-persistent

mode with viruses acquired within minutes to hours and retained for several hours; the persistent mode with viruses that require minutes to hours for acquisition and that can be retained for very long periods, often until the vector dies (Brault *et al.*, 2010). *Aphis craccivora* is a primary pest of cowpea but may also attack beans while *A. fabae* attacks common bean. *A, craccivora* causes direct damage together with transmission of viruses especially CABMV, CMV and BCMV - BlCM in cowpea.

2.7.2.1 Aphid's biology and damage

Aphis craccivora is medium sized, shiny black aphid whose biology varies depending on climate and soil. Under favourable conditions a generation may take only 13 days. Adults live from 6 to 15 days and may produce more than 100 progeny. On cowpea, aphids normally feed on the under surface of young leaves, on young stem tissues and on pods of mature plants. When present in large numbers, they cause direct feeding damage. The plant becomes stunted leading to leaf distortion, premature defoliation and death of seedlings (Singh and Allen, 1979; Allen *et al.*, 1996).

2.7.3 Foliage beetles

Foliage beetles are widely distributed in Africa where they are an important foliage feeder of cowpea seedlings. They are of the order Coleopteran, family Chrysomelidae. *Ootheca mutabilis* and *Ceratoma spp.* are common in West Africa and America causing damage and transmitting cowpea viruses. Some beetle-borne viruses are circulative while others are non-circulative. For instance, *Epilachna varivestis* retains CPSMV for one day while *Ceratoma trifurcata* retains the same virus for several days (Raccah and Fereres, 2009). Also, *O. mutabilis* transmits CPSMV and CPMoV and while *Ceratoma trifurcata* transmits SBMV in cowpea. In East Africa a related species, *Ootheca bennigseni*, is also found (Singh and Allen, 1979; Allen, *et al.*, 1996)

2.7.3.1 Foliage beetle's (*Ootheca mutabilis*) biology and damage

Adults are about 6 mm long, oval, and normally shiny reddish brown although this varies considerably and black or brown adults may occur. Yellow egg masses are laid in the soil and there are three larval instals. Adults feed interveinally on the leaves and later enlarging damage into feeding holes. High beetles populations can totally defoliate cowpea seedlings and kill them. The larva feeds on cowpea roots but seldom cause serious damage but adult beetles are effective vectors of cowpea viruses.

2.8 Seed transmission of viruses

Seed transmission plays an important role in virus diseases. It was not initially considered to play an important role in the epidemiology of *Zucchini yellow mosaic virus* (ZYMV, genus *Potyvirus*), a devastating pathogen of cucurbits causing yield losses up to 99 % until 2002, where up to 5 % of the seeds of *Cucurbita pepo* var. *styriaca* (oil pumpkin) were reported to have transmissible virus (Riedle-Bauer *et al.*, 2002)**.** Seed-borne viruses have been distributed to most cowpea producing regions of the world through the exchange of seeds (Hampton *et al*., 1997). The increasingly recognized importance of seed transmission in plant virus ecology has led to the strengthening of seed-health testing for viruses in certification and quarantine agencies internationally.

Thirty percent seed transmission of BCMV - BlCM has been reported (Frison *et al*., 1990) while incidence of seed-borne as high as 50 % BCMV - BlCM was observed by Gillaspie *et al.*, (1993). In Nigeria, SBMV has been reported to be seed borne at rates of 3 – 4 % (Thottappilly and Rossel, 1988) and 30 % seed transmission rate has been reported in CMV (Abdullahi *et al.*, 2001). However, seed transmission of viruses under mixed viral infections has not been adequately reported and mixed infections are naturally more common than single infections on the field.

2.9 Economic importance of cowpea viruses

Virus diseases have been reported to cause substantial yield reduction in cowpea production in West and Central Africa (VanBoxtel *et al.*, 2000). Estimated losses due to virus infection have been variously put at between 10 and 100 % depending on the virus - host-vector relationships as well as prevailing epidemiological factors (Shoyinka, 1974). A yield loss of 13 – 87 % due to natural infection of cowpea by CABMV was reported in Iran (Kaiser and Mossahebi, 1975) and 48-60 % loss in cowpea was reported in Zambia (Kannaiyan and Haciwa, 1993). Several studies also revealed economic loss in cowpea in Nigeria as a result of virus diseases. CPMoV has been reported to cause 75 % decline in yield of cowpea in Nigeria (Taiwo and Shoyinka, 1988). Similarly, Shoyinka (1974) reported that cowpea mosaic virus (CPMV) caused yield losses of between 60 and 100%. Taiwo *et al.*, (2007) reported apical necrosis and a total yield loss in some commercial cowpea cultivars with a multiple viral infection of CAbMV + CPMoV + SBMV while yield loss of 32 – 85 % was reported when cowpea was infected with mixed infection of BCMV - BlCM + CMV (Kuhn, 1990).

2.10 Plant virus disease management

There are no economically feasible chemical agents similar to fungicides and bactericides that are effective against plant viruses. Thus, strategies aimed at plant virus disease management are largely directed at preventing virus infection. These include: 1) eradicating the source of infection to prevent the virus from reaching the crop such as, elimination of weeds that harbor the viruses, rouging of infected plants to prevent the spread of the viruses and plant quarantine, 2) minimizing the spread of the disease by controlling the biotic vectors either chemically or by other means, 3) utilizing virus-free planting materials and 4) the use of host-plant resistance (Khetarpal *et al*., 1998; Naidu and Hughes, 2003). Integrated disease management based on combination of genetic resistance and crop management components such as vector control, rouging as well as use of plant quarantine is usually more effective.

2.11 Prevention of cowpea diseases by the Nigeria Agricultural Quarantine Service

Due to the increased world-wide movement of germplasm through seed and other propagative materials in global trade and agriculture, diagnosis of pathogens in these materials assumes greater importance for national quarantine services to ensure safe movement of germplasm across the borders (Naidu and Hughes, 2003). For importing cowpea seed into Nigeria, the conditions of importation must be fulfilled. These conditions vary with country, based on the presence and type of cowpea pests.

Some of these conditions are: 1) The consignment must be accompanied by a Phytosanitary Certificate issued by the appropriate authority of the country, 2) Additional declarations that the parent plants where the seeds were harvested were inspected during active growth and found to be free from cowpea quarantine pest/diseases which will be listed per country. For instance, cowpea from South Africa as at 2014, must be declared free from *Alfalfa mosaic virus*, *Bean leaf roll virus*, *Bean yellow mosaic virus*, *Broad bean wilt virus*, *cowpea chlorotic mottle virus*, *cowpea severe mosaic virus*, *cowpea stunt virus*, *Phytophthora vignae*, *Perenospora viciae* (downy mildew) and *Heterodera cajani*, 3) The seed must be treated with metalaxyl and also fumigated with phostoxin at the rate of $2.5g/m^3$ for 5 days against storage pests, 4) Seed from some countries must not be GMO or LMO product and 5) consignment must be delivered on arrival to the Post-entry Quarantine Station, NAQS, Ibadan with enclosed labels where samples are to be subjected

to laboratory test before release and follow-up field inspection by the NAQS officials at the importers farm (NAQS, 2012).

For export, import permit documents may be required from the country of export. Cowpea seeds, after graded free of debris, will be subjected to fumigation with phostoxin for 72 hrs at 33 g/1000 cc. The seeds are packaged in transparent polythene bags and sealed. The exporter is issued a Phytosanitary Certificate which contains statements to the effect that quarantine inspection has been carried out by accredited officer of the NAQS and the consignment of plants /plant products is pest-free at the time of examination (NAQS, 2010).

2.12 Host plant's response to infections

In order to survive, plants developed a broad range of defense mechanisms to pathogen infections. Responses to pathogen infections vary from immunity to resistance, tolerance or even susceptibility of the host plant to the pathogens.

2.12.1 Host resistance

Host resistance occurs when genetic polymorphism for susceptibility is observed in the plant taxon, that is, some genotypes show heritable resistance to a particular virus whereas other genotypes in the same gene pool are susceptible. In resistant individuals, the virus may or may not multiply to some extent but spread of the pathogen through the plant is demonstrably restricted relative to susceptible hosts, and disease symptoms generally are highly localized or not evident. Resistance to the pathogen typically leads to the resistance to the disease. Plants are resistant to certain pathogens because they belong to taxonomic groups that are outside the host range of these pathogens (non-host resistance), because they possess genes for resistance (R genes) directed against the avirulence genes of the pathogen (true, race-specific, cultivar-specific, or gene-for-gene resistance) or because, for various reasons, the plants escape or tolerate infection by these pathogens (Agrios, 2005).

Another important category of host resistance is systemic acquired resistance. This response can be activated in many plant species by diverse pathogens that cause necrotic cell death (Ross, 1961) resulting in diminished susceptibility to the later pathogen attack. Virus-induced gene silencing is another induced defense mechanism to virus disease. Transgenic approaches to plant virus resistance have been widely explored since the

earliest experiments where by transgenic tobacco plants expressing TMV coat protein were challenged with Tobacco mosaic virus (TMV) and shown to be resistant (Roger, 2002). It is now possible to engineer resistance and tolerance to plant viruses using transgenes derived from a wide range of organisms including plant-derived natural R genes, pathogen-derived transgenes and even non-plant or non-pathogen-derived transgenes.

2.12.2 Tolerance

Tolerance to disease is the ability of plants to produce good crop even when they are infected with a pathogen. Tolerance results from specific, heritable characteristics of the host plant that allow the pathogen to develop and multiply in the host while the host, either by lacking receptor sites for or by inactivating or compensating for the irritant excretions of the pathogen, still manages to produce a good crop. Tolerant plants are, obviously, susceptible to the pathogen, but they are not killed by it and generally show little damage (Agrios, 2005). In case of tolerance to viral disease, the virus may move through the host in a manner that is indistinguishable from that in susceptible hosts, but disease symptoms are not observed (Kang *et al.*, 2005). Tolerant plants show lesser degree of symptom expressions, while allowing virus multiplication and spread and they produce significantly better yield and quality than the susceptible plants.

The genetics of tolerance to disease are not well understood. Tolerant plants, whether because of exceptional vigor or a hardy structure, probably exist in most host–parasite combinations. Tolerance to disease is observed most commonly in many plant–virus infections in which mild viruses, or mild strains of virulent viruses, infect plants such as potato and apple systemically and yet cause few or no symptoms and have little discernible effect on yield (Agrios, 2005). This host response is very prevalent in nature and has been used to considerable benefit in some crops e.g. the control of *Cucumber mosaic virus* in cucumber (Roger, 2002).

2.13 Natural resistance mechanisms

Viruses have been reported to undergo a multistep process to complete their life cycles, these include entry into plant cells, uncoating of nucleic acid, replication of viral nucleic acid, assembly of progeny virions, cell-to-cell movement, systemic movement, and plant-to-plant movement (Carrington *et al.* 1996). Plant viruses typically initiate infection by

penetrating through the plant cell wall into living cell through wounds caused by mechanical abrasion or by vectors such as insects and nematodes. Unlike animal viruses, there are no known specific mechanisms for entry of plant viruses into plant cells (Shaw, 1999). When virus particles enter a susceptible plant cell, the genome is released from the capsid, typically in the plant cytoplasm. Later, the virus faces various constraints imposed by the host and also requires the involvement of many host proteins, typically diverted for function in the viral infection cycle. Successful infection of a plant by a virus therefore requires a series of compatible interactions between the host and a limited number of viral gene products. Absence of a necessary host factor or mutation to incompatibility has long been postulated to account for recessively inherited disease resistance in plants termed "passive resistance" (Fraser, 1986).

In contrast, dominant resistance has been shown in a number of plant pathosystems to result from an active recognition event that occurs between host and viral factors, resulting in the induction of host defense responses. The biochemistry of this recognition event is still not thoroughly understood. Genes that contribute to this response are likely to be dominant or incompletely dominant, unless resistance response occurs as a result of derepression of a defense pathway. In theory, passive or active resistance can function at any stage of the virus life cycle, although most known viral resistance mechanisms target virus replication or movement. For instance, several resistant cowpea genotypes with symptomless or mild mosaic reactions to SBMV have been shown to restrict virus accumulation to levels lower than those of susceptible cultivars (Hobbs *et al.*, 1987).

2.14 Genetics of disease resistance

Over the past decade, the cloning and analysis of numerous plant R genes have stimulated attempts to develop unifying theories about mechanisms of resistance and susceptibility and co- evolution of plant pathogens and their hosts (Martin, *et al.* 2003). Resistance may be controlled by any number of genes and the effects of resistance genes vary from large to minute. Resistance genes may interact epistatically or additively and for relationships between biographic parasites and host plants, resistance and virulence genes often operate on a gene-for-gene basis (Day, 1974). Vertical or race-specific resistance is inherited through oligogenes with relatively large effects. Resistance genes in many biotrophic pathogen-host plant combinations are typically of a vertical nature and these host-pathogen systems are proven or assumed to operate on a gene-for-gene basis. The

inheritance of horizontal or race-nonspecific resistance is more complicated and some avoidance mechanisms can be expected to be race nonspecific. Horizontal resistance can arise in two ways: 1) when the host genes do not operate in a gene-for-gene way with the pathogen genes, no differential interactions are possible (Van der Plank, 1975) and 2) when several host genes with small effects operate on a gene-for-gene basis with an equivalent number of genes in the pathogen population, differential effects are so small as to be undetectable and the result appears to be horizontal resistance (Parlevliet and Zadoks, 1977).

2.15 Genetics of virus resistance in nature

More than 80 per cent of reported viral resistance is monogenically controlled while the remainder shows oligogenic or polygenic control (Kang *et al.*, 2005). Only slightly more than half of reported monogenic resistance traits show dominant inheritance. In most but not all cases (Fraser, 1986), dominance has been reported as complete and where incomplete dominance is observed, there are important implications for mechanism that may involve gene dosage effects. The relatively high proportion of recessive viral R genes is in marked contrast to fungal or bacterial resistance, where most of reported resistance is dominant (Kang *et al.*, 2005). When multiple loci control the same virus or viral pathotype, the mode of inheritance of the resistance may be similar, as expected if the loci had arisen via duplicative processes that have generated the high degree of redundancy observed in plant genome or the mode of inheritance may be different.

There are a number of examples of dominant and recessive genes that appear to control a relatively wide range of viral genotypes that span multiple viral species, according to current delineation of viral taxa. The most dramatic examples appear to involve members of Potyviridae, e. g. *I* gene in *Phaseolus vulgaris* now appears to control a dominant resistance to ten different related potyviruses some of which include: *Bean necrotic mosaic virus,* BCMV - BlCM, CABMV *and Soybean mosaic virus* (Fisher and Kyle, 1994). Conversely, there are cases where resistance alleles at two or more loci are required to observe the resistance response (Kang *et al.*, 2005).

Dominant resistance is often, although not always, associated with the hypersensitive response (HR) (Fraser, 1986), possibly due to the frequent use of HR as a diagnostic indicator for field resistance by plant breeder. HR, induced by specific recognition of the

virus, localizes virus spread by rapid programmed cell death surrounding the infection site, which results in visible necrotic local lesions. HR- mediated resistance is a common resistance mechanism for viruses and other plant pathogens. However, since the extent of visible HR may be affected by gene dosage (Collmer *et al.*, 2000), genetic background, environmental conditions such as temperature, viral genotype and so on, schemes that classify or name virus R genes based on presence or absence of HR may obscure genetic relationships.

Meanwhile, many recessive R genes appear to function at a single cell level or affect cell-to-cell movement. More than half of the recessive R genes identified confer resistance to potyviruses, members of the largest and perharps the most economically destructive family of plant viruses (Shukla *et al.*, 1994). Considerably less is known regarding mechanisms that account for recessively inherited resistance mechanisms however, trends are noted in the types of genetic resistance available to control viruses belonging to specific plant virus family. For example, resistance to CMV often shows a complex inheritance. Despite the enormous host range of CMV and its economic impact, most resistance or tolerance of economic significance to this virus is quantitatively inherited. In contrast, resistance to tobamoviruses is widespread and is often monogenic dominant.
For some viral families of extreme agricultural importance, most notably the Geminiviridae, naturally occurring genetic resistance can be difficult to locate and is often highly strain-specific and or quantitatively inherited (i.e. each gene has a relatively slight positive effect on host response), making resistant varieties extremely difficult to develop without molecular markers or transgenic approaches.

2.16 Sources of resistance to viruses

Identification of the sources of resistance is the first step in breeding for disease resistance. Thus, the initial phase of cowpea improvement programme at IITA involved concerted efforts to collect a large number of cowpea germplasm lines and screen these for resistance to various diseases. Through systematic screening of large number of germplasm lines, several sources of disease resistance have been identified and over the years planned efforts have been made to incorporate disease resistance into new breeding lines. By adopting a combination of field screening with natural infection and artificial screening in glasshouse, several cowpea breeding lines have been developed which possess multiple disease resistance (Singh *et al.*, 1982). Large numbers of improved

cowpea lines have also been screened for virus resistance (Singh and Hughes, 1999). More sources of resistance are being produced for effective cowpea improvement programmes.

2.17 Inheritance of viral disease resistance in cowpea

Genetic resistance is one of a number of approaches to protect crops from virus infection. To date, hundreds of naturally occurring genes for resistance to plant viruses have been reported from studies of both monocot and dicot crops, their wide relatives and the plant model Arabidopsis. The isolation and characterization of a few of these genes in the past decade have resulted in detailed knowledge of some of the molecules that are critical in determining the outcome of plant viral infection (Kang *et al.*, 2005).

The information on genetics of resistance in edible legumes reveals that most resistance is inherited in an oligogenic manner (Meiners, 1981). Modes of inheritance of resistance to several cowpea viruses have been reported. Different inheritance of the same virus was reported from different cowpea varieties. Umaharan *et al.*, (1997) reported that P1, P2, F1, F2, BC1 and BC2 generations of four resistant × susceptible crosses and three resistant × resistant crosses of cowpea were screened for resistance to CPSMV. Resistant lines used include; TVu 1984, TVu 382, TVu 3961 and CNCx-102 with Bush Sitao as susceptible parent. The segregation ratio showed a ratio of 63 susceptible: 1 resistant in the F2 generation indicating that resistance is governed by three major genes and backcross tests and the F3 test confirmed this. Meanwhile, Vale and Lima (1995) studied the resistance to the same CPSMV using cowpea variety Macaibo as the resistant parent and Pitiuba as the susceptible parent. In this study, the F1 plants were uniformly susceptible and F2 segregated into a ratio of three susceptible to one resistant, indicating involvement of a single recessive gene pair for resistance and the variety Macaibo was reported to be immune to CPSMV. This result was supported by Vale and Lima (1995) and Jimenez *et al.*, (1989) who attributed control of immunity to CPSMV in variety Macaido to a single recessive gene.

In a collaborative study, knowledge of plant genetics (Bruening *et al.*, 1987) was effectively integrated with viral molecular genetics (Kiefer *et al.*, 1984) and molecular mechanism of virus resistance (Ponz *et al.*, 1988) and in this classical effort, an inhibitor of CPMV polyprotein processing was found to be coinherited with immunity to CPMV in cowpea cultivar Arlington. The data showed that immunity to CPMV was conferred by a

specific *V. unguiculata* proteinase inhibitor in the cultivar. Without cleavage by a CPMV-encoded proteinase, the polyprotein product CPMV RNA translation was rendered functionless and virus synthesis was thus precluded.

Arshad *et al.* (1998) studied the inheritance of resistance to BCMV - BlCM in six cowpea varieties: IT86F-2089-5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2065-5, and PB1CP3 and the segregation pattern in F2, and backcross populations suggested that the resistance to BCMV - BlCM is controlled by single recessive gene pair in each cowpea line and he designated *bcm* as the gene symbol. In another study by Taiwo *et al.* (1981), crosses between the resistant cowpea line TVU 2480 from IITA, Ibadan and the susceptible domestic cultivar "Early Ramshorn: were used to study the inheritance of resistance to BCMV - BlCM. Evaluation of F1, F2, and reciprocal backcross populations clearly indicated that a single recessive gene controls the high level of resistance.

Genetic studies of SBMV also revealed that one dominant gene with symbol (*SBM*) conditioned the virus resistance in cowpea (Brantley and Kuhn, 1970; Fery, 1980). However, inheritance of non-necrotic resistance to SBMV in cowpea depended on the cowpea line. The moderate resistance of "Early Pinkeye" was conferred by a single gene with partial dominance that of "Iron" appears to be controlled by multiple genes with incomplete dominance while the extreme resistance of "PI 186465" was largely controlled by one gene with partial dominance for resistance (Hobbs *et al.*, 1987). In another study, Melton *et. al*. (1987) reported that resistance to SBMV-CP is conditioned by two recessive genes.

Rogers *et al*. (1973) reported that resistance to CCMV is exhibited by PI 255811 and is conditioned by a single recessive gene, which was symbolized cc and this report was supported by Singh *et al*. (1982). In contrast to this, Goodrick *et al*. (1991) reported that inheritance of non-necrotic resistance to CCMV is conditioned by two recessive genes.

Several workers have also reported that one dominant gene controls resistance of CMV in cowpea (DeZeeuw and Crum, 1963; Fery, 1980, Khalf-Allah, *et al.*, 1973). Inheritance studies on resistance to CMV in Mungbean (*Vigna radiata*) by Sittiyos *et al*., (1979) also indicated a single dominant gene, designated *Cmm.*

There are insufficient reports on inheritance studies of multiple viral infections probably because of virus-virus interaction. Pio-Ribeirio *et al.* (1980) studied the inheritance of the synergistic necrotic reaction associated with cowpea stunt using plant populations inoculated with both Bean common mosaic virus - blackeye cowpea mosaic strain (BICMV) and *Cucumber mosaic virus* (CMV) and they concluded that the reaction is conditioned by an incompletely dominant gene, symbolized by *Nv*.

2.18 Cowpea breeding

IITA has a global mandate for cowpea improvement. Thus, to meet the regional preferences for specific seed types and adaptability to different environments, her general strategy is to develop a range of breeding lines with diverse maturity, plant type and seed type with combined resistance to major diseases, insect pests, Striga, Alectra, and broad based adaptability. Some of the cowpea varieties developed by IITA and released in Nigeria include: TVX-3236, IT81D-994, IT84S-2246-4, IT89KD-374 and IT90K-76 (NCVLBRRC, 2013), while many others were released in collaboration with the national agricultural research institutes. The Bean/Cowpea Collaborative Research Support Programme (CRSP) is also taking active roles in supporting research on cowpea improvement in USA, Cameroon and Senegal. Research on various aspects of cowpea improvement is also being done in Brazil, Nigeria, Burkina Faso, Senegal, Mali and India and in some other countries (Singh, 2006).

In Nigeria, efforts of the National Agricultural Research systems and universities have produced several improved cowpea varieties (Table 2.2). At the Institute for Agricultural Research (IAR) Samaru, of the Ahmadu Bello University, Zaria, development of new crop varieties and improved cultural practices are important aspects of research aimed at improving production and utilization systems. Most the research areas involve introgression of genes of resistances/tolerance to biotic and abiotic production constraints into Nigerian popular cowpea land races and varieties, as well as enhancement of consumer-preferred quality traits. Significant progress has been made in the development and release of high yielding, disease and pest resistant varieties with good quality and adaptation as well as acceptability to consumers

Nine varieties of cowpea for different ecologies have been developed and released for production at IAR, Samaru. The most popular are SAMPEA 6 and SAMPEA 7 with yield potential of 2.5t/ha and resistance to many stress factors. SAMPEA 6 is one of the parents

of the American black eye beans. SAMPEA 8 is extra-early in maturity while SAMPEA 9 is dual purpose (high grain and fodder yields). Other varieties released were SAMPEA 13 (dual purpose) and SAMPEA 14. In addition to high grain yields, SAMPEA 13 and SAMPEA 14 are resistant to Striga and Alectra which are serious constraints to cowpea production especially in the dry savanna agro-ecological zones. Many of the cowpeas eaten in most of Nigerian households are products of IAR with several new improved cowpea varieties released jointly with IITA Scientists (ABU, 2011; NCVLBRRC, 2013).

Institute for Agricultural Research and Training (IAR&T) Moor plantation under the Obafemi Awolowo University (OAU), Ile Ife, has also developed and released various cowpea varieties of good agronomic traits some of which are resistant /tolerant to pest and diseases. Some of these varieties are Ife brown, Ife Bimpe, IFH-101, Popse-1 and SAMPEA 13 (NCVLBRRC, 2013). National Cereal Research Institute (NCRI) Badeggi is another organization that has developed and released some cowpea varieties namely Kudi, K-28 and L-25 with characteristics of pest and disease resistance, good cooking value and good for processing into canned beans respectively (NCVLBRRC, 2013).

The breeder seeds from the research institutes are passed on to the National Seed Service (NSS) now National Agricultural Seed Council (NASC) for foundation seed production. The NASC provides Foundation Seeds to the ADPs and private seed companies (NSS, 2000). Both the ADPs and the private seed companies produce certified seeds, either from their own farms, or through contract farmers/out-growers, or both. This structure is appropriate for effective performance as it not only ensures linkages between the research institutes and NASC, but also provides alternative sources of certified seeds to the farmers (NSS, 2000).

2.18.1 Methods of breeding for disease resistance in cowpea

As reported by Buddenhagen (1984), breeding in self-fertilized crops is generally based on crossing two parental varieties possessing different characters. Plants of self-fertilized varieties are essentially homogenous. So selection begins with the F2, followed by a number of generations of line selection to fix the desired combination of characters in a pure line. This line can either become a variety or be used for further crossing. In many cases, only one or a few characters are to be added to an existing good variety, so repeated backcrossing to the variety is carried out. The general approach had been to sequentially

Table 2.2 Some pest and disease resistant cowpea varieties released in Nigeria

Variety Name	Original name	National Code	Origin/ Source	Developing Institute	Breeder/ collaborating scientists	Outstanding Characteristics	Year of Release
Kudi	K-59	NGVU-91-5	Nigeria (local selection)	N.C.R.I Badegi	O.A. Ojomo, S.O. Olafare, M.A. Adenihun & J.A. Raji	Uniformity in flowering and maturity, pest &disease resistant.	1984
IT90K-76	IT90K-76	NGVU-96-20	I.A.R Samaru	IITA Ibadan	Dr. B.B. Singh	Early maturity, multiple disease and pest resistance.	1991
IFH-101	IFH-101	NGVU-96-21	I.A.R.&T Moor Plantation Ibadan.	I.A.R.&T Moor Plantation Ibadan	Dr. I. Fawole, N. O. Afolabi & Dr. B.A. Ogunbodede	High yielding, insensitive to photoperiod, resistant to important cowpea diseases and tolerant to common pest.	1985
Popse-1	Popse-1	NGVU-96-22	I.A.R.&T Moor Plantation Ibadan.	I.A.R.&T Moor Plantation Ibadan	Dr. I. Fawole, N. O. Afolabi & Dr. B. A. Ogunbodede	High yielding, resistant to anthracnose and tolerant to other common cowpea diseases and pests.	1985
SAMPEA 14	IT99K-573-1-1	NGVU-96-29	IITA Ibadan.	IITA Ibadan I.A.R ABU, Zaria	Singh, B.B, Ishiyaku, M.F. Fatokun, C., Ousmane, B. Omoigui, L.O., Zaria, A.A. Ajeigbe, H.A., Olufajo, O. O.. Kamara, A.Y. Adeleke, R.	Multiple disease resistance especially Fusarium wilt, drought tolerance, striga and Alectra resistance.	2011
SAMPEA 15	IT99K-573-2-1	NGVU-96-30	IITA Ibadan	IITA Ibadan I.A.R ABU, Zaria	Singh, B.B, Ishiyaku, M.F. Fatokun, C., Ousmane, B. Omoigui, L.O., Zaria, A.A. Ajeigbe, H.A., Olufajo, O. O.. Kamara, A.Y. Adeleke, R	Multiple disease resistance, drought tolerance, striga and Alectra resistance.	2011

*I.A.R. &T. = Institute for Agricultural Research and Training, ABU = Ahmadu Bello University, N.C.R.I. = National Cereal Research Institute, I.A.R = Institute for Agricultural Research. **Source** = National Crops Varieties and Livestock Breeds Registration and Release Committee (2013)

inoculate each F2 plant with several diseases and advance the progeny of the resistant plant only. The F3 progeny rows were advanced and re-screened for the diseases. The selected F4 progenies were then included in preliminary trials conducted at several locations ranging from humid tropics to Sudan savanna zones and evaluated for agronomic characters as well as disease resistance. The best lines from these were finally selected for advanced trial (Singh *et al.* 1984).

2.18.2 Breeding virus resistant cowpea

Breeding for virus resistance can be achieved through screening of improved cowpea lines or landraces for virus resistance and multiplying the resistant genotypes having good agronomic traits for release as a variety. Sources of resistance without desired agronomic characters are usually employed in cowpea improvement by hybridization using pedigree or backcross method to incorporate the virus resistance genes into other cowpea with such qualities like high yielding, good cooking value, desirable seed-coat colour, early maturity, drought tolerance or any other desirable character depending on the breeding objectives (Singh, 2006).

Singh and Hughes (1999) reported several cowpea breeding lines to be completely resistant to CYMV, BCMV - BlCM and CABMV and out of these lines, IT96D-659, IT96D-660, IT97K-1068-7, and IT95K-52-34 are most promising in terms virus resistance and yield potential. Bashir *et al.*, (1995) screened several cowpea varieties from IITA and observed that IT86F 2089-5, IT86D-880, IT90K-284-2, IT90K-76, IT86D-611-3 were immune to BCMV - BlCM. Van-Boxtel *et al.* (2000) artificially screened 14 cowpea varieties with three isolates of BCMV - BlCM and 10 isolates of cowpea aphid borne mosaic virus in order to identify lines with multiple strain resistance. They observed that cowpea breeding lines IT86D-880 and IT86D-1010 were resistant to all the three isolates of blackeye cowpea mosaic and five strains of cowpea aphid borne mosaic. Cowpea varieties IT82D-889, IT90K-277-2, and TVu201 showed resistance to one or the other of the five remaining isolates and thus by using the above mention five cowpea varieties as parental lines, it is possible to breed new varieties with combined resistance to all the 13 strains of the viruses.

Lima *et al.*, (1986), in a study that involved 248 genotypes, identified four new genotypes (TVu 379, TVu 382, TVu 966 and TVu 3961) as being immune to CSMV and CABMV.

Lima *et al.* (1998), in another study that involved 44 genotypes, confirmed the immunity of genotypes TVu 379, TVu382, TVu 966, and TVu 3961 to three strains of CSMV. These resistance sources have been used in cowpea improvement in Brazil. Several varieties that have been released commercially and breeding lines that are still under evaluation were developed from crosses with the varieties CNC0434, Macaibo, and TVu 612. Resistance to CSMV, CABMV, and CGMV has already been incorporated in some of the released varieties like BR 10-Piaui (Santos *et al.*, 1987), BR 14-Mutalo (Cardoso *et al.*, 1990), and BR17-Gurgueia (Freire Filho *et al.*, 1994).

CHAPTER THREE

MATERIALS AND METHODS

Five distinct experiments carried out in this study were conducted at the experimental fields, screen houses and laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan between 2009 and 2013. The Institute lies on latitude 70 31' N and longitude 30 45' E and 210 m above sea level in forest-savannah transition agro-ecological zone, with bimodal rainfall distribution averaging about 1,500 mm and temperatures of about 25 to 32 ^{o}C during the wet season (April to October) and 19 to 35 ^{o}C during the dry season (November to April).

3.1 Sources of cowpea lines and virus isolates

The nine cowpea genotypes evaluated were obtained from the Cowpea Breeding Unit of IITA, Ibadan. They consisted of eight improved cowpea genotypes developed by IITA and Ife brown cultivar (cv.). These improved genotypes were selected based on their resistance status to five cowpea viruses namely: CPMoV, CABMV, CPMMV, CYMV and BCMV - BlCM observed from screening of IITA developed 50 improved cowpea genotypes, previously conducted by IITA scientists in 2008 before this study (Table 3.1). The 50 improved genotypes have been subjected to National multi-locational and international trials and they possessed some important agronomic characteristics, some of which are in the process of being released as varieties. The nine cowpea genotypes evaluated and their characteristics are shown in Table 3.2. Ife brown cv. was used as a positive control in this study due to its susceptibility to all the viruses studied. The three economically important cowpea viruses used in this study are: 1) Bean common mosaic virus – Blackeye cowpea mosaic strain (BCMV-BlCM) 2) *Southern bean mosaic virus* (SBMV) and 3) *Cucumber mosaic virus* (CMV). Isolates of these viruses were obtained from the Virology and Molecular Diagnostic Unit of IITA, Ibadan.

3.2 Establishment and maintenance of pure virus isolates

Pure isolates of each of the three viruses were obtained from Calcium Chloride preserved infected cowpea leaves kept at 4 ^{o}C. These isolates were established and maintained by mechanical inoculation on healthy susceptible cowpea genotype and other virus test plants in an insect-proof screen house of the Virology and Molecular Diagnostics Unit of IITA. BCMV - BlCM was maintained on Ife brown cv. and cowpea accession TVU 2657, SBMV on Ife brown, TVU 2657 and *Chenopodium ammaranthicolor* while CMV was

Table 3.1 Resistance status of 50 improved cowpea genotypes to virus infections obtained from the initial evaluation conducted by IITA in 2008

Genotype	CPMoV	CABMV	CPMMV	CYMV	BICMV
IT98K-692 (Striga)	S	S	MR	R	HS
*IT98K-133-1-1 (early)	R	R	R	R	MR
ITK99K-216-24-2 (Dual)	HS	HS	MR	HS	HS
IT99K-1122 (Early)	R	HS	R	R	HS
IT98K-1103-13 (Medium)	HS	S	R	HS	HS
IT99K-377-1 (Early)	S	S	S	R	HS
IT96D-610 (Early)	HS	HS	MR	HS	HS
IT99K-529-1 (Striga)	R	S	MR	R	HS
IT00K-1263 (Early)	R	S	MR	R	HS
IT98D-1399 (Medium)	HS	MR	S	HS	HS
IT04K-227-4-(Striga)	S	S	MR	R	HS
IT97K-390-2 (Striga)	R	MR	R	R	MR
IT03K-316-1 (Early)	HS	MR	MR	MR	HS
*IT98K-1092-1 (Striga)	R	MR	MR	R	R
IT98K-166-4 (Dual)	HS	MR	S	S	MR
*IT97K-1069-6 (Medium)	R	MR	R	R	R
IT97K-568-18 (Early)	S	S	MR	R	MR
IT98K-131-2 (Medium)	S	MR	R	R	MR
IT99K-494-6 (Striga)	HS	MR	S	R	HS
IT00K-835-45 (Striga)	HS	HS	R	HS	HS
IT98K-491-4 (Early)	HS	HS	MR	MR	HS
*IT98K-5O3-1 (Striga)	HS	HS	S	HS	HS
IT89KD-288 (Dual)	HS	HS	MR	S	HS
IT98K-628 (Striga)	HS	MR	R	R	HS
IT99K-529-2 9 (Striga)	MR	HS	S	R	MR
IT98K-1111-1 (Striga)	HS	R	R	S	S
IT99K-216-44 (Striga)	HS	MR	MR	R	S
IT98K-1263 (Medium)	HS	R	S	R	HS
IT03K-351-1 (Early)	HS	MR	S	R	HS
*IT97K-1042-3 (Early)	HS	MR	S	HS	HS
IT98K-311-8-2 (Dual)	HS	R	HS	R	S
IT98K-506-1 (Early)	S	MR	MR	R	HS
*IT04K-405-5 (Dual)	R	R	HS	R	S
IT00K-901-5 (Early)	MR	MR	MR	R	HS
IT98K-412-13 (Dual)	MR	MR	MR	R	R
IT97K-819-118 (Striga)	MR	S	S	R	S
*IT99K-1060 (Early)	S	MR	S	HS	S
*IT99K-573-1-1 (Striga)	R	R	S	R	MR

Table 3.1 Continued

Genotype	CPMoV	CABMV	CPMMV	CYMV	BICMV
IT03K-324-9 (Early)	HS	R	S	R	R
IT99K-7-21-2-2 (Dual)	HS	R	S	MR	HS
IT00K-1207 (Striga)	R	MR	MR	R	R
IT98K-128-3 (Medium)	HS	MR	MR	HS	R
IT99K-573-2-1 (Striga)	S	MR	MR	R	R
IT98K-1092-2 (Dual)	MR	R	S	R	S
IT98K-589-2 (Early)	HS	MR	S	HS	R
IT03K-378-4 (Striga)	HS	HS	HS	HS	MR
IT93K-452-1 (Early)	HS	R	S	HS	HS
IT00K-898-5 (Early)	HS	MR	S	HS	S
IT97K-499-35 (Striga)	HS	MR	MR	R	MR
IT98K-205-8 (Striga)	HS	MR	R	R	MR

CPMoV, *Cowpea mottle virus;* CABMV, *Cowpea aphid-borne mosaic virus*; CPMMV, *Cowpea mild mottle virus*; CYMV, *Cowpea yellow mosaic virus;* BCMV - BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; R= Resistance; MR=Moderately resistance; S=Susceptible; HS = Highly susceptible; Striga= striga resistant; Early= early maturing; Medium= medium maturity; Dual= dual purpose; *Genotypes selected and evaluated in this study.

Source: Cowpea Breeding Unit, IITA-Ibadan.

Table 3.2 Some characteristics of the cowpea genotypes evaluated in the study

Genotype	Seed Colour	Seed coat[1] texture	Seed[2] Size	Days to 50 % Flowering	Days to Maturity	Growth[3] habit	Other characteristics
IT98K-133-1-1	Brown	S	M	41	65	P	Early maturing
IT98K-1092-1	Black	S	M	43	67	S.E	Striga resistant
IT97K-1069-6	Brown	S	M	44	68	S.E	Medium maturing
IT98K-503-1	Cream	R	M	41	65	S.E	Striga resistant
IT97K-1042-3	Brown	S	M	37	61	E	Early maturing
IT04K-405-5	Brown	S	L	47	72	P	Dual purpose
IT99K-1060	Brown	R	M	39	63	S.E	Early maturing
IT99K-573-1-1	White	R	M	40	63	P	Striga resistant
Ife brown cv.	Brown	R	M	38	63	S.E	Early maturing

[1]S = Smooth; R = Rough; [2]M = Medium; L = Large; [3]P = Prostrate; S.E = Semi-erect; E = Erect. **Source**: Cowpea Breeding Unit, IITA-Ibadan

cultured on Ife brown cv., TVU 76 and *Nicotiana glutinosa*. These maintenance and diagnostic host plants were kept in the screen house at about 25 ^{o}C to 36 ^{o}C during rainy season and 26 ^{o}C to 39 ^{o}C in the dry season under weekly insecticide spray with Lambdacyalothrin (Karate 5 % EC) at 4 mls per litre of water. Antigen coated plate - enzyme linked immunosorbent assay (ACP-ELISA) was used as described in section 3.4.1.6 to detect the viruses and confirm their purity in the hosts. These host plants were tested with specific antibodies against eight common Nigerian cowpea viruses namely BCMV - B1CM, SBMV, CMV, CABMV, CPMoV, CYMV, BPMV and CPMMV (Table 3.3) in a single infection check in which each sample was tested for the presence of any of the eight viruses. This check was carried out occasionally to ensure and maintain isolate's purity. Sap inoculations were carried out on new susceptible hosts at 3 - 4 weeks intervals to maintain the virus cultures in a highly infective state. The virus isolates were occasionally preserved at -20 ^{o}C and on Calcium Chloride or Silica gel at 4 ^{o}C and re-cultivated when necessary.

3.3 Soil sterilization

Soil used in all the pot experiments was sandy loam collected from a regrown rainforest land at the West bank of IITA dam. This was sterilized by heating in Terra Force Soil sterilizer (Terra Force, Division of H.E., Reed LMD, Horticultural Engineers, Tonbridge Road, Wateringbury, Kent) at a temperature of 93 ^{o}C and at a pressure of 40 psi. The soil was fed into the machine at a regular speed of one shovel load at a time and allowed to cool for at least 24 hours after sterilization before use.

3.4 Screening eight IITA improved cowpea genotypes for resistance to single and multiple BCMV - BlCM, SBMV and CMV infections

3.4.1 Screen-house evaluation for virus resistance

Eight IITA improved cowpea lines, with Ife brown cv. as a check, were evaluated for resistance to BCMV - BlCMV, SBMV and CMV both singly and in mixed inoculations. The screening experiments were conducted in the screen-house of Virology and Molecular Diagnostics Unit of IITA between July and October 2009 and repeated between April and June 2011. Confirmatory screening experiment to validate the resistance status of few lines that showed viral infections in 2009 and not in 2011 was conducted between July and October, 2013.

Table 3.3 Antibodies for cowpea viruses at the IITA antiserum bank used for ACP-ELISA

Virus*	Genus	Antibody dilution ratio (v/v)
BCMV - BlCM	*Potyvirus*	1: 5, 000
SBMV	*Sobemovirus*	1: 10, 000
CMV	*Cucumovirus*	1: 3, 000
CABMV	*Potyvirus*	1: 5, 000
CPMoV	*Carmovirus*	1: 10, 000
CYMV	*Comovirus*	1: 3, 000
CPMMV	*Carlavirus*	1: 10, 000
BPMV	*Comovirus*	1: 1, 000

*BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain;* SBMV, *Southern bean mosaic virus;* CMV, *Cowpea mosaic virus;* CABMV, *Cowpea aphid-borne mosaic virus;* CPMoV, *Cowpea mottle virus;* CYMV, *Cowpea yellow mosaic virus;* CPMMV, *Cowpea mild mottle virus and* BPMV, *Bean pod mottle virus*; ACP-ELISA = Antigen coated plate-enzyme linked immunosorbent assay.

3.4.1.1 Mechanical inoculation of test plants and viral treatments

Virus inocula were prepared by grinding systemically infected leaves from susceptible cowpea maintenance hosts on which the viruses were established, at ratio 1:10 (w/v) infected leaf using Mettler balance (Denver Instrument Company, weighing balance model SE 04510, New Jersey, USA) to inoculation buffer in a chilled sterilized mortar with pestle using 0.05 M Phosphate buffer (2.4 g KH_2PO_4, 5.4 g K_2HPO_4 and 0.04 ml β-mercaptoethanol in 1litre distilled water, adjusted to pH 7.5). Leaves of the test plants to be inoculated were dusted sparingly with carborundum (600 mesh) before inoculation to create micro-wounds. Cowpea seedlings were mechanically inoculated by gently rubbing the inocula on the leaves dusted with carborundum, with fingers protected with disposable gloves. After inoculation, carborundum was rinsed off the leaves to improve light interception. Sap inoculation was performed on the eight IITA improved cowpea genotypes and Ife brown cultivar as a positive control at the primary leaf stage (6-8 days after planting: DAP) using inocula of the three viruses singly and in all possible combinations. The viral treatments are as follows:

1. BCMV-BlCM
2. SBMV
3. CMV
4. BCMV-BlCM + SBMV
5. BCMV-BlCM + CMV
6. SBMV + CMV
7. BCMV-BlCM + SBMV + CMV
8. Healthy (control)

For mixed virus treatments, grinded leaf saps from the relevant inocula were mixed in ratio 1:1 (v/v) respectively just before inoculation. The nine cowpea genotypes were also mock inoculated with only buffer as a negative (non-inoculated) control. Daily watering was carried out in the screen house but there was no fertilizer application. The inoculated plants were kept in a screen-house under weekly insecticide spray with Lambdacyalothrin (Karate 5 % EC) at 4 mls per litre of water to control insect vectors. Sticky insect traps were also hung above the potted plants to further check insect infestation.

3.4.1.2 Experimental design

Eight virus treatments were applied on nine cowpea genotypes. Experimental pots were arranged in 8 by 9 Factorial Experiment laid-out in a Completely Randomized Design

(CRD), in an insect-proof screen house. There were three replications, making 216 experimental pots. Six seeds were sown in each 8″ plastic pots filled with 4.5 Kg top soil and the seedlings were thinned down to four per pot before inoculation.

3.4.1.3 Symptomatology

Disease incidence and severity were determined by post-inoculation disease symptom severity scores. The severity scores were taken weekly till eight weeks post inoculation (WPI) using a symptom severity scale of 1-5 according to Thottapilly *et al.*, (1994), Shoyinka *et al.*, (1997) and Singh *et al.* (1982). Scale 1-5 was used for the two isometric viruses (SBMV and CMV) that showed varying levels of mosaic, mottling, vein clearing and puckering symptoms. The same scale (1 – 5) was also used for the filamentous virus (BCMV- BlCM) that showed mosaic, mottling, as well as different levels of vein banding which are characteristic symptoms of the virus (Plates 3. 1 – 3. 2). Mixed infections were also scored using the same severity scale.

3.4.1.4 Virus detection and plant evaluation for virus resistance

Infections of the virus inoculated plants were studied to evaluate the resistance and susceptibility of the eight IITA improved genotypes to the single and multiple viruses. Resistance status was determined by symptomatology, serological detection using ACP-ELISA, analysis of area under disease progress curves (AUDPC) and assessment of reduction in yield parameters due to viral infections. Classification into resistance status was carried out by the combination of infection severity score 1 – 5 and ACP-ELISA result (Table 3.4) according to Kumar, (2009) and Ogunsola *et al.* (2010). Plants with no visible symptom (disease severity score of 1) and with negative ELISA result verified by RT-PCR were classified as resistant. Those with mild symptoms (severity scores 2) with plant recovery and positive to ELISA were classified as moderately resistant. Plants showing no or mild symptoms (severity score 1 – 2) that are positive to the virus by ELISA were referred to as tolerant. All plants with symptom severity scale from 3 to 5 and virus positive by ELISA were regarded as moderately to severely susceptible (Table 3.4).

Similar method was used for cowpea plants under mixed infections. Plants with severity scale 1 and ELISA negative to all the viruses involved in the mixed infection were categorized as resistant. Those with severity scale 1 to 2 and ELISA positive to the

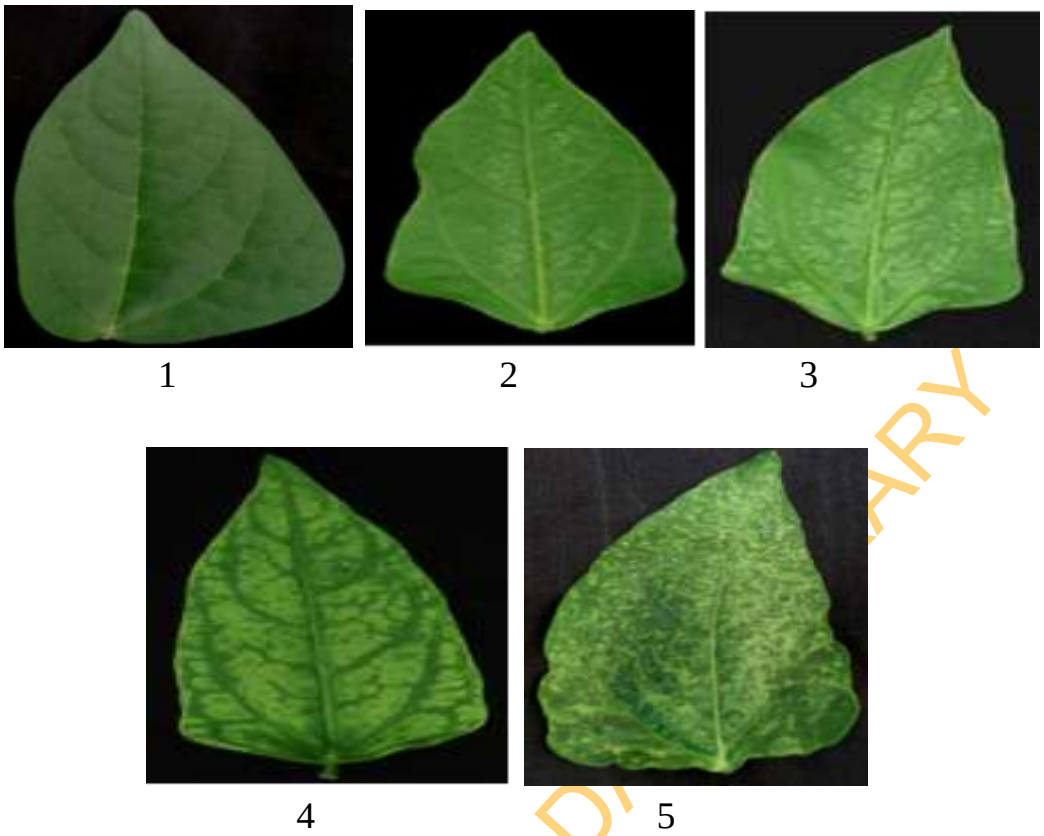

Plate 3.1 Infection symptom severity scale 1 - 5 of Bean common mosaic virus - blackeye cowpea mosaic strain. 1 = symptomless leaf of IT98K-1092-1, 2 - 4 = symptoms on Ife brown cv., 5 = severe symptom on cowpea line IT99K-1060

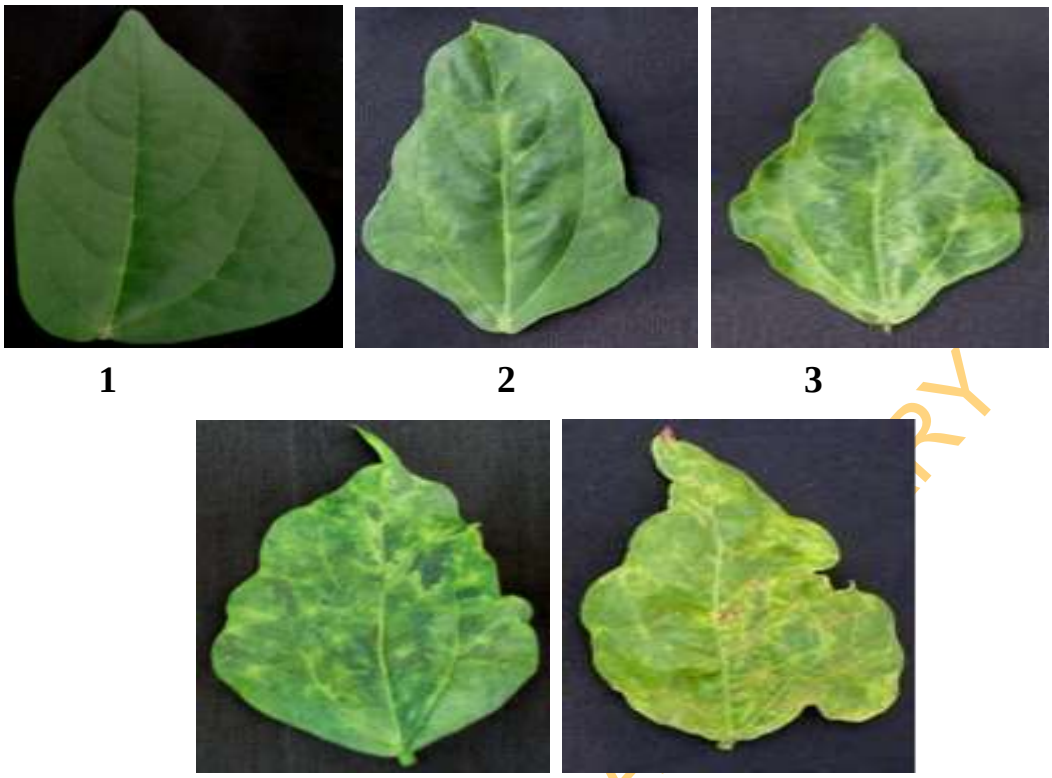

Plate 3.2 Infection symptom severity scale 1-5 for *Southern bean mosaic virus* and *Cucumber mosaic virus.* 1 = symptomless leaf of IT98K-1092-1, 2 and 3 = symptoms on Ife brown cv., 4 = symptom on cowpea line IT04K-405-5, 5 = severe symptom on IT99K-1060

Table 3.4 Criteria for classification of virus resistance in cowpea

Severity Score	Symptom	Relative virus concentration by ELISA*	Classification
1	No symptom	< 2 x healthy control; ELISA negative; PCR negative	Immune/resistant
2	No symptom or mild mosaic or mottling on leaves with symptom recovery (with no marked effect on growth, vigour and yield)	≥ 2 x healthy control; Elisa positive (only in symptomatic tissues)	Moderately resistant / Tolerant
3	Mosaic or mottling on many leaves	≥ 2 x healthy control; Elisa positive (only in symptomatic tissues)	Moderately susceptible
4	Severe mosaic, puckering and mild stunting	≥ 2 x healthy control; Elisa positive	Susceptible
5	Severe mosaic, puckering, leaf distortion, severe stunting with necrosis or death of leaves or plants	≥ 3 x healthy control ELISA positive	Highly susceptible

*ELISA reading at 405nm Absorbance value, ELISA positive = ELISA values ≥ 2 x Absorbance value of healthy control. **Sources**: Kumar (2009), Ogunsola, *et al.*, (2010)

co-inoculated viruses were regarded as tolerant. Plants with severity scale 2 to 4 and ELISA positive to all or any of the viruses involved in the co-infections were categorized as susceptible while those with severity scale of 3 to 5 and with ELISA result moderately (++) to highly positive (+++) to all or any of the viruses involved were classified as highly susceptible to viruses.

3.4.1.5 Area under disease progress curves

Mean data from disease severity scores observed weekly successively for periods of eight weeks in both 2009 and 2011 screen-house evaluations were used in computing AUDPC. AUDPC was calculated for each genotype as described by Anilkumar *et al*., (1994):

$$AUDPC = \sum_{i=1}^{n-1} \left[\frac{(Xi + Xi+1)}{2}\right] (ti + 1- ti)$$

Where n = the total number of observations

Xi = disease severity at the ith observation

t = time in days after virus inoculation at ith observation

ti + 1-ti = interval between two consecutive observations

AUDPC values were computed using a statistical analysis system (SAS, 2008) package, version 9.2. The AUDPC values were ranked and the grand mean of the rank as well as deviations from the rank grand mean were calculated and used to classify cowpea genotypes into their levels of resistance or susceptibility to the viruses.

3.4.1.6 Virus detection by Antigen Coated Plate-Enzyme Linked Immunosorbent Assay

Virus detection was carried out serologically with ACP-ELISA as described by Kumar (2009) since the three viruses are highly immunogenic. Leaf samples were collected from the youngest well-expanded trifoliate leaves of each plant at four and eight weeks post inoculation (WPI) and subjected to ACP-ELISA using three antisera specific for BCMB - BICM, SBMV and CMV from the antiserum bank of the Virology and Molecular diagnostic unit of IITA. Purity check of the plant viruses was also carried out occasionally, taking plant samples at random and testing them for other cowpea viruses as in section 3.2. The ACP- ELISA was performed (Kumar, 2009) as follows:

Test leaves were grinded with a mortar and pestle in coating buffer (Na2CO3 1.59 g, NaHCO3 2.93 g and Sodium diethyldithiocarbamate 10 g in 1 litre of distilled water at pH adjusted to 9.6) at a ratio of 0.1 g/ml (1:10 w/v) leaf sample to buffer. Hundred microlitres (100 µl) of the extract was tested per well of the ELISA plate. The plate was covered and incubated inside a humid box at 37 ºC for 1 hour or at 4 ^{o}C overnight. After incubation, the plate was washed with three changes of Phosphate buffer saline-Tween (PBS-T: $Na2HPO_4$ 22 g, KH_2 PO_4 4 g, KCl 4 g, NaCl 160 g and 10 ml of Tween-20, made up to 2 litres with distilled water, at pH 7.4), allowing three minutes for each wash. After washing, the plate was tap-dried on paper towel to drain the wells. The same washing procedure was carried out after each successive incubation step apart from blocking with milk. Then, blocking was done by adding 200 µl of dried skimmed (non-fat) milk (3 % in 100 ml PBS-T) to each well.

Polyclonal antibodies were further purified by cross-absorption by grinding 1g of healthy cowpea leaf into 20 ml of conjugate buffer (0.05 g of Albumin, 0.5 g of Polyvinyl Pyrrolidone (PVP) and 12.5 ml of 10 X PBS-without Tween-20 made up to 250 ml with distilled water). Appropriate antibody dilution ratio was used (Table 3.3) and the antibody and the leaf sap was incubated for 30 mins. 100 µl of this was dispensed into each well of the ELISA plate and then incubated 37 ^{o}C for 1 hr. The plate was washed as above. Then, Alkaline phosphatase (ALP) conjugated anti-rabbit (goat) antibody (Sigma, USA) was diluted using 1 µl anti-rabbit alkaline in 15 ml conjugate buffer and mixed thoroughly. 100 µl of this was dispensed into each well of the ELISA plate and then incubated at 37 ^{o}C for 1 hr. After that, *p*-nitro phenyl phosphate (PNP) substrate solution was prepared at a concentration of 1mg / ml in substrate buffer (10 % diethanolamine in distilled water, at pH 9.8). 100 µl of PNP was added to each well and the plate incubated in the dark, for 1 hour at room temperature and overnight (approximately 16 hrs) at 4 oC to allow colour development. Lastly, Optical density (OD) values were read at 405 nm using a BIO-RAD Microplate Reader (ELx 800, Universal Microplate Reader). Readings were taken 1 hour and 4 hours after incubation at 37 ^{o}C or overnight at 4 ^{o}C. Samples were considered positive for a virus when an OD value is greater than two times the mean of the negative controls (Thottappilly *et al.,* 1998). For virus detection under mixed infections, same grinded leaf sample was tested singly for each virus in different wells of ELISA plate using the same ELISA procedures.

3.4.1.7 Virus detection by Reverse Transcription-Polymerase Chain Reaction

Negative ACP-ELISA results were verified by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Samples were taken from inoculated cowpea genotypes under screening for virus resistance that have shown negative results to ELISA, from which total RNAs were isolated and subjected to RT-PCR to further confirm the resistance status of the plants.

3.4.1.8 Isolation of total RNA for Reverse Transcription-Polymerase Chain Reaction

Total RNA was extracted from the leaf tissues of the plants using modified Cetyltrimethyl ammonium bromide (CTAB) method described by Abarshi *et al.*, (2010). Young fresh leaf tissues (100 mg) were homogenized 1:10 (w/v) in 1000 µl CTAB buffer [2% CTAB w/v, 1.4 M NaCl, 0.2 % β-mercaptoethanol (added just before use), 20 mM EDTA and 100 mM Tris-HCl at pH 8.0] using chilled sterile mortar and pestle. 750 µl of the homogenate was transferred into new sterile 2.0 ml capacity eppendorf tubes, vortexed and incubated at 60 ^{o}C for 10mins in the water bath. Equal volume (750 µl) of phenol: chloroform: isoamyl alcohol prepared in the ratio 25:24:1 were added, vortexed and centrifuged at 12, 000 g for 10 mins. The supernatant was then transferred to a fresh tube and the nucleic acid was precipitated by adding ice-cold (-20 ^{o}C) isopropanol, 2/3 of the volume of the supernatant. The precipitated nucleic acids were incubated at -70 ^{o}C for 15 mins and centrifuged at 12, 000 g for 10 mins. Decanting the supernatant, the pellets were washed twice in 500 µl of 70 % ethanol and centrifuged for 5 mins at 12, 000 g. Nucleic acid pellets were air-dried at 37 ^{o}C for about 15 mins to drain off the ethanol, dissolved in appropriate amount of sterile distilled water (40 - 50 ml) and stored at – 20 ^{o}C until use.

Quality of the extracted nucleic acid was analyzed by agarose gel electrophoresis described by (Kumar, 2009) and RNA concentration was estimated with NanoDrop (2000) spectrophotometer (Thermo scientific Tegrant Corporation, SE 06629). Agarose gel (1.5 %) was prepared using 0.5x TAE (242 g Tris base, 57.1 ml Glacial acetic acid, 100 ml of 0.5 M EDTA in 1 litre distilled water at pH 8.3) buffer, stained with 5 µl 5 % ethidium bromide and casted on electrophoresis tank. Then, 3 µl of the total RNA mixed with 3 µl of gel loading dye (0.25 % bromophenol blue, 0.25 % xylene cyanol and 30 % glycerol) were loaded into the wells. The gel was run for 45 min at 120 V and the nucleic acid fragments were visualized under UV- transilluminator at 302 nm and then photographed using a digital camera. Purity of the RNAs were estimated by the NanoDrop

spectrophotometer by determining A260/280 and A260/230 ratio and yield estimate by measuring absorbance at 260 nm.

3.4.1.9 Reverse Transcription-Polymerase Chain Reaction and Gel Electrophoresis

RT-PCR was performed by the procedure described by Kumar (2009) using primer pairs in the primer bank of the Virology and Molecular Diagnostics unit at IITA, Ibadan (Table 3.5). The total RNA extracted (in section 3.4.1.8) was diluted in ratio 1:50 and 12.5 µl PCR reaction mixture was prepared which comprised of 10x reaction buffer (flexi), 0.75µl of 25 mM MgCl2, 0.25 µl mixture of 10 mM dNTPs (dATP, dCTP, dGTP and dTTP), 0.25 µl of respective primers, 0.06 µl Taq DNA polymerase (Promega Corporation, USA), 0.06 µl M-MLV Reverse transcriptase (RT) (Promega Corporation, USA), 2.0 µl of 10 ng/µl genomic DNA and sterile distilled water.

PCR amplification was performed with Applied Biosystems (GeneAmp® PCR System 9700) Cycler machine using lyophilized PCR micro tubes (Promega Corporation, USA). Amplification of BCMV - BlCM RNA was done using the following parameters: one cycle of reverse transcription to complementary DNA (cDNA) for 30 min at 42 ^{o}C, one cycle of initial denaturation at 94 ^{o}C for 3 min, followed by 40 cycles of amplification by denaturation at 94 ^{o}C for 30 sec, primer annealing at 40 ^{o}C for 30 sec and primer extension at 68 ^{o}C for 1 min and final incubation at 72 ^{o}C for 10 min for extension. SBMV RNA was amplified using one cycle of reverse transcription for 30 min at 44 ^{o}C, one cycle of initial denaturation at 95 ^{o}C for 5 min, followed by 35 cycles of amplification by denaturation at 95 ^{o}C for 45 sec, primer annealing at 54 ^{o}C for 45 sec and primer extension at 72 ^{o}C for 45 sec and final incubation at 72 ^{o}C for 7 min for extension. For CMV, RNA amplification was carried out by one cycle of reverse transcription for 10 min at 50 ^{o}C, one cycle of initial denaturation at 95 ^{o}C for 5 min, followed by 35 cycles of amplification by denaturation at 95 ^{o}C for 30 sec, primer annealing at 55 ^{o}C for 1 min and primer extension at 72 ^{o}C for 1 min and with final incubation at 72 ^{o}C for 10 min for extension. The RT-PCR product was analyzed by agarose gel electrophoresis according to Kumar (2009) as earlier described (section 3.4.1.8), by loading the well with the 12.5 µl amplicons using 4 µl 100 bp DNA marker.

Table 3.5 Primers used in the RT-PCR and their predicted amplicon sizes for detected viruses*

Virus	Primer	Primer position	Primer sequence (5' ⟶ 3')	Band size (bp)	Tm (°C)
BCMV - BlCM	CI	CI F	CGIVIGTIGGIWSIGGIAARTCIAC	700	67.7
		CI B	ACICCRTTYTCDATDATRTTIGTIGC		59.5
CMV	CMV	CMV 1	GCC GTA AGC TGG ATG GAC AA	500	57.6
		CMV 2	TAT GAT AAG AAG CTT GTT TCG CG		53.4
SBMV	SBMV	SBMV For	TGGTCCTTCGACGCAATCT	500	56.5
		SBMV Rev	GTCTGCTTCAGCTGCAGGACA		59.9

*BCMV – BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus; CMV, Cucumber mosaic virus;* CI, cylindrical inclusion; Tm, melting temperature

3.4.1.10 Data collection and statistical analyses

Data were collected from the nine cowpea lines on the disease incidence, disease severity, ACP-ELISA absorbance (A405) values and area under disease progress curve (AUDPC), to determine their resistance or susceptibility status to the single and mixed virus infections. Samples of inoculated genotypes that tested negative to ELISA were verified by RT-PCR. AUDPC was analyzed from symptom severity scores taken over the period of 8 WPI. Data were analyzed by Analysis of Variance (ANOVA) using SAS (2008) package. Means with significant differences were separated using Duncan's Multiple Range Test (DMRT) at 1 % or 5 % level of probability.

3.4.1.11 Effects of single and multiple-viral infections on yield parameters of inoculated cowpea

The nine cowpea genotypes were inoculated with the viruses in eight virus treatments namely: 1) BCMV - BlCM, 2) SBMV, 3) CMV, 4) BCMV - BlCM + SBMV, 5) BCMV - BlCM + CMV, 6) SBMV + CMV, and 7) BCMV - BlCM + SBMV + CMV, and 8) un-inoculated control. This experiment was a continuation of the screen house evaluation of virus resistance described earlier (section 3.4.1). The yield parameter data of the inoculated plants were evaluated in comparison with non-inoculated plants to investigate the effects of the viruses on the seed yield reduction of the plants.

3.4.1.12 Evaluation of yield reduction and data analysis

Yield parameter data recorded in the first screening in 2009 were: number of pods per plant, pod length, number of seeds per pod and 100 seed weight, while number of productive peduncles and total seed weight per plant were added to these in the second screening carried out in 2011. Data were taken on the infected plants and healthy controls. Number of pods per plant, productive peduncles and seeds per pod were determined by counting. Pod lengths were measured with meter rule while 100-seed and total seed weights were measured using Mettler balance (Denver Instrument Company, weighing balance model SE 04510, New Jersey, USA). Data on yield parameters were recorded from 10 WPI. Number of productive peduncles was first recorded after which the dry mature pods were harvested and dried till stable weight in the screen house. Shelling of pods was done manually. Data from yield parameters were correlated with incidence and severity scores. Data were analyzed by ANOVA using SAS (2008). Means with

significant differences were separated using Duncan's Multiple Range Test (DMRT) at 5 % confidence levels.

3.4.2 First field screening for virus resistance

Two field experiments were conducted at different locations in IITA cowpea fields to screen the eight cowpea breeding lines for resistance or susceptibility to BICMV, SBMV and CMV under natural field conditions. First field screening experiment was performed between September and December 2010. Cowpea cultivar "Ife brown" was planted as border plants and the test lines were exposed to natural field viral infection without infector or spreader rows.

3.4.2.1 Experimental design and field layout

The experimental field was ploughed, harrowed and ridged according to standard practice. The field was laid-out in a Randomized Complete Block Design (RCBD) with four replications. The plot was 26 m by 16.75 m making 435.5 m^2 land areas. Each of the four blocks was 4 m by 12.75 m separated by 2 m alley. Each block comprised of 9 plots on which the cowpea genotypes were randomly planted in two rows. Each plot was 4m by 0.75 m separated by 0.75 m alley. Planting was done with spacing of 0.4 m intra-row and 0.75 m inter-row with each plot containing 2 rows of the test lines. Four seeds were sown per hole and later thinned to two making 22 test plants per row and 44 test plants per plot. Two rows of Ife brown were planted as border plants 1m away from the test plants at spacing of 1 m by 0.4 m.

3.4.2.2 Field management

Missing stands were supplied one week after sowing and seedlings were thinned to two per stand three weeks after planting (WAP). The soil was sandy loam and no fertilizer application was carried out. Manual weeding was performed at four and eight WAP. Insectide spray was carried out weekly from four WAP using cypermethrin ('Cyperforce', 10 % EC) and lambdacylothrin ('Karate', 10 % EC) using knapsack sprayer at 5 ml per litre of water for both chemicals. Fungicide spray with 'Benlate' at 2 g per litre of water was carried out eight WAP. Dry pods were harvested at ten to twelve WAP and dried till stable weight in the green-house before evaluation of yield parameters.

3.4.2.3 Virus detection and evaluation of resistance

Leaf samples were taken from the youngest well expanded trifoliate leaves of each plant 8 WAP and analyzed by ACP-ELISA to confirm infection with the viruses in symptomatic plants and to detect latent infection in asymptomatic plants. All plants showing viral symptom were tested by ELISA and from asymptomatic plants, leaf samples were taken from randomly selected four plants per genotype in each plot for ACP-ELISA. Thus, different number of plant samples were tested per genotype which comprised of 16 asymptomatic samples (4 samples from 4 replicates) plus varying number of symptomatic samples observed on the field. The four sampled asymptomatic plants were tagged accordingly from which yield parameter data were taken from 10 WAP. ACP-ELISA was carried out as described by Kumar (2009).

3.4.2.4 Data collection and analysis

Data were collected on disease incidence, symptom severity and virus titre values. Disease severity was assessed visually using a descriptive scale of 1 - 5 according to Kumar (2009) (Table 3.4). Yield parameters recorded include: number of productive peduncles per plant, number of pods per plant, pod length per plant, number of seeds per pod, 100-seed weight, and total seed weight. Data from yield parameters were correlated with incidence and severity scores. Data were analyzed by ANOVA as earlier described in section 3.4.1.12.

3.4.3 Second field screening experiment

The second field trial was carried out on IITA cowpea experimental site, about 2 Km away from the first field, between December 2010 and March 2011. The field was under overhead sprinkler irrigation three times per week for duration of 4 hours per day. This experiment entailed planting the nine cowpea lines in which the virus susceptible Ife brown was mechanically inoculated with BCMV - BlCM, SBMV and CMV as spreader rows planted in-between rows of the test lines in each plot. This was to facilitate insect transmission of the viruses.

3.4.3.1 Experimental design and field layout

The field experiment was laid-out in a Randomized Complete Block Design (RCBD) with four replications. The plot was 26 m by 29.5 m making 767 m^2 land areas. Each of the four blocks was 4 m by 22.5 m separated by 2 m alley. Each block comprised of nine plots on which the cowpea genotypes were randomly planted. Each plot was 4m by 1.5 m

separated by 1.5 m alley (Figure 3.1). Planting was done with spacing of 0.4 m intra-row and 0.75 m inter-row with each plot containing three rows, two rows of test crops and the middle rows containing the virus inoculated infector plants. Four seeds were sown per hole and later thinned to two making 22 test plants per row and 44 test plants per plot. Two rows of Ife brown were planted at spacing of 1 m by 0.4 m as border rows 1m away from the test plants.

3.4.3.2 Mechanical inoculation of infector and border lines

Ife brown variety was planted as infector line in-between rows of test lines in each plot and also in two-line border rows, 1m away from the test lines. Ife brown was planted two weeks before planting the test lines. The infector and the border plants were artificially inoculated (as described in 3.4.1.1) but with BCMV - BlCM, SBMV and CMV in single infections nine days after planting (DAP). Four to five plants of the infector lines were inoculated with each virus and one-third of the plants in each border row were inoculated by a virus.

3.4.3.3 Field Management

Field management practices were performed as described in the first field experiment. Also, to enhance natural field virus infection by insect vector transmission of viruses to the test lines, insecticidal spray was delayed till six WAP, after which weekly spraying was carried out as described (section 3.4.2.2) in the first field screening.

3.4.3.4 Virus detection and resistance evaluation

Virus infection and concentration were confirmed with ACP-ELISA as described by Kumar (2009). Both symptomatic and asymptomatic plants were sampled and tested as described in section 3.4.2.3. Resistance status of the plants was determined from disease incidence, symptom severity and ACP-ELISA, using a classification criteria described by Kumar (2009) and Ogunsola *et al.* (2010). Yield parameter data were also taken as described (section 3.4.2.4) in the first screening.

3.4.3.5 Data collection and statistical analysis

Data were collected on disease incidence, symptom severity and virus titre values. Disease incidence was determined by expressing the number of plants with virus symptoms as a percentage of the 44 plants in each plot. Severity scores were taken weekly from four WPI

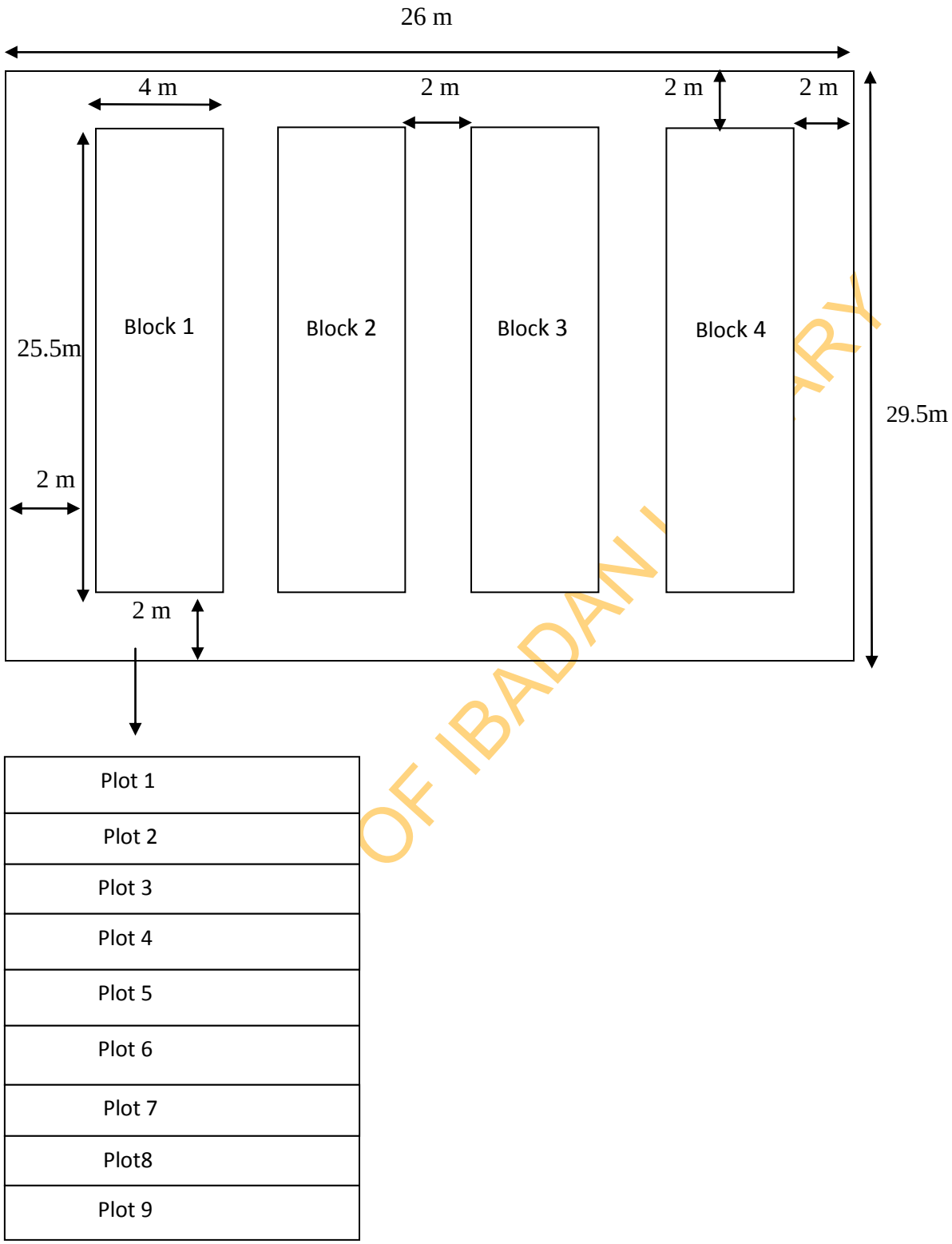

Figure 3.1 Field layout of second field screening experiment

and this was carried out on all the plants per plot using a scale of 1 – 5 described earlier (section 3.4.1.3). Severity scores were taken for a period of six weeks. Data were analyzed by ANOVA using SAS (2008); PROC GLM package and means with significant differences were separated using DMTR (p = 0.05).

3.5 Nucleic acid sequencing for confirmation of virus identity

3.5.1 Nucleic acid purification for sequence analysis

Purification of nucleic acid from the viruses for sequence analysis was done using both Ethanol purification and QIAquick gel elusion kit's protocol.

3.5.1.1 Ethanol method of nucleic acid purification

After running agarose gel electrophoresis to affirm the DNA bands, the PCR product meant for sequencing was transferred into sterile 1.5 ml eppendorf tubes and ethanol (95 %) was added (1: 2 v/v PCR product: ethanol) to the tubes, which were inverted gently and kept at – 70 ^{o}C for 10 min. The tubes were centrifuged at 13,000 revolutions per minute (rpm) for 10 min after which the solution was carefully decanted, leaving the pellet. Exactly 500 µl of 70% ethanol was added to the tubes, centrifuged at 13,000 rpm for four min and after which the ethanol was decanted. The nucleic acid pellets were dried at 37 ^{o}C for 15 to 20 min to completely drain the alcohol and pellets were dissolved in sterile distilled water (30 – 50 µl) and stored at -20 ^{o}C until they were sent for sequencing.

3.5.1.2 QIAquick gel elusion kit protocol

The method was adopted to obtain pure quality nucleic acids mainly from PCR products that could not show sharp clear band sizes on gels. The nucleic acid fragment (band) from agarose gel was neatly excised with a sterile sharp scalpel (minimizing the size of the gel slice by removing extra agarose) and transferred to 1.5 ml eppendorf tubes. This was weighed after which QG buffer was added (3:1 v/w). The samples were incubated at 50 ^{o}C for 10 min (or till when the gel slice had completely dissolved). Tubes contents were mixed by vortexing every 2-3 minutes during the incubation to dissolve the gel. Complete dissolution of the gel was confirmed when colour of the mix turned yellow, particularly at pH $\leq$ 7.5 indicating efficient adsorption of nucleic acid to QIAquick membrane. Then, isopropanol (1:1 v/w isopropanol to gel weight) was added to the sample and mixed to increase yield of nucleic acid fragment. QIAquick spin column was placed in a 2 ml collection tube to which the nucleic acid was added and centrifuged at 10,000 x g for 1 min to facilitate nucleic acid binding. The flow-through was discarded and QIAquick spin

column placed back in the same collection tube. Exactly 0.5 ml of QG buffer was added to QIAquick column and centrifuged for 1minute to remove all traces of agarose. After that, 0.75ml of wash (PE) buffer was added to QIAquick column and centrifuged at 10,000x g for 1min. After discarding the flow-through, the QIAquick column was centrifuged for additional 1min. The QIAquick column was then placed into 1.5 ml micro centrifuge tube. Exactly 50 µl of Elution Buffer (EB) (10 mM Tris-Cl, pH 8.5) or water was added to the center of the QIAquick membrane and the column centrifuged for 1min to elute the nucleic acid. Also, for increased nucleic acid concentration, 30 µl elution buffer was added to the center of the QIAquick membrane and the column was allowed to stand for 1min and then centrifuged for 1min. The purified pellets obtained were kept at -20 ^{o}C till they were sent for sequencing.

3.5.2 Sequencing of PCR products

Amplified cDNAs of BCMV-BICM, SBMV and CMV, extracted from the cowpea maintenance hosts and the source of isolates, were purified as described in section 3.5.1 and shipped to IOWA State University, USA for sequencing. Purified PCR products were sequenced in both directions using appropriate primers (Table 3.5) in an automated sequencer (ABI). Nucleotide sequences were edited and consensus sequence for each isolate was made. Sequence similarity searches were made in GenBank databases using the BLAST program (NCBI) to further ascertain the identity of the three viruses.

3.6 Genetic studies to determine the mode of inheritance of resistance to BCMV-BICM, SBMV and CMV infections in cowpea

These experiments were conducted in the screen houses of the Cowpea Breeding Unit and Virology and Molecular Diagnostics Unit of IITA between November 2009 and April 2012. Patterns of inheritance of resistance to BCMV - BlCM, SBMV and tolerance to CMV in the sources of resistance and tolerance genes were investigated. From the results (section 4.1.1.5), cowpea line IT98K-1092-1 was found to be resistant to BCMV - BlCM and SBMV and tolerant to CMV. Also, IT97K-1042-3 is resistant to BCMV - BlCM and SBMV, Line IT99K-1060 is highly susceptible to the three viruses while IT99K-573-1-1 is highly susceptible to BICMV and CMV. The two resistant/tolerant cowpea lines (IT98K-1092-1 and IT97K-1042-3) and two highly susceptible lines (IT99K-1060 and IT99K-573-1-1) were selected and crossed to investigate the mode of inheritance of the resistance/tolerance to the three viruses in the selected cowpea lines.

3.6.1 Plant establishment for hybridization

All experiments conducted under inheritance studies were carried out in insect-proof screen houses. All hybridizations to produce the six generations P_1, P_2, F_1, F_2, BCP_1 and BCP_2 were carried out in the screen house of Cowpea Breeding Unit while screening of cowpea generations for each of the virus treatments was conducted in the screen houses of Virology and Molecular Diagnostics unit of IITA. To generate F1 hybrids, 36 plants of each of the resistant: IT98K-1092-1 and IT97K-1042-3 and susceptible parents: IT99K-1060 and IT99K-573-1-1 were raised in well labeled 10" plastic pots at 3 plants per pot, using sterilized sandy loam soil. Seeds were sown at two-week intervals to ensure synchronized flowering of the parental lines.

Seeds were treated with fungicide (Benlate) at 1 g per 40 seed before sowing and sowing was carried out fortnightly to ensure availability of flowers for the hybridization process. To obtain adequate F_2 plants for the inheritance studies, some F_1 plants were vegetatively propagated through vine cuttings at four weeks after planting (WAP) at two vines per cowpea lines. Watering was carried out regularly and NPK 15.15.15 fertilizer was applied in each pot at three WAP. Insecticide application was carried out using Thionex (EC) at 2 ml per litre and Vertimec (018 EC) at 0.75 ml per litre of water at 10 days intervals from four WAP till maturity. Weeding was carried out manually and the prostrate and semi erect plants were staked to enhance crossing.

3.6.2 Crossing procedures

The emasculation and hand pollination procedure for cowpea described by Myers (1991) was followed. Crosses were made between lines resistant and susceptible (P_1 x P_2) to each virus. Flowers on the male parents were carefully opened early in the morning of the day before anthesis to enable removal of anthers. Flower buds of the female parents that have reached their maximum unopened size (a day prior to opening) were carefully emasculated. A cut approximately 4.0 mm on the concave part of the un-opened flower was carefully made and the upper part of the cut segment was gently lifted with forceps, exposing the style and stamens. The anthers were carefully removed. Male flowers that opened were plucked and their pollen dusted by rubbing the anthers on the stigma and hairy segment of the style of emasculated female flower. Appropriately labeled tags were carefully fixed to the base of the pollinated female flower and allowed to develop to pod maturity. The hybridizations were carried out in the screen house between January 2010

and September 2011. The resulting F1 seeds were selfed to generate F_2 while some of the F_1 were backcrossed to their respective parental lines. Mature pods of the generations P_1, P_2, F_1, F_2, BCP_1 and BCP_2 from each cross combination were harvested and allowed to dry in the screen house before manual shelling. The well dried seeds were kept at 4 ^{0}C till when ready for virus resistance screening

3.6.3 Screening methods and criteria for determining plant resistance and susceptibility

Crosses were tested to confirm successful hybridization using some morphological characters that distinguished parental lines. To determine whether F_1 plants are true hybrids or not, traits like shape, length and width of the terminal leaflets and internodes length of the seedlings, and sometimes pod length and shape of the parental lines were compared with those of the hybrids. The segregating (F_2, BCP_1 and BCP_2) cowpea generations were evaluated for virus resistance to determine the segregation patterns for each virus. Screening was carried out by sap inoculation of each of the virus isolates in the screen house as earlier described (Section 3.4.1). Each population was screened on an individual plant basis and each pot was labeled in a way that ensured the identity of each plant. The six cowpea generations were screened for each of the three viruses (BCMV - BlCM, SBMV and CMV) with two reciprocal crosses in five experiments. The number of plants screened per cross depend on the number of available seeds generated from each crosses per virus. Sixteen plants of Ife brown were inoculated as positive control while 10 plants of each parent were used as negative (non-inoculated) control. The temperature in the screen house during the period ranged between 27 ^{o}C and 38 ^{o}C. Plant management in the screen house was as described in section 3.4.1.1.

Virus inoculated P_1, P_2, F_1, F_2, BCP_1 and BCP_2 were observed for disease symptoms appearance and the date of first symptom appearance was noted. Symptom severity scores were taken weekly for a period of seven weeks according to Kumar (2009) as described earlier (section 3.4.1.3). At five WPI, plants were tested for viruses on individual plant basis using ACP-ELISA as earlier described (in 3.4.1.4). A two class classification into resistant and susceptible lines was employed using severity scores and ELISA as described by Kumar (2009) and Ogunsola *et al*., (2010) in Table 3.2. Plants showing moderate to severe systemic symptoms and positive to ELISA were considered susceptible while symptomless, ELISA and PCR negative plants were classified as resistant. Those without

visible symptoms or with mild symptoms but ELISA positive were classified as tolerant. Segregation ratio of resistant and susceptible plants was thus determined from this classification.

3.6.4 Inheritance of resistance to Bean common mosaic virus - blackeye cowpea mosaic strain in cowpea

Cowpea line IT97K-1042-3, which was resistant to BCMV - BlCM was crossed with a susceptible line IT99K-1060. The F_1 progenies were advanced to generate F2 and backcrossed to each of the parents to produce BCP_1 and BCP_2. Reciprocal crosses were also made and the P_1, P_2, F_1, F_2, BCP_1 and BCP_2 populations of both direct and indirect crosses were screened for resistance to BCMV – BlCM.

3.6.5 Inheritance of resistance to *Southern bean mosaic virus* in cowpea

A SBMV resistant line IT98K-1092-1 was crossed to the susceptible line IT99K-1060, with the resistant line used as male parent. This is because of the very low rate of successful crosses when the resistant line was used as female parent. Hence, there was no reciprocal cross made in the study of resistance to SBMV. Some of the F_1 hybrids were advanced to F_2 and also backcrossed to produce BCP_1 and BCP_2 generations.

3.6.6 Inheritance of tolerance to *Cucumber mosaic virus* in cowpea

The CMV tolerant cowpea breeding line IT98K-1092-1 and susceptible IT99K-573-1-1 were crossed. The F_1 progeny was advanced to F_2 and some of them were used to generate backcrosses BCP_1 and BCP_2. Reciprocal crosses were made and the generations resulting from the direct and reciprocal crosses were evaluated for tolerance to CMV

3.6.7 Data collection and statistical analysis

Data from severity scores taken over a period of seven WPI and from ELISA and RT-PCR virus detection were used to determine the segregation patterns in each experiment. The qualitative traits were classified into two phenotypic classes of resistant and susceptible plants and the segregation patterns tested for goodness-of-fit to appropriate Mendelian segregation ratios using the chi-square analysis. The formula for the Chi-square ($\chi 2$) test according to Gomez and Gomez, (1984) is:

$$\chi^2 = \Sigma \frac{(Oi - Ei)^2}{Ei} = \frac{(O1 - E1)^2}{E1} + \frac{(O2 - E2)^2}{E2} \ldots\ldots\ldots + \frac{(On - En)^2}{En}$$

Where O = number of observations within a class

E = expected number in the class

n = number of classes

3.7 Determination of seed transmission of single and mixed viruses in cowpea

This experiment was performed in the screen house of Virology and Molecular Diagnostics Unit of IITA. Six virus susceptible genotypes were selected from the nine cowpea genotypes infected with BCMV - BlCM, SBMV and CMV singly and in mixed infections in the screen-house evaluation for virus resistance (section 3.4.1). Seeds obtained from harvested pods of the infected cowpea lines were used in this experiment. The six genotypes evaluated for virus transmission were:

1. IT98K-133-1-1
2. IT97K-1069-6
3. IT98K-503-1
4. IT99K-1060
5. IT99K-573-1-1
6. Ife brown

Fifty seeds of each of the six genotypes were sown for each virus treatment, making a total of 300 seeds in each experiment except in some virus treatments on three highly susceptible lines IT98K-503-1, IT99K-573-1-1 and IT99K-1060, where less than fifty seed (27 – 45 seeds) were available. In these three lines, while 50 seeds were used in other treatments, 37, 40 and 45 seeds of IT98K-503-1 infected with SBMV, BCMV - BlCM and CMV respectively were used and under mixed infections, 27 and 30 seeds infected with BCMV – BlCM + SBMV + CMV and SBMV + CMV were used. Forty seeds each of IT99K-573-1-1 infected with BCMV - BlCM and BCMV - BlCM + CMV were used while 32 and 43 seeds of IT99K-1060 infected with SBMV+CMV and BCMV – BlCM + SBMV + CMV respectively were used. Seeds from un-inoculated plants of each of the six genotypes were sown as control. Seed were sown in 60 cm by 45 cm plastic trays each with 35 wells at one seed per well (Figure 3.2). There were seven experiments in all to

Figure 3.2 Layout of seed transmission experiments

investigate the seed transmission of the three viruses under single and mixed infections. The seven experiments comprised seed transmission of viruses under the seven virus treatments namely: 1) BCMV - BlCM 2) SBMV 3) CMV 4) BCMV - BlCM + SBMV, 5) BCMV - BlCM + CMV, 6) SBMV + CMV and 7) BCMV - BlCM + SBMV + CMV. These experiments were conducted between August and October 2011.

3.7.1 Crop management

Sterilized sandy loam soil was used. Seeds were treated with Benlate, a fungicide at 1 g per 40 seed before sowing. Watering was done regularly and there was no application of fertilizer. Insect infestation was controlled using insecticides Lambdacyalothrin (Karate; 5% EC) at 4mls per litre of water and Vertimec (018 EC) at 0.75ml per litre of water. Sticky insect traps were also hung above the plants for further pest control. Each experiment was terminated after six WAP.

3.7.2 Data collection and analysis

Percentage seed germination was determined. Symptoms of seed transmitted viruses were recorded as observed. Virus detection was carried out using ACP-ELISA according to by Kumar (2009) as described earlier (section 3.4.1.3.3). ACP-ELISA was carried out at five WAP to confirm the presence of seed transmitted viruses which also determined any latent infection. Virus infections were determined by symptom appearance and virus detection by ACP-ELISA. Percentage seed transmission was calculated from the number of infected seedlings over the total number of seedlings.

CHAPTER FOUR

RESULTS

4.1 Evaluation of eight IITA improved cowpea breeding lines for resistance to single and mixed infections of three economically important cowpea viruses.

4.1.1 Screen-house evaluation of cowpea for resistance to BCMV - BlCM, SBMV and CMV

4.1.1.1 Symptomatology

Symptoms observed on the evaluated cowpea genotypes from BCMV - BlCM, SBMV and CMV infections varied according to genotype, type of virus and mode of infection either single or mixed. The three viruses induced very severe disease symptoms on the susceptible and highly susceptible genotypes under artificial inoculations. Mild symptoms were observed on moderately resistant lines, mild to no symptom on tolerant genotypes while the more resistant lines were symptomless. The three viruses induced severe symptoms on the Ife brown cv. used as a susceptible check and all the non-inoculated controls from each of the cowpea genotypes were asymptomatic.

Under single infections, BCMV - BlCM produced systemic foliar symptoms of varied levels of mosaic and mottling, inter-veinal chlorosis and vein banding. Days of first symptom appearance of BCMV - BlCM in inoculated cowpea ranged between eight days post inoculation (DPI) in Ife brown to 16 DPI in IT98K-133-1-1. SBMV induced chlorotic local lesions on inoculated leaves of some cowpea genotypes and produced systemic symptoms of mild to severe mosaic, inter-veinal chlorosis, mottling, mild puckering and leaf deformation. SBMV symptoms appeared from eight to 14 DPI depending on cowpea line. However, symptoms incited by SBMV are generally milder than those of other two viruses. CMV infection produced chlorotic local lesions which later resulted in abscission of inoculated leaves in highly susceptible genotypes. This progressed into systemic symptoms of mild mosaic, mottling, veinal and midrib chlorosis, puckering, leaf distortion and stunted growth. Incubation period of seven days was observed for CMV in the susceptible cowpea lines. However, CMV symptoms faded from three or four WPI in most of the lines especially in IT99K-1060, IT99K-573-1-1 and Ife brown. Each of the viruses generally induced severe symptoms on Ife brown (susceptible check) and IT99K-1060 (Plate 4.1). BCMV - BlCM induced moderate to severe symptoms on IT97K-1069-6, IT99K-1060, IT99K-573-1-1, IT98K-133-1-1, IT98K-503-1 and IT04K-405-5. SBMV produced severe symptoms on IT98K-503 and IT99K-1060 only while CMV induced

moderate to severe symptoms in all the genotypes but with mild symptoms in IT98K-1092-1.

Mixed infections produced more severe symptoms on the susceptible lines in both 2009 and 2011 trials, severity of which depended on the type and number of viruses and the cowpea genotype involved in the co-infection. These co-infections resulted in abscission of some inoculated leaves, reduced leaf areas, severe mosaic, stunted growth, few or no pod formation and premature death in highly susceptible lines. Co-infections involving CMV produced more severe symptoms. Inoculation with BCMV - BlCM + CMV generally produced more severe symptoms than BICMV + SBMV and SBMV + CMV. The triple infection produced the most severe symptoms when compared with single and double infections (Plate 4.2). Triple infection (BCMV - BlCM + SBMV + CMV) induced defoliation of the first trifoliate leaves in IT99K-1060 and Ife brown within seven DPI and resulted in systemic symptoms of mosaic, puckering, reduced leaf area, leaf distortion, apical necrosis, stunted growth, few or no pod formation and premature death in some plants of highly susceptible genotypes (Plate 4.2 and 4.3).

4.1.1.2 Disease incidence and severity

In both 2009 and 2011 trials, 100% incidence with highest severity was observed in cv. Ife brown (susceptible control) in both single and mixed infections, while no infection was observed in all the eight genotypes mocked-inoculated with inoculation buffer (healthy control). Incidence of infections by the three viruses differed significantly ($p < 0.05$) among the cowpea genotypes. Disease incidences of the three viruses under single infections are presented in Table 4.1. In both trials, mean incidence of BCMV - BlCM was significantly ($p < 0.01$) higher in IT98K-503-1 (100 %), IT04K-405-5 (10 0%), IT99K-1060 (100 %), IT98K-133-1-1 (95.8 %) and IT99K-573-1-1 (87.5 %) than in IT99K-1060 (81.3 %) and IT97K-1069-6 (66.7 %). However, cowpea lines IT98K-1092-1and IT97K-1042-3 had 0.0 % incidence of BICMV infection. There was a significant difference ($p = 0.01$) in the incidence of SBMV which were higher in IT99K-1060 (100 %) and IT98K-503-1 (87.5 %) while IT98K-1092-1, IT97K-1042-3 and IT04K-405-5 had 0 % incidence of SBMV in both years. Unlike the other two viruses, CMV infection was observed in all the eight cowpea genotypes evaluated. Hundred percent CMV incidence was observed in IT97K-1069-6 and IT99K-1060 in both trials.

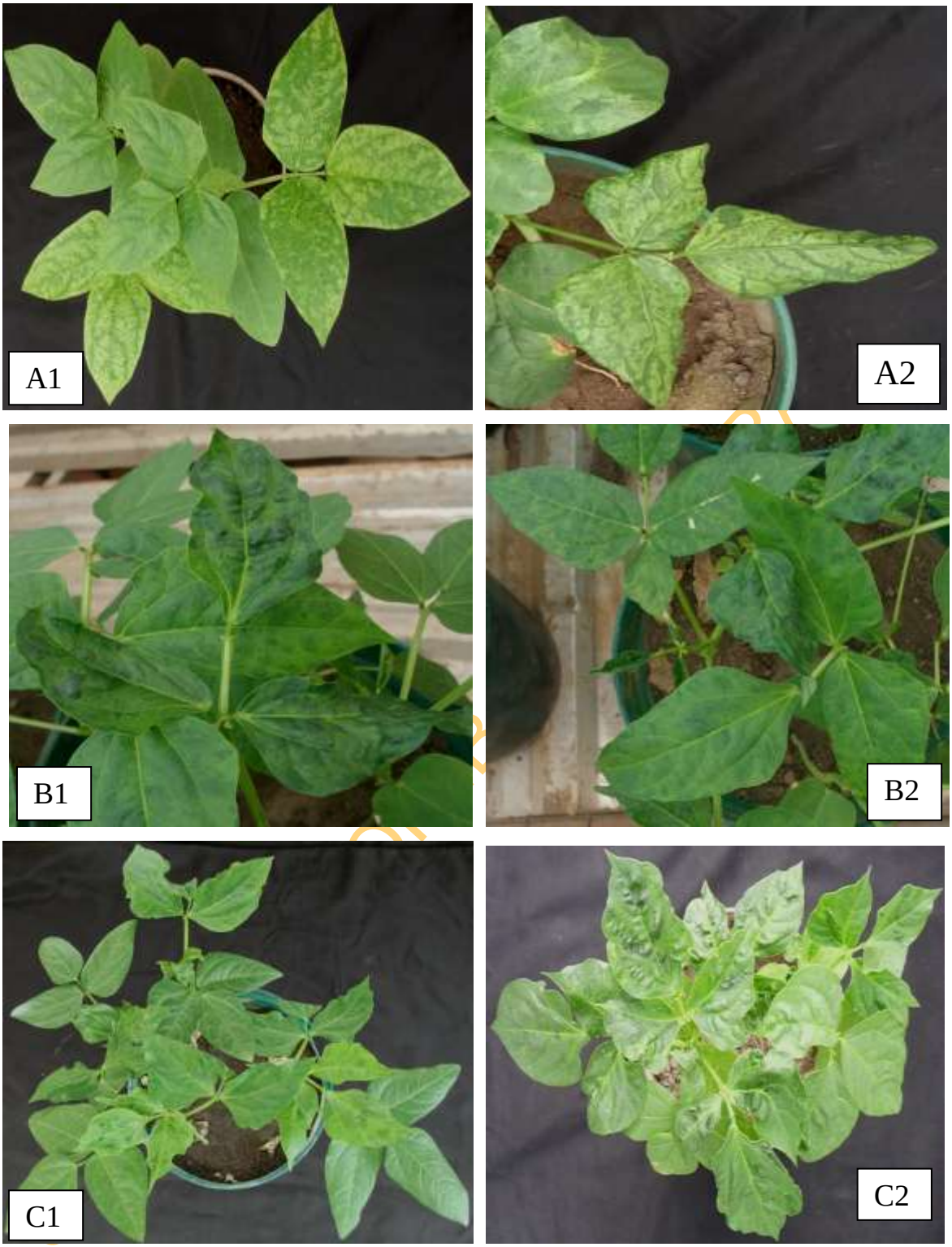

Plate 4.1 Symptoms induced on cowpea genotypes by single infection of *Bean common mosaic virus - blackeye cowpea mosaic strain* (BCMV - BlCM), *Southern bean mosaic virus* (SBMV) and *Cucumber mosaic virus* (CMV) at 4 weeks post inoculation. A1= BCMV-BlCM on Ife brown (WPI), A2 = BCMV-BlCM on IT99K-1060, B1 = SBMV on IT99K-1060 4 WPI, B2 = SBMV on Ife brown, C1 = CMV on IT99K573-1-1, C2, = CMV on line TVu76

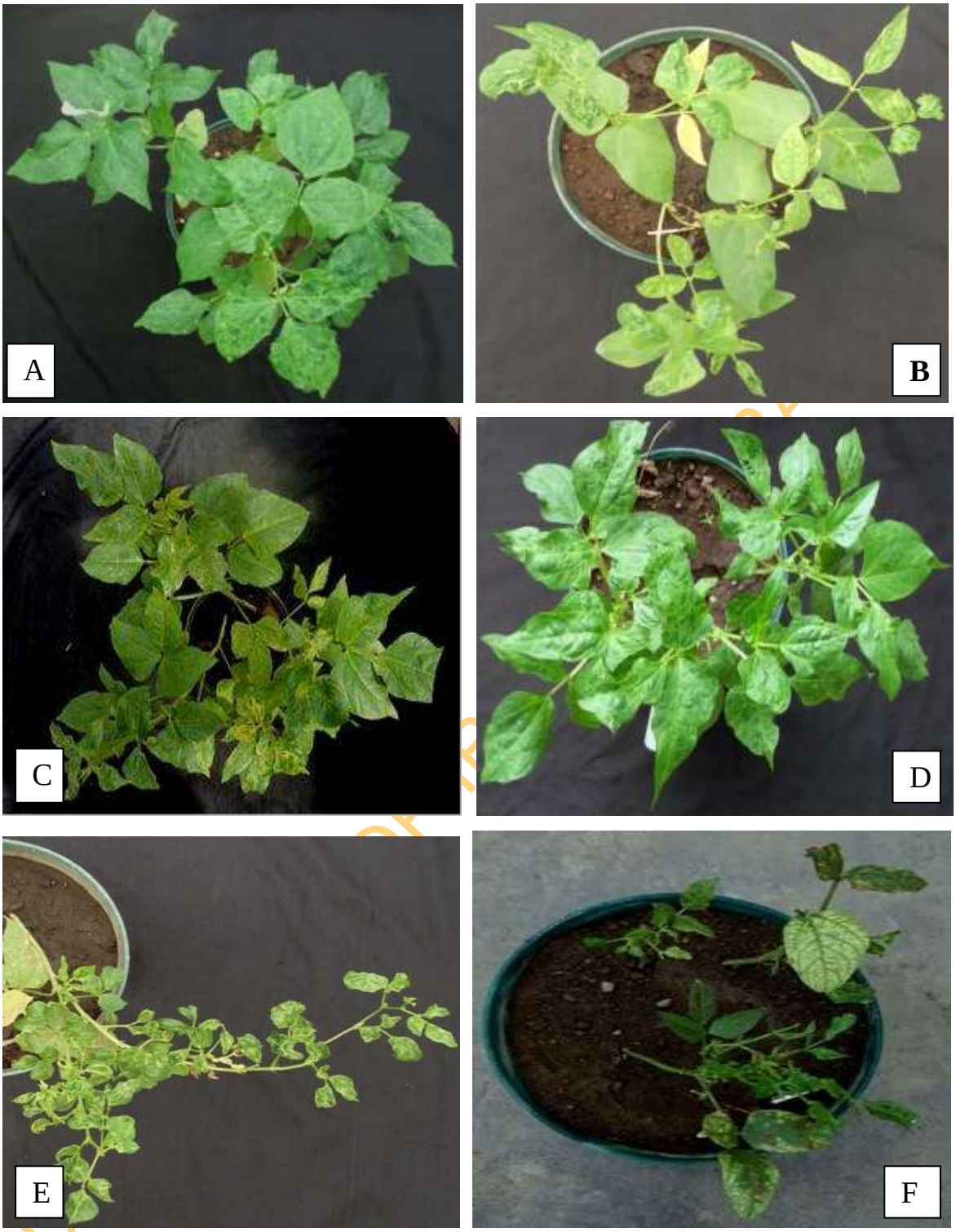

Plate 4.2 Symptoms induced in cowpea genotypes mixed infected with Bean common mosaic virus - Blackeye cowpea mosaic strain (BCMV - BlCM), *Southern bean mosaic virus* (SBMV) and *Cucumber mosaic virus* (CMV) at 5 weeks post inoculation. A= BCMV-BlCM + SBMV on Ife brown, B = BCMV-BlCM + CMV on IT99K-1060, C = BCMV -BlCM + CMV on IT04K-405-5, D = SBMC + CMV on Ife brown at 5 WPI, E = BCMV-BlCM + SBMV + CMV on Ife brown, F = BCMV -BlCM + SBMV + CMV on IT99K-1060.

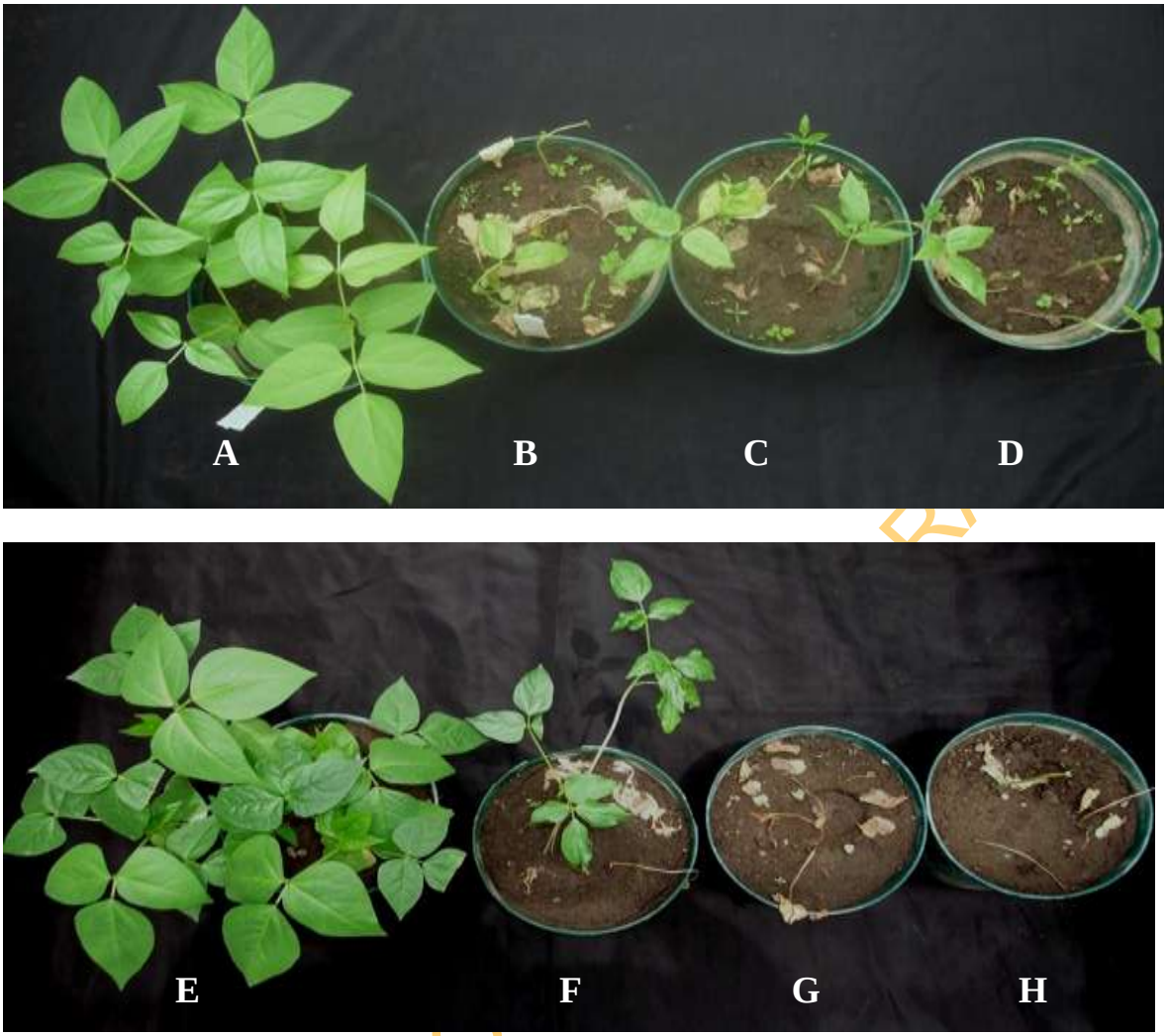

Plate 4.3 Symptom severity of cowpea lines mix-inoculated with Bean common mosaic virus - blackeye cowpea mosaic strain (BCMV - BlCM), *Southern bean mosaic virus (SBMV)* and *Cucumber mosaic virus* (CMV), 2 weeks post inoculation. A = Buffer (control) on Line IT99K-1060, B = BCMV-BlCM + SBMV + CMV on IT99K-1060, C = SBMV + CMV on IT99K-1060, D = BCMV-BlCM + CMV on IT99K-1060, E = BCMV-BlCM + SBMV + CMV on IT98K1092-1 (resistant line), F = BCMV-BlCM + SBMV + CMV on IT97K-1069-6, G = BCMV-BlCM + SBMV + CMV on IT97K-1042-3 and H = BCMV-BlCM + SBMV + CMV on Ife brown.

Table 4.1 Disease incidence (%) of BICMV, SBMV and CMV on inoculated cowpea lines under screen house conditions in 2009 and 2011

Genotype	BCMV-BICM			SBMV			CMV		
	2009	2011	Mean	2009	2011	Mean	2009	2011	Mean
IT98K-133-1-1	100.0a	91.7a	95.8ab	0.0d	8.3c	4.2c	100.0a	91.7a	95.8ab
IT98K-1092-1	0.0d	0.0b	0.0d	0.0d	0.0c	0.0c	66.7b	75.0a	70.8c
IT97K-1069-6	50.0c	83.3a	66.7c	12.5d	16.7c	14.6c	100.0a	100.0a	100.0a
IT98K-503-1	100.0a	100.0a	100.0a	75.0b	100.0a	87.5a	75.0b	100.0a	87.5ab
IT97K-1042-3	0.0d	0.0b	0.0d	0.0d	0.0c	0.0c	100.0a	75.0a	87.5ab
IT04K-405-5	100.0a	100.0a	100.0a	0.0d	0.0c	0.0c	100.0a	91.7a	95.8ab
IT99K-1060	62.5bc	100.0a	81.3bc	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
IT99K-573-1-1	75.0b	100.0a	87.5ab	37.5c	58.3b	47.9b	75.0b	88.7a	81.8bc
Ife brown[b]	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a

BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; b, susceptible check. Means followed by the same letter in each column are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Significantly ($p < 0.01$) high incidence was similarly observed in IT98K-133-1-1 (95.8%), IT98K-503-1 (87.5%), IT97K-1042-3 (87.5%) and IT04K-405-5 (95.8%) with lowest CMV incidence (70.8%) in IT98K-1092-1. Generally, IT98K-503-1 and IT99K-1060 showed significantly high incidences of the three viruses while IT99K-573-1-1 had high incidence of BICMV and CMV. Also, IT98K-1092-1 showed low incidences of the three viruses compared with the susceptible line and lines IT97K-1042-3 and IT98K-1092-1 had 0% incidence of BCMV - BlCM and SBMV.

Disease severity of the viruses (Table 4.2 and 4.4) differed significantly ($p = 0.01$) among the cowpea genotypes studied and in similar trend with disease incidence. Means of the two trials showed that severity of single infection of BICMV was significantly ($p = 0.01$) higher in IT98K-503-1 (4.7±0.7) than in IT99K-1060 (4.0±0.6) and IT99K-573-1-1 (3.7±0.6). Cowpea lines IT98K-133-1-1 and IT97K-1069-6 showed intermediate severity of BCMV - BlCM (2.7±0.5 and 2.3±0.5) while there was no visible symptom of the virus (severity score 1.0±0.0) in lines IT98K-1092-1 and IT97K-1042-3. Severity of SBMV disease was most pronounced ($p < 0.05$) in IT98K-503-1 and IT99K-1060 (3.7±1.0 and 3.2±1.0) and very low (1.0±0.0 – 1.6±0.4) in all other evaluated cowpea genotypes. However, four lines (IT98K-133-1-1, IT98K-1092-1, IT97K-1042-3 and IT04K-405-5) did not produce any symptom (1.0±0.0) to SBMV inoculations in both trials. CMV was the most aggressive of the three viruses by inciting mild to severe symptoms in all the test lines. Meanwhile, line IT98K-1092-1 showed tolerance response to CMV infection by producing significantly ($p < 0.01$) mild symptoms (1.8±0.3) without marked reduction in plant vigor and yield though the virus was able to multiply in this line as in other susceptible lines as determined ACP-ELISA.

Incidence of mixed infections of the viruses also differed significantly ($p \leq 0.05$) among the cowpea lines and was generally higher than that of the single infections. Mean incidences of 2009 and 2011 trials showed significantly lower ($p < 0.05$) incidence of BCMV-BlCM + SBMV in IT98K-1092-1and IT97K-1042-3 than in the other six genotypes (Table 4.3). While incidence of BCMV-BlCM + CMV was high in the eight evaluated lines similarly to the susceptible check, significantly lower incidence of SBMV + CMV occurred in IT98K-1092-1 than in the other genotypes. Lines IT97K-1069-6 and IT99K-1060 showed 100% incidence of the three viruses in both the double and triple

Table 4.2 Disease severity of BCMV - BlCM, SBMV and CMV infections on inoculated cowpea genotypes under screen house conditions in 2009 and 2011

Genotype	BCMV-BlCM			SBMV			CMV		
	2009	2011	Mean	2009	2011	Mean	2009	2011	Mean
IT98K-133-1-1	3.0bc	2.3b	2.7±0.5d	1.0d	1.1d	1.0±0.1c	2.5c	3.1bc	2.8±0.3bc
IT98K-1092-1	1.0d	1.0c	1.0±0.0e	1.0d	1.0d	1.0±0.0c	1.7d	2.0d	1.8±0.3d
IT97K-1069-6	2.5c	2.2b	2.3±0.5d	1.1d	1.2d	1.1±0.1c	2.7c	3.3bc	3.0±0.4bc
IT98K-503-1	4.9a	4.5a	4.7 ±0.7a	2.9b	4.6a	3.7±1.0b	2.7c	4.3a	3.5±1.0ab
IT97K-1042-3	1.0d	1.0c	1.0±0.0e	1.0d	1.0d	1.0±0.0c	2.6c	2.7cd	2.6±0.3c
IT04K-405-5	4.6a	3.0b	3.7±1.0c	1.0d	1.0d	1.0±0.0c	4.5a	3.1bc	3.8±0.5a
IT99K-1060	3.5b	4.5a	4.0±0.6bc	2.3c	4.1b	3.2±1.0b	3.4b	4.3a	3.8±0.5a
IT99K-573-1-1	3.4b	3.9a	3.7±0.6c	1.4d	1.8c	1.6±0.4c	3.6b	2.9c	3.2±0.5abc
Ife brown[b]	5.0a	4.2a	4.6±0.5ab	4.8a	4.2ab	4.5±0.4a	3.7b	3.9ab	3.8±0.6a

BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; severity 1-5, severity scale 1-5, (1= No visible symptom and 5= severe foliage symptom); b, susceptible check. Means followed by the same letter in each column are not significantly different (p = 0.01) according to Duncan's multiple range test.

Table 4.3 Disease incidence (%) of mixed infections of BICMV, SBMV and CMV on inoculated cowpea genotypes under screen house conditions in 2009 and 2011

Genotype	BCMV – BlCM + SBMV			BCMV – BlCM + CMV			SBMV+CMV			BCMV-BICM+SBMV+CMV		
	2009	2011	Mean	2009	2011	Mean	2009	2011	Mean	2009	2011	Mean
IT98K-133-1-1	75.0b	83.3a	79.2b	62.5b	100.0a	81.3a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
IT98K-1092-1	0.0d	16.7c	8.3d	58.3b	91.7a	75.0a	33.3b	91.2a	62.5b	58.3b	75.0a	66.7b
IT97K-1069-6	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
IT98K-503-1	87.5ab	100.0a	93.8ab	75.0ab	100.0a	87.5a	100.0a	100.0a	100.0a	87.5a	100.0a	93.8a
IT97K-1042-3	50.0c	50.0b	50.0c	100.0a	83.3a	91.7a	100.0a	75.0b	87.5a	100.0a	83.3a	91.7a
IT04K-405-5	100.0a	91.7a	95.8a	62.5b	83.3a	72.3a	100.0a	75.0b	87.5a	62.5b	100.0a	81.3ab
IT99K-1060	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
IT99K-573-1-1	87.5ab	91.7a	89.6ab	100.0a	83.3a	91.7a	100.0a	88.7ab	94.3a	87.5a	91.7a	89.6a
Ife brown[b]	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a

BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; b, susceptible check. Means followed by the same letter in each column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 4.4 Disease severity of mixed infections of BICMV, SBMV and CMV infections on inoculated cowpea genotypes under screen house conditions in 2009 and 2011

Genotype	BCMV – BlCM + SBMV			BCMV – BlCM + CMV			SBMV+CMV			BCMV – BlCM + SBMV+CMV		
	2009	2011	Mean	2009	2011	Mean	2009	2011	Mean	2009	2011	Mean
IT98K-133-1-1	2.2d	2.7c	2.4±0.5c	2.2c	3.0cd	2.6±0.5bc	3.8b	2.2c	3.0±0.9c	2.4e	3.2bc	2.8±0.5bc
IT98K-1092-1	1.0e	1.2d	1.1±0.2d	1.6d	2.1d	1.8±0.3c	1.3c	2.2c	1.8±0.5d	1.6f	2.5c	2.0±0.7c
IT97K-1069-6	4.5a	2.4c	3.5±1.2b	5.0a	3.8bc	4.4±0.9a	3.7b	3.3b	3.5±0.6c	5.0a	3.5bc	4.3±0.8a
IT98K-503-1	3.2c	4.9a	4.0±0.9b	3.0b	4.8ab	3.9±1.0a	4.4ab	4.8a	4.6±0.5a	3.8c	4.8a	4.3±0.7a
IT97K-1042-3	1.5e	1.6d	1.6±0.1d	5.0a	3.5c	4.3±0.9a	5.0a	3.6b	4.3±0.8ab	5.0a	3.6bc	4.3±1.1a
IT04K-405-5	4.5a	2.9bc	3.7±0.9b	2.3c	3.3c	2.8±1.1b	4.4ab	3.1b	3.8±0.8bc	2.9d	3.6b	3.2±0.6b
IT99K-1060	3.9b	4.8a	4.3±0.6ab	4.6a	5.0a	4.8±0.3a	5.0a	4.4a	4.7±0.4a	5.0a	4.8a	4.9±0.1a
IT99K-573-1-1	3.9b	3.4b	3.6±0.3b	4.5a	3.9bc	4.2±0.6a	3.7b	3.1b	3.4±0.6c	4.3b	4.0ab	4.2±0.4a
Ife brown[b]	5.0a	4.8a	4.9±0.1a	5.0a	4.7ab	4.8±0.2a	5.0a	4.4a	4.7±0.5a	5.0a	4.8a	4.9±0.1a

BCMV - BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; b, susceptible check; severity 1-5, severity scale 1-5, (1= No visible symptom and 5= severe symptoms). Means followed by the same letter in each column are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

infections. High mean incidences of BCMV - BlCM + SBMV, BCMV - BlCM + CMV, SBMV + CMV and BCMV - BlCM + SBMV+CMV were also found in IT98K-503-1 (93.8%, 87.5%, 100.0% and 93.8%), IT99K-573-1-1 (89.6%, 91.7%, 94.3% and 89.6%), IT04K-405-5 (95.8%, 72.3%, 87.5% and 81.3%) and IT98K-133-1-1 (79.2%, 81.3%, 100.0% and 100.0%). However, similar to single infections of BCMV - BlCM and SBMV, significantly lower ($p \leq 0.05$) mixed infections of BCMV - BlCM + SBMV were observed in IT98K-1092-1 (8.3%) and IT97K-1042-3 (50.0%).

Disease severity of mixed viral infections showed significant differences ($p < 0.05$) among the cowpea genotypes (Table 4.4) following similar trend with incidence of mixed infections. More severe infections of the three viruses were observed in mixed infections than single ones (Plate 4.4). Cowpea line IT99K-1060 relatively has the highest mean disease severity (4.3±0.6, 4.8±0.3, 4.7±0.4 and 4.9±0.1) of BCMV - BlCM + SBMV, BCMV - BlCM + CMV, SBMV + CMV and BCMV - BlCM + SBMV + CMV. The triple and BCMV - BlCM + CMV infections resulted in higher disease severity in most of the susceptible lines, with least severity caused by BCMV - BlCM + SBMV. Comparatively, IT98K-1092-1 relatively produced lower (1.1±0.2, 1.8±0.3, 1.8±0.5 and 2.0±0.7) disease severity than other lines (Plate 4.4a). Similar to single infections, very high viral disease severities were observed in IT98K-503-1 and IT99K-573-1-1 which were not different ($p \leq 0.05$) from those observed in IT99K-1060 in all the mixed infections. Cowpea line IT97K-1042-3 also produced mild symptoms of BCMV - BlCM + SBMV next to IT98K-1092-1.

4.1.1.3 Detection and determination of relative titre values of BCMV - BlCM, SBMV and CMV in cowpea as determined by enzyme linked immunosorbent assay

All Ife brown (susceptible control) plants were highly positive to the three viruses when tested with ACP-ELISA while all healthy control plants of all the cowpea genotypes tested negative. Results of virus detection and relative titre values of BCMV - BlCM, SBMV and CMV under single, double and triple inoculations at eight WPI are shown in Tables 4.5, 4.6 and 4.7 respectively. Mean values of ELISA readings in the two trials were used as the final results. High titre values of the three viruses were detected in all the three cowpea genotypes with high disease incidence and severity (IT99K-1060, IT98K-503-1 and IT99K-573-1-1) under single, double and triple infections. Cowpea lines IT98K-133-3-1,

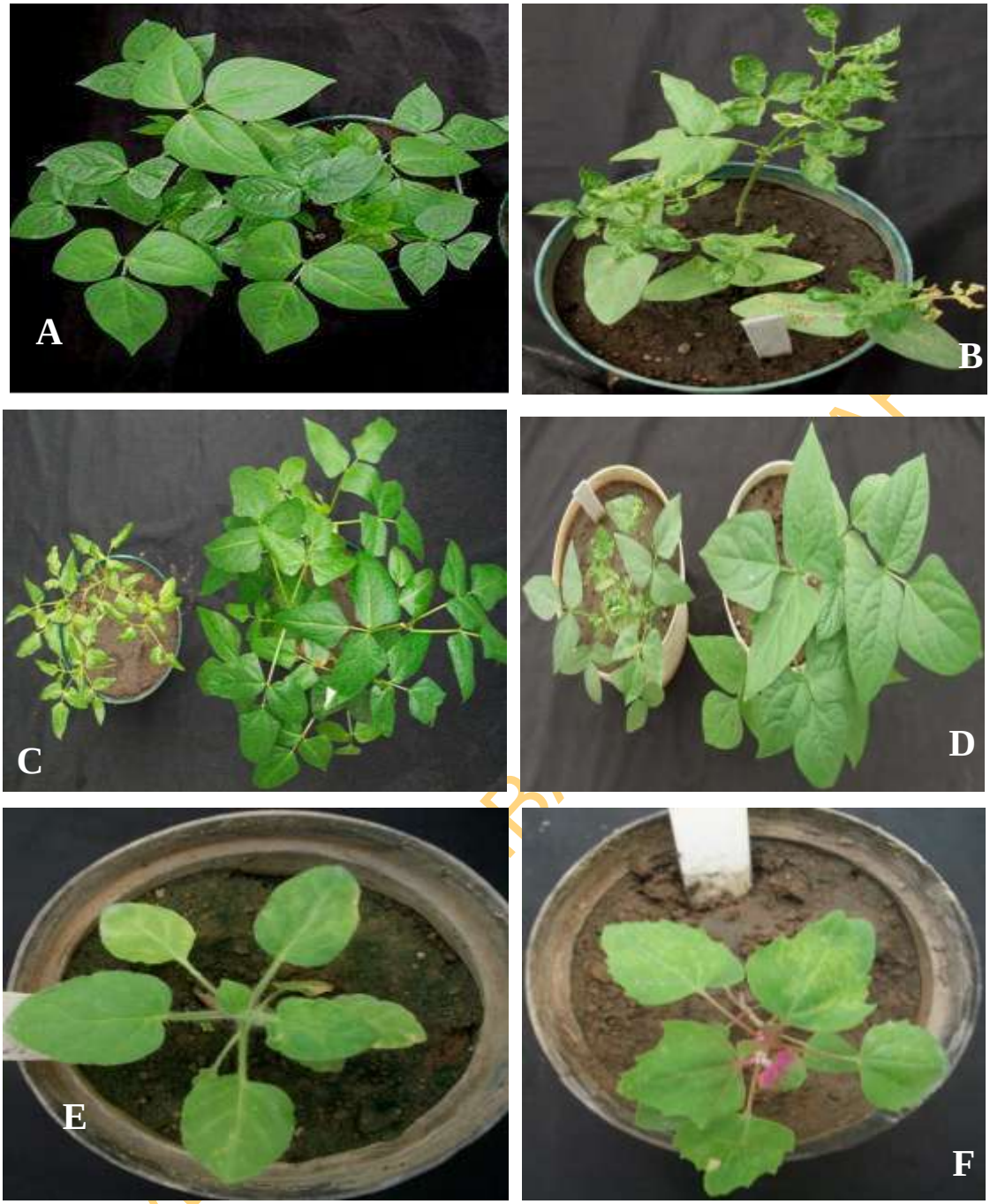

Plate 4.4 Symptoms induced on cowpea lines at 6 weeks post inoculation (WPI) and virus indicator plants at 3 WPI singly or mixed infected with Bean common mosaic virus - blackeye cowpea mosaic strain (BCMV-BlCM), *Southern bean mosaic virus* (SBMV) and *Cucumber mosaic virus* (CMV). A = BCMV-BlCM + SBMV + CMV on Cowpea line IT98K-1092-1(resistant line) showing no visible symptoms, B = BCMV-BlCM + CMV showing mosaic, leaf distortion, puckering, reduced leaf area and inter-veinal chlorosis on Ife brown, C = BCMV-BlCM + SBMV+ CMV on IT97K-1042-3 and its healthy control, D = BCMV-BlCM + CMV on Ife brown and healthy control, E = CMV showing systemic chlorosis on *Nicotiana glutinosa*, F = SBMV showing chlorotic local spots on *Chenopodium ammaranthicolor*.

Table 4.5 Detection and determination of the relative titre values of BCMV - BlCM, SBMV and CMV in cowpea genotypes as determined by enzyme linked immunosorbent (ELISA) under screen house conditions in 2009 and 2011

Genotype	BCMV – BlCM			SBMV			CMV		
	2009	2011	Mean	2009	2011	Mean	2009	2011	Mean
IT98K-133-1-1	1.95++++	1.23+++	+++	0.27-	0.16-	-	0.39+	0.39+	+
IT98K-1092-1	0.17-	0.20-	-	0.48-	0.20-	-	0.38+	0.94++	++
IT97K-1069-6	1.90+++	1.77+++	+++	0.29-	0.21-	-	0.66++	1.28+++	++
IT98K-503-1	2.19+++	2.52+++	+++	1.61+++	1.48+++	+++	0.57++	1.30+++	++
IT97K-1042-3	0.38-	0.15-	-	0.44-	0.20-	-	1.21+++	0.43++	++
IT04K-405-5	1.16+++	1.40+++	+++	0.34-	0.19-	-	2.06++	1.27+++	++
IT99K-1060	3.00+++	2.55+++	+++	0.77++	1.38+++	++	0.40+	1.75+++	++
IT99K-573-1-1	0.63+++	1.58+++	+++	0.99++	0.77+++	++	1.30+++	2.01+++	+++
Ife brown[b]	1.52+++	1.58++	++	2.62+++	2.39+++	+++	0.85+++	0.60+++	+++

BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; b, susceptible check; - = negative [ELISA value (read at 405nm Absorbance) ≤ H; H = absorbance value of healthy leaf]; + = positive (ELISA values ≥ 2 x H); ++ = moderately positive (≥ 3 x H); +++ = highly positive (≥ 4 x H)

Table 4.6 Detection and determination of titre values of doubly infected BCMV - BlCM, SBMV and CMV in cowpea genotypes under screen house conditions as determined by enzyme linked immunosorbent assay (ELISA) in 2009 and 2011a

Genotype	BCMV – BlCM + SBMV				BCMV – BlCM + CMV				SBMV+CMV			
	Isolate	2009	2011	Mean	Isolate	2009	2011	Mean	Isolate	2009	2011	Mean
IT98K-133-1-1	BlCM	0.44-	0.15-	-	BICM	0.62+	0.34-	+	SBMV	0.80+++	0.16-	++
	SBMV	0.22-	0.28-	-	CMV	0.56+	1.06+++	++	CMV	1.03+++	0.45++	++
IT98K-1092-1	BlCM	0.14-	0.14-	-	BICM	0.12-	0.16-	-	SBMV	0.20-	0.16-	-
	SBMV	0.20-	0.23-	-	CMV	0.47+	0.84+++	++	CMV	0.97+++	1.28+++	+++
IT97K-1069-6	BICM	1.75+++	0.17-	++	BICM	2.34+++	0.59++	+	SBMV	0.29-	0.16-	-
	SBMV	0.18-	0.30-	-	CMV	2.77+++	2.67+++	+++	CMV	2.60+++	1.90+++	+++
IT98K-503-1	BICM	1.52+++	0.21-	++	BICM	0.87+	0.14-	+	SBMV	3.00++	1.10+++	+++
	SBMV	2.80+++	2.04+++	+++	CMV	2.66+++	1.12+++	+++	CMV	2.05+++	0.42++	++
IT97K-1042-3	BICM	0.12-	0.13-	-	BICM	0.31-	0.14-	-	SBMV	0.22-	0.17-	-
	SBMV	0.19-	0.24-	-	CMV	1.25+++	0.12-	++	CMV	0.72++	0.44++	++
IT04K-405-5	BICM	0.94++	0.80+++	++	BICM	0.75+	0.48+	+	SBMV	0.36-	0.15-	-
	SBMV	0.23-	0.22-	-	CMV	2.01+++	0.82+++	+++	CMV	2.08+++	2.43+++	+++
IT99K-1060	BICM	1.34+++	0.17-	++	BICM	2.79+++	0.16-	++	SBMV	1.67+++	0.54+++	+++
	SBMV	0.92++	2.92+++	++	CMV	2.74+++	0.65+++	+++	CMV	0.54+	2.90+++	++
IT99K-573-1-1	BICM	2.59+++	0.73+++	+++	BICM	0.59+	0.15-	+	SBMV	0.47-	0.41++	+
	SBMV	1.66+++	1.52+++	+++	CMV	1.10+++	1.35+++	+++	CMV	0.54+	2.05+++	++
Ife brown[b]	BICM	2.50+++	0.52++	++	BICM	2.84+++	0.15-	++	SBMV	2.95+++	1.73+++	+++
	SBMV	2.06+++	2.88+++	+++	CMV	2.97+++	0.77+++	+++	CMV	2.92+++	1.95+++	+++

BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; b, susceptible check;- = negative [ELISA value (read at 405nm Absorbance) ≤ H; H = absorbance value of healthy leaf]; + = positive (ELISA values ≥ 2 x H); ++ = moderately positive (≥ 3 x H); +++ = highly positive (≥ 4 x H)

Table 4.7 Detection and determination of relative titre values of triply infected cowpea genotypes with BCMV-BlCM, SBMV and CMV under screen house conditions as determined by enzyme linked immunosorbent assay (ELISA) in 2009 and 2011

Genotype	BCMV-BlCM + SBMV + CMV			
	Isolate	2009	2011	Mean
IT98K-133-1-1	BCMV - BlCM	0.59+	0.65++	+
	SBMV	0.29-	0.25-	-
	CMV	0.68++	0.34+	++
IT98K-1092-1	BCMV - BlCM	0.12-	0.18-	-
	SBMV	0.30-	0.15-	-
	CMV	1.43+++	1.72+++	+++
IT97K-1069-6	BCMV - BlCM	2.16+++	0.16-	++
	SBMV	0.29-	0.22-	-
	CMV	1.55+++	1.77+++	+++
IT98K-503-1	BCMV - BlCM	0.61+	0.18-	+
	SBMV	1.64+++	0.62+++	+++
	CMV	1.40+++	2.07+++	+++
IT97K-1042-3	BCMV - BlCM	0.33-	0.18-	-
	SBMV	0.26-	0.14-	-
	CMV	0.85+++	0.56+	++
IT04K-405-5	BCMV - BlCM	0.63+	0.33+	+
	SBMV	0.28-	0.17-	-
	CMV	1.07+++	2.07+++	+++
IT99K-1060	BCMV - BlCM	3.00+++	0.13-	++
	SBMV	2.27+++	1.72+++	+++
	CMV	2.57+++	0.42++	++
IT99K-573-1-1	BCMV - BlCM	0.73+	0.14-	+
	SBMV	0.45-	1.40+++	++
	CMV	0.52+	1.23+++	++
Ife brown[b]	BCMV - BlCM	2.47+++	0.69++	++
	SBMV	1.79+++	1.52+++	+++
	CMV	2.60+++	1.46+++	+++

BCMV-BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; b, susceptible check; - = negative [ELISA value (read at 405nm Absorbance) ≤ H; H = absorbance value of healthy leaf]; + = positive (ELISA values ≥ 2 x H); ++ = moderately positive (≥ 3 x H); +++ = highly positive (≥ 4 x H)

IT97K-1069-6 and IT04K-405-5 were ELISA negative to SBMV but positive or highly positive to BCMV - BlCM and CMV under single, double or triple infections. Under single infections, BCMV - BlCM was detected in high titres (0.63 – 3.00) in six genotypes while SBMV tested positive with high virus concentrations (0.77 – 1.61) in three genotypes. However, CMV was detected in moderate to high concentrations (0.34 – 2.90) in all the cowpea genotypes under single or mixed infections. Conversely, BCMV - BlCM and SBMV were not detected in IT98K-1092-1 and IT97K-1042-3 under single and mixed infections in the two trials.

Mixed infection seemed to influence the presence of the viruses in the plants. For instance, in cowpea line IT98K-133-1-1, BCMV - BlCM detected under single infection was not found under BCMV - BlCM + SBMV. The titre value of this virus was also higher (+++) under single infection than in co-infections BCMV - BlCM + CMV and BCMV - BlCM + SBMV + CMV.

4.1.1.4 Use of Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for confirmation of ELISA negative infected cowpea plants

The BCMV - BlCM and SBMV inoculated plants that tested negative to ELISA were further examined using RT-PCR to confirm the absence of viruses and test for the possibility of minute virus titre that might have escaped serological assay. Results showed that all ELISA negative plants also tested negative to RT-PCR (Plates 4.5 and 4.6). Under single, double or triple inoculations which included BCMV - BlCM, all samples of IT98K-1092-1 inoculated with BCMV - BlCM, BCMV - BlCM + SBMV, BCMV - BlCM + CMV and BCMV - BlCM + SBMV + CMV and those of IT97K-1042-3 inoculated with BCMV - BlCM, BCMV - BlCM + SBMV, BCMV - BlCM + CMV and BCMV - BlCM + SBMV + CMV tested negative to BCMV - BlCM (Plate 4.5). Similarly, for inoculations involving SBMV, all samples of IT98K-1092-1 inoculated with SBMV, BCMV - BlCM + SBMV, SBMV + CMV and BCMV - BlCM + SBMV + CMV and that of IT97K-1042-3 inoculated with SBMV, BCMV - BlCM + SBMV, SBMV + CMV and BCMV - BlCM + SBMV + CMV tested negative to SBMV (Plate 4.6).

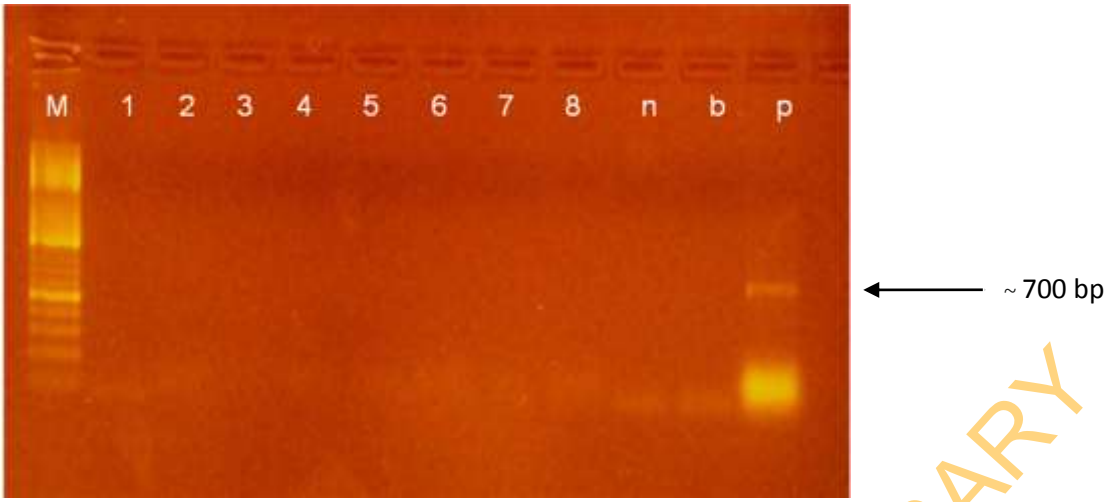

Plate 4.5: No amplification detected in cowpea plants negative to ELISA following RT-PCR using CIF/CIR primers for BCMV - BlCM. Electrophoresis with Ethidium-bromide stained 1.5% agarose gel; M, DNA size marker (100 bp; Promega, USA); lanes 1 - 4, extracts from cowpea line IT98K-1092-1 inoculated with: 1) BCMV -BlCM, 2) BCMV-BlCM + SBMV, 3) BCMV- BlCM + CMV and 4) BlCM + CMV + SBMV respectively; lanes 5 - 8, extract from IT97K-1042-3 inoculated with: 1) BCMV -BlCM, 2) BCMV - BlCM + SBMV , 3) BCMV - BlCM + CMV and 4) BCMV - BlCM + CMV + SBMV; n = negative control consisting of extract from healthy cowpea, b = buffer control, p = positive control consisting of extract from BCMV-BlCM infected susceptible cowpea.

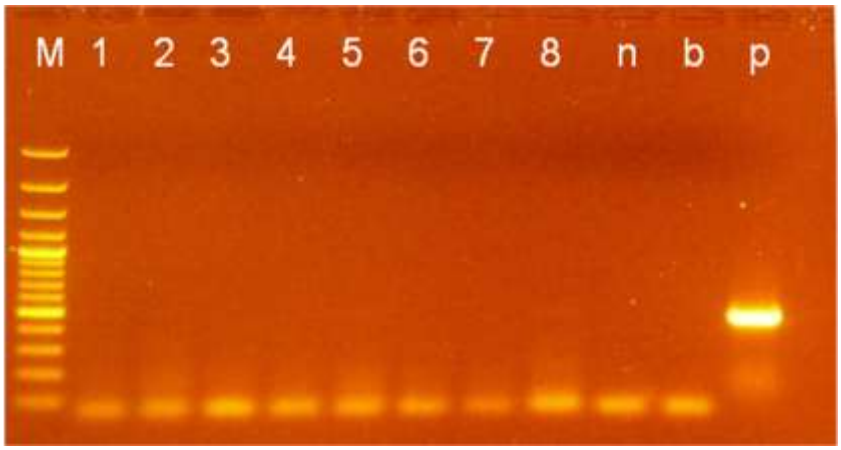

Plate 4.6: No amplification detected in cowpea plants negative to ELISA following RT-PCR using SBMVF / SBMVR primers for SBMV; Electrophoresis with Ethidium-bromide stained 1.5% agarose gel; M, DNA size marker (100 bp; Promega, USA); lanes 1 - 4, extracts from cowpea line IT98K-1092-1 inoculated with 1) SBMV, 2) BCMV - BlCM + SBMV, 3) SBMV + CMV and 4) BCMV - BlCM + CMV + SBMV respectively; lanes 5 - 8, extracts from IT97K-1042-3 inoculated with 1) BCMV - BlCM, 2) BCMV - BlCM + SBMV , 3) SBMV + CMV and 4) BCMV - BlCM + CMV + SBMV; n = negative control consisting of extract from healthy cowpea, b = buffer control, p = positive control consisting of extract from SBMV infected susceptible cowpea.

4.1.1.5 Resistance classes of cowpea genotypes to BCMV - BlCM, SBMV and CMV as determined by disease severity and enzyme-linked immunosorbent assay

Classification of the cowpea genotypes to viral disease resistance status from the screen-house evaluations was carried out using the combination of disease severity, serological and RT-PCR detections.

The ACP-ELISA results were positive to the inoculated virus in all symptomatic cowpea genotypes including Ife brown and negative in non-inoculated control plants. The results of these evaluations, presented in Table 4.8, indicated that IT98K-1092-1 was the most resistant of the genotypes. It was resistant to BCMV - BlCM and SBMV and showed tolerance to CMV. The next was IT97K-1042-3, which showed resistance to BICMV and SBMV but susceptible to CMV. Cowpea lines IT99K-1060 and IT98K-503-1 were highly susceptible to the three viruses while IT99K-573-1-1 was highly susceptible to BCMV - BlCM and CMV but tolerant to SBMV. Lines IT98K-133-1-1, IT97K-1069-6 and IT04K-405-5 were to resistant SBMV and susceptible to highly susceptible to the other two viruses.

Results of screening for resistances to mixed virus infections are presented in Table 4.9. Depending on cowpea line and type and combination of virus, most of the plants had similar reactions to each of the viruses both under single and mixed inoculations. For instance, lines IT99K-1060 and IT98K-503-1 that are most susceptible to single infections of the three viruses remained highly susceptible to the viruses under double or triple infections. The two resistant lines IT98K-1092-1 and IT97K-1042-3 to BCMV - BlCM and SBMV under single infections were also resistant under co-infections, these lines retained their resistance to the two viruses and susceptibility or tolerance status to CMV regardless of either single double or triple infections.

4.1.1.6 Tolerance response

Tolerance, a host response identified by the presence and multiplication of virus determined serologically in the plant but with mild or no symptom expression (severity 1 – 2), was observed in IT98K-1092-1 to CMV under single infection and also in all co-infections with CMV. The same host response was also observed in IT99K-573-1-1 to single infection of SBMV (Table 4.8).

Table 4.8 Resistance classes of cowpea genotypes to BCMV - BlCM, SBMV and CMV infections obtained from mean 2009 and 2011 screen house evaluations, determined by disease severity and enzyme linked immunosorbent assay(ELISA)

Genotype	BCMV – BlCM			SBMV			CMV		
	DS	ELISA	Class	DS	ELISA	Class	DS	ELISA	Class
IT98K-133-1-1	2.7±0.5d	+++	S	1.0±0.1c	-	R	2.8±0.3bc	+	S
IT98K-1092-1	1.0±0.0e	-	R	1.0±0.0c	-	R	1.8±0.3d	++	T
IT97K-1069-6	2.3±0.5d	+++	S	1.1±0.1c	-	R	3.0±0.4bc	++	S
IT98K-503-1	4.7 ±2.7a	+++	HS	3.7±1.0b	+++	HS	3.5±1.0ab	++	HS
IT97K-1042-3	1.0±0.0e	-	R	1.0±0.0c	-	R	2.6±0.3c	++	S
IT04K-405-5	3.7±1.0c	+++	HS	1.0±0.0c	-	R	3.8±0.5a	++	HS
IT99K-1060	4.0±0.6bc	+++	HS	3.2±1.0b	++	HS	3.8±0.5a	++	HS
IT99K-573-1-1	3.7±0.6c	+++	HS	1.6±0.4c	++	T	3.2±0.5abc	+++	HS
Ife brown[b]	4.6±0.4ab	++	HS	4.5±0.4a	+++	HS	3.8±0.6a	+++	HS

BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; DS, disease severity (severity scale1-5: 1= No visible symptom and 5 = severe foliar symptoms); b, susceptible check; -, ELISA negative (overnight ELISA reading at 405nm Absorbance); +, ELISA positive (+, ≥ 2 x H; H represents absorbance value of healthy control); ++ = moderately positive (≥ 3 x H); +++ = highly positive (≥4 x H); R = resistant, T = tolerant; S = susceptible, HS = highly susceptible. Means followed by the same letter in each column are not significantly different (p = 0.01) according to Duncan's multiple range test.

Table 4.9 Resistance classes of cowpea genotypes to mixed infections of BCMV - BlCM, SBMV and CMV from mean 2009 and 2011 screen house evaluations, determined by disease severity and enzyme linked immunosorbent assay ELISA

Genotype	BCMV – BlCM + SBMV				BCMV – BlCM + CMV				SBMV + CMV				BCMV – BlCM + SBMV + CMV				
		ELISA				ELISA				ELISA				ELISA			
	DS	BCMV - BlCM	SBMV	Class	DS	BCMV - BlCM	CMV	Class	DS	SBMV	CMV	Class	DS	BICMV	SBMV	CMV	Class
IT98K-133-1-1	2.4±0.5c	-	-	R	2.6±0.5bc	+	++	S	3.0±0.9c	++	++	S	2.8±0.5bc	+	-	++	S
IT98K-1092-1	1.1±0.2d	-	-	R	1.8±0.3c	-	++	T	1.8±0.5d	-	+++	T	2.0±0.7c	-	-	+++	T
IT97K-1069-6	3.5±1.2b	++	-	S	4.4±0.9a	+	+++	HS	3.5±0.6c	-	+++	HS	4.3±0.8a	++	-	+++	HS
IT98K-503-1	4.0±0.9b	++	+++	HS	3.9±1.0a	+	+++	HS	4.6±0.5a	+++	++	HS	4.3±0.7a	+	+++	+++	HS
IT97K-1042-3	1.6±0.1d	-	-	R	4.3±0.7a	-	++	HS	4.3±0.8ab	-	++	S	4.3±1.1a	-	-	++	S
IT04K-405-5	3.7±0.9b	++	-	S	2.8±1.1b	+	+++	S	3.8±0.8bc	-	+++	S	3.2±0.6b	+	-	+++	HS
IT99K-1060	4.3±0.6ab	++	++	HS	4.8±0.3a	++	+++	HS	4.7±0.4a	+++	++	HS	4.9±0.1a	++	+++	++	HS
IT99K-573-1-1	3.6±0.3b	+++	+++	HS	4.2±0.6a	+	+++	HS	3.4±0.6c	+	++	S	4.2±0.4a	+	++	++	HS
Ife brown[b]	4.9±0.1a	++	+++	HS	4.8±0.2a	++	+++	HS	4.7±0.5a	+++	+++	HS	4.9±0.1a	++	+++	+++	HS

BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; DS, disease severity (severity scale 1-5: 1= No visible symptom and 5= severe symptoms), b = susceptible check, - = ELISA negative (overnight ELISA reading at 405nm Absorbance), + = ELISA positive (+, ≥ 2 x H; H represents absorbance value of healthy control), ++ = moderately positive (≥ 3 x H), +++ = highly positive (≥4 x H), R = resistant, T = tolerant, S = susceptible, HS = highly susceptible. Means followed by the same letter in each column are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

4.1.1.7 Interactions between viruses under co-infections

Virus-virus interaction was suspected in co-infection involving BCMV - BlCM and SBMV where different reactions were observed compared with single infections. Cowpea genotype IT98K-133-1-1 was observed to be susceptible to BCMV - BlCM under single inoculation but the virus was not detected serologically in the genotype under co-infection with SBMV. In the same cowpea genotype, resistance to SBMV under single infection was maintained under BCMV - BlCM + SBMV and BCMV - BlCM + SBMV + CMV but not under SBMV + CMV. In three cowpea lines, virus detected in one trial was not detectable in the other trial (Table 4.6). This was observed for only BCMV - BlCM in lines IT98K-503-1 and IT99K-1060 in all mixed infections except SBMV + CMV and in IT99K-573-1-1for BCMV - BlCM in BCMV - BlCM + CMV, SBMV in SBMV + CMV and both viruses in BCMV - BlCM + SBMV + CMV (Table 4.7).

4.1.1.8 Resistance classes of cowpea genotypes to BICMV, SBMV and CMV using disease severity scores as categorized by area under disease progress curves (AUDPC)

Disease severity scores taken over a period of eight WPI was analyzed by AUDPC and the result further confirmed the resistance status of cowpea lines. There was an agreement in the categorization of the cowpea resistance classes when based on AUDPC analysis and disease severity with ELISA in most of the cowpea genotypes, especially the highly resistant and susceptible ones. Under single infections (Table 4.10), IT99K-1060 and IT98K-503-1) were either susceptible or highly susceptible to the three viruses. The susceptibility of these lines to the three viruses, BCMV - BlCM, SBMV and CMV was evident from disease incidence and disease severity scores with ACP-ELISA. Two other lines (IT04K-405-5 and IT99K-573-1-1) were also categorized to be either susceptible or moderately susceptible to the three viruses. Results obtained from disease incidence, disease severity and serological test showing IT98K-1092-1 to be most resistant among the cowpea lines to the three viruses was also confirmed by AUDPC.

Under mixed infections (Table 4.11), line IT99K-1060 was either susceptible or highly susceptible to both double and triple infections of the three viruses. Line IT99K-573-1-1 was next in this category, showing susceptibility or moderate susceptibility to mixed infections while IT98K-503-1 showed susceptibility to BCMV - BlCM + SBMV and SBMV + CMV. Resistant genotypes were also observed to follow the same trend as in single inoculations.

Line IT98K-1092-1 was highly resistant to all the combinations of BCMV - BlCM, SBMV and CMV and IT97K-1042-3 was resistant to BCMV - BlCM + SBMV and susceptible to others.

AUDPC however, showed some limitations and could not effectively distinguish between tolerance and susceptibility of the cowpea lines. As a result, some differences were observed in the result obtained from resistance classification by AUDPC when compared with that of disease severity with ELISA and RT-PCR especially as pertain to IT98K-133-1-1, IT98K-503-1, IT97K-1069-6 and IT04K-405-5 in either single and mixed infections. For instance, susceptibility to CMV by all except one of the test lines and that of lines IT98K-133-1-1 and IT97K-1069-6 to BCMV - BlCM under single or mixed infections were not demonstrated by AUDPC analysis. Despite these limitations however, AUDPC analysis confirmed the host response of the susceptible and highly susceptible lines and the most resistant genotypes to the three viruses as obtained from virus disease incidence, severity, serological and RT-PCR virus detection methods used in determination of cowpea resistance status.

4.1.1.9 Effects of single and mixed viral infections on yield parameters of cowpea genotypes under screen house conditions

Effects of single and mixed infections of BCMV - BlCM, SBMV and CMV on yield parameters of cowpea genotypes under screen house conditions in 2009 and 2011 are presented in Tables 4.12 and 4.13 respectively. In IT98K-133-1-1, which is susceptible to CMV and BCMV - BlCM (Table 4.8), all the viral treatments caused significant reduction in the number of pods in 2009 trial but had no effect on pod length except SBMV + CMV. The viral treatments with the exception of SBMV produced reduction in weight of hundred seed while only BCMV - BlCM and SBMV + CMV reduced number of seeds. The 2011 trial showed significant reduction ($p < 0.01$) in pod length, seed per pod and total seed weight only by mixed infections especially those involving CMV. Only CMV in both single and mixed infections caused significant reductions in number of seeds per pod. Unlike the 2009 trial, no significant reduction was observed in number of pods and 100-seed weight in this genotype. The 2009 screening experiment revealed that in cowpea line IT98K-1092-1, with high resistances to BCMV - BlCM and SBMV and tolerance to CMV (Table 4.8), neither single nor mixed infections with the three viruses had significant effect on mean number of pods, pod length per plant and number of seeds per pod relative to the controls.

Table 4.10 Resistance classes of cowpea genotypes to single infections of BICMV, SBMV and CMV from mean disease severity scores of 2009 and 2011 screen house evaluations as categorized by area under disease progress curves (AUDPC)

Genotype	BCMV – BlCM			SBMV			CMV		
	AUDPC	D	Class	AUDPC	D	Class	AUDPC	D	Class
IT98K-133-1-1	12.70	-0.8	R	6.18	-3.0	HR	10.32	-2.3	HR
IT98K-1092-1	6.03	-3.0	HR	6.27	-2.3	HR	9.35	-3.0	HR
IT97K-1069-6	10.12	-1.5	R	6.82	-1.5	R	16.33	-0.8	S
IT98K-503-1	24.50	2.3	HS	16.32	2.3	HS	18.73	0.8	S
IT97K-1042-3	6.92	-2.3	HR	7.78	-0.8	R	15.65	-1.5	R
IT04K-405-5	17.72	0.0	MS	7.82	0.0	MS	19.67	2.3	HS
IT99K-1060	20.32	1.5	S	15.70	1.5	S	20.87	3.0	HS
IT99K-573-1-1	18.63	0.8	S	10.22	0.8	S	17.70	0.0	MS
Ife brown[b]	25.75	3.0	HS	21.37	3.0	HS	19.22	1.5	S

BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic Virus*, D = deviation from general mean of rank score = HR, highly resistant, R = resistant, MS = moderately susceptible, S = susceptible, HS = highly susceptible, b = susceptible check.

Table 4.11 Resistance classes of cowpea genotypes to mixed infections of BICMV, SBMV and CMV from mean disease severity scores of 2009 and 2011 screen house evaluations as categorized by area under disease progress curves (AUDPC)

Genotype	BCMV-BlCM + SBMV			BCMV-BlCM + CMV			SBMV+CMV			BlCM+SBM+CMV		
	AUDPC	D	Class	AUDPC	D	Class	AUDPC	D	Class	AUDPC	D	Class
IT98K-133-1-1	10.57	-1.5	R	11.85	-2.3	HR	15.35	-2.3	HR	10.32	-2.3	HR
IT98K-1092-1	6.22	-3.0	HR	8.93	-3.0	HR	8.85	-3.0	HR	8.68	-3.0	HR
IT97K-1069-6	15.42	-0.8	R	23.07	0.8	S	18.10	-1.5	R	23.10	0.0	MS
IT98K-503-1	19.12	1.5	S	19.53	-0.8	R	22.23	0.8	S	21.55	-0.8	R
IT97K-1042-3	9.67	-2.3	HR	24.23	2.3	HS	23.67	1.5	S	23.95	1.5	S
IT04K-405-5	18.05	0.0	MS	15.48	-1.5	R	18.17	-0.8	R	16.17	-1.5	R
IT99K-1060	22.02	2.3	HS	23.92	1.5	S	24.47	2.3	HS	25.02	2.3	HS
IT99K-573-1-1	18.17	0.8	S	22.60	0.0	MS	19.60	0.0	MS	23.33	0.8	S
Ife brown[b]	27.22	3.0	HS	26.27	3.0	HS	25.45	3.0	HS	27.25	3.0	HS

BCMV - BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; D = deviation from general mean of rank score, HR = highly resistant, R = resistant, MS = moderately susceptible, S = susceptible, HS = highly Susceptible, b = susceptible check.

However, viral treatments apart from BCMV - BlCM and BCMV - BlCM + SBMV caused reduction in the weight of hundred seed (Table 4.12). Similar reactions to viral infections was observed in pod number, number of productive peduncles, seed numbers, weight of 100 seed and total seed weight per plant in 2011(Table 4.13).

Results of 2009 trial showed that, in line IT97K-1069-6 which is susceptible to BCMV - BlCM and CMV but resistant to SBMV (Table 4.8), viral infection produced a significant reduction in the pod numbers with mixed infection involving CMV causing the highest reduction. Only the mixed infections involving CMV caused significant reduction in the remaining three yield parameters. In 2011 trial however, viral infections did not cause any significant reduction ($p < 0.01$) in number of productive peduncles, seed number and pod length except by BCMV - BlCM + CMV. There were reduction in pod numbers and total seed weight while only CMV and double infections involving CMV had significant reduction in weight of hundred seeds

In cowpea line IT98K-503-1 which was observed to be highly susceptible to the three viruses (Table 4.8), viral infections led to a significant ($p = 0.05$) reduction in pods number except for BCMV - BlCM + SBMV. However, only BCMV - BlCM caused reduction in pods length and seed numbers while BCMV - BlCM and SBMV reduced the weight of hundred seeds. In the 2011 trial however, both single and multiple infections with BCMV - BlCM, SBMV and CMV produced significant reductions in all the yield parameters measured. Meanwhile, there was no difference in the yield parameters by either single or mixed inoculations except for hundred seed weight, where single BCMV - BlCM and CMV infections induced lower yield parameters than other virus treatments (Table 4.12 and 4.13).

The 2009 screening revealed that in line IT97K 1042-3, observed to be resistant to BCMV - BlCM and SBMV and susceptible to CMV, mixed infections produced reduction in all the yield parameters studied than singles. Single infections could not reduce number of pods and pod length apart from CMV. In 2011 trial also, only mixed infections caused reduction in pod and seed numbers. However, there was a significant reduction in number of productive peduncles by the single and mixed inoculation and in total seed weight with the exception of BCMV - BlCM + SBMV. Most mixed inoculations involving CMV resulted in reduction in yield parameters.

The numbers of pods per plant, pod length with the exception of BCMV - BlCM + CMV infection and number of seeds per pod were not significantly different between all the virus treatments and the healthy control in line IT04K-405-5. However, significant reductions ($p < 0.01$) were observed in weight of 100 seed (Table 4.12). Results of 2011 trial also showed that unlike in mixed infections, single infection of BCMV - BlCM and SBMV could not reduce most of the yield parameters except for seed number and total seed weight. Similar to 2009 trial, significant reductions in weight of 100 seeds were produced by the viral treatments except for SBMV while significantly higher reduction was caused by CMV and co-infections involving CMV in most of the yield parameters.

In IT99K-1060 there was no significant difference between the infected and healthy cowpea in pod length except by the triple infection and number of seeds per pod in 2009 trial (Table 4.12). Meanwhile, SBMV and the mixed infections other than SBMV + CMV reduced pods number and all the viral treatments caused significantly smaller weight of hundred seeds. Significant reduction occurred between the viral treatments and the healthy controls in all the yield parameters measured in 2011. However, the same yield reduction was induced on IT99K-1060 by all viral inoculations, either single of mixed in all the yield parameters except 100-seedweight (Table 4.13).

The 2009 results indicated that for cowpea line IT99K-573-1-1, virus treatments other than BCMV - BlCM caused reduction ($p < 0.05$) in pod length while BCMV - BlCM + CMV and triple inoculation significantly ($p < 0.01$) reduced the weight of 100 seed (Table 4.12). Meanwhile, inoculation of the three viruses either singly or mixed did not result in any significant reduction in the number of pods per plant. The reduction in pod length and weight of 100 seed was confirmed by the 2011 trial. However, unlike in the 2009 trial, viral treatment produced reductions in number of pods per plant and that of seed per pod Reduction was also observed in the number of productive peduncles and total seed weight. Also, BCMV - BlCM + CMV and the triple infections resulted in similar reduction in all the yield parameters studies except in weight of hundred seeds. In Ife brown (susceptible check), almost all the viral treatments produced significant reductions in all the yield parameters studied in both trials. Total or near total yield losses were observed with greater reduction from mixed than single infections.

Table 4.12 Effects of single and mixed infections of BCMV - BlCM, SBMV and CMV on yield parameters of cowpea genotypes under screen house conditions in 2009

Genotype	Virus isolates	Pod no	Pod length (cm)	Seed no / pod	100 seed wgt (g)
IT98K-133-1-1	Healthy control	1.75±0.00a	14.15±0.00a	10.88±0.00a	16.00±0.00a
	BICMV	1.13±0.18b	15.12±0.17a	13.40±1.56a	9.50±0.70bc
	SBMV	1.13±o.18b	12.67±1.32a	11.19±1.15ab	13.56±3.57ab
	CMV	1.00±0.00b	14.29±0.33a	11.65±0.92ab	7.00 ± 0.00c
	BICMV+SBMV	1.25±0.00b	14.22±0.43a	9.25±0.21b	8.50±0.70bc
	BICMV+CMV	1.13±0.18b	13.42±1.61a	10.95±2.05ab	8.50±2.12bc
	SBMV+CMV	0.42±0.12c	2.62±1.11b	3.13±1.95c	7.60±5.01c
	BICM+SBMV+CMV	1.13±0.18b	11.93±3.74a	10.30±2.12ab	7.00±0.00c
IT98K-1092-1	Healthy control	2.25±0.00a	9.85±0.00a	7.71±0.00a	15.40±0.00a
	BICMV	1.63±0.18a	9.82±0.22a	9.94±0.85a	14.88±0.18ab
	SBMV	1.13±0.18a	8.99±0.78a	9.80±1.13a	10.00±0.00d
	CMV	1.33±0.18a	9.85±0.45a	10.47±2.17a	12.55±0.35bc
	BICMV+SBMV	2.25±0.71a	10.22±0.92a	10.49±0.82a	15.73±0.61a
	BICMV+CMV	1.50±0.71a	8.81±0.74a	8.23±0.32a	11.50±0.00cd
	SBMV+CMV	1.44±0.09a	8.86±0.04a	10.72±0.75a	12.08±0.81cd
	BICM+SBMV+CMV	1.38±0.18a	9.95±0.60a	10.72±0.75a	12.70±2.55bc
IT97K-1069-6	Healthy control	2.25±0.00a	14.47±0.00a	10.75±0.00a	17.60±0.00a
	BICMV	1.38±0.18b	12.07±0.45ab	6.40±0.85abc	12.00±0.00ab
	SBMV	1.25±0.00b	11.13±1.44abc	6.05±0.35abc	13.00±0.00ab
	CMV	1.38±0.18b	10.96±0.21abc	5.90±0.71abc	13.50±0.71ab
	BICMV+SBMV	1.25±0.35b	10.95±5.27abc	7.91±03.67ab	13.40±4.38ab
	BICMV+CMV	0.38±0.53c	3.67±5.19cd	2.63±3.71cd	4.13±5.83cd
	SBMV+CMV	0.56±0.09c	5.09±1.28bcd	4.56±0.97bcd	9.73±1.80bc
	BICM+SBMV+CMV	0.25±0.35c	2.75±3.88d	1.00±1.41d	1.60±2.26d
IT98K-503-1	Healthy control	3.33±0.00a	9.96±0.00a	6.17±0.00a	17.40±0.00a
	BICMV	0.75±1.00c	2.14±3.01b	1.49±2.11b	5.63±2.95b
	SBMV	1.38±0.18bc	6.83±0.32a	4.60±0.00a	8.50±0.71b
	CMV	1.75±0.35bc	6.77±0.69a	6.25±0.21a	10.00±0.00ab
	BICMV+SBMV	1.63±0.5bca	9.68±1.89a	6.60±2.12a	10.00±1.41ab
	BICMV+CMV	1.88±0.18bc	7.95±0.28a	6.95±1.34a	10.50±2.12ab
	SBMV+CMV	2.00±0.00b	9.03±0.92a	5.70±0.57a	11.50±0.71ab
	BICM+SBMV+CMV	2.00±0.35b	7.01±2.22a	5.70±0.57a	11.00±1.41ab
IT97K-1042-3	Healthy control	2.50±0.00a	12.52±0.00a	10.00±0.00a	16.50±0.00a
	BICMV	2.00±0.00a	12.92±0.30a	7.25±0.21b	13.00±1.41b
	SBMV	2.25±1.06a	12.77±1.12a	6.10±0.00c	12.50±2.12b
	CMV	2.13±0.53a	11.53±0.18b	4.45±0.21e	11.50±0.71b
	BICMV+SBMV	2.50±0.35a	10.67±0.06b	5.45±0.50d	12.00±1.41b

Table 4.12 Continued

Genotype	Virus isolates	Pod No	Pod length (cm)	Seed no / pod	100 seed wgt (g)
	BICMV+CMV	0.00±0.00b	0.00±0.00c	0.00±0.00f	0.00±0.00c
	SBMV+CMV	0.00±0.00b	0.00±0.00c	0.00±0.00f	0.00±0.00c
	BICM+SBMV+CMV	0.00±0.00b	0.00±0.00c	0.00±0.00f	0.00±0.00c
IT04K-405-5	Healthy control	1.00±0.00a	15.30±0.00a	12.75±0.00a	22.40±0.00a
	BICMV	1.06±0.27a	11.96±1.65a	10.06±0.57a	16.25±1.77abc
	SBMV	1.34±0.47a	14.30±0.35a	13.40±0.57a	14.50±4.94bcd
	CMV	1.02±0.03a	12.44±3.62a	12.13±4.10a	18.05±2.89ab
	BICMV+SBMV	1.25±0.18a	12.38±1.41a	9.81±1.14a	11.75±4.60bcd
	BICMV+CMV	0.75±0.35a	6.23±3.85b	5.60±4.10a	8.00±0.00d
	SBMV+CMV	0.75±0.35a	9.76±3.01ab	9.09±1.55a	14.65±1.92bcd
	BICM+SBMV+CMV	1.25±0.35a	9.95±0.77ab	11.60±1.56a	10.50±0.71cd
IT99K-1060	Healthy control	3.00±0.47a	9.92±0.83a	5.80±0.57a	20.00±1.41a
	BICMV	1.88±0.18ab	10.59±0.36a	4.50±0.71a	15.00±0.00b
	SBMV	1.63±0.18b	10.55±0 12a	4.85±1.34a	14.50±0.71bc
	CMV	2.00±0.71ab	9.57±0.18a	4.05±0.35a	13.50±0.71bc
	BICMV+SBMV	1.75±0.35b	7.87±2.49a	4.80±0.42a	13.50±0.71bc
	BICMV+CMV	1.63±0.18b	8.86±1.15a	4.40±0.57a	9.50±2.12cd
	SBMV+CMV	2.00±0.00ab	8.57±0.26a	4.40±0.57a	13.50±0.71bc
	BICM+SBMV+CMV	0.88±0.88b	3.35±0.92b	4.40±0.57a	5.50±4.95d
IT99K-573-1-1	Healthy control	1.88±0.18a	13.89±1.97a	6.15±0.71a	19.00±1.41a
	BICMV	1.63±0.53a	11.28±0.56ab	4.90±1.41ab	15.50±2.12a
	SBMV	2.00±0.35a	9.54±1.19bc	3.85±0.78b	16.00±0.00a
	CMV	1.38±0.18a	10.07±0.14bc	5.25±0.07ab	16.00±0.00a
	BICMV+SBMV	1.75±0.71a	10.40±0.15bc	6.05±0.35a	15.50±0.71a
	BICMV+CMV	1.75±0.71a	7.56±2.67c	4.65±0.92ab	11.00±1.41b
	SBMV+CMV	1.38±0.53a	10.71±0.32bc	4.10±0.14b	17.00±1.41a
	BICM+SBMV+CMV	1.38±0.88a	8.03±0.13c	4.10±0.14b	10.50±0.71b
Ife brown[b]	Healthy control	2.13±0.18a	9.81±0.36a	5.71±1.02a	16±0.42a
	BICMV	0.00±0.00c	0.00±0.00c	0.00±0.00b	0.00±0.00c
	SBMV	1.25±0.35b	5.53±0.82b	5.78±1.02a	10.18±1.95ab
	CMV	1.25±0.00b	6.83±0.14b	5.88±0.18a	12.63±0.18ab
	BICMV+SBMV	0.25±0.25c	2.25±3.18c	2.75±3.88ab	5.50±2.77bc
	BICMV+CMV	0.00±0.00c	0.00±0.00c	0.00±0.00b	0.00±0.00c
	SBMV+CMV	0.13±0.18c	0.47±0.66c	0.44±0.62b	2.15±2.04c
	BICM+SBMV+CMV	0.00±0.00c	0.00±0.00c	0.00±0.00b	0.00±0.00c

BCMV - BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; b = susceptible check. Means followed by the same letter in each column for each cowpea genotype are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

Table 4.13 Effects of single and mixed infections of BCMV - BlCM, SBMV and CMV on yield parameters of cowpea genotypes under screen house conditions in 2011

Genotype	Virus isolates	No productive Peduncles	Pod no	Pod length (cm)	Seed no / pod	100 seed wgt (g)	Total seed wgt (g)
IT98K-133-1-1	Healthy control	1.67±0.14a	1.75±0.00a	14.63±0.68a	12.07±0.97a	13.93±0.81a	2.51±0.54a
	BICMV	1.25±0.25a	1.83±1.83a	11.68±0.62ab	11.07±0.69ab	11.8±0.00a	1.77±0.66ab
	SBMV	1.50±0.50a	1.25±0.25a	12.46±1.91ab	9.31±2.09ab	10.85±2.61a	1.89±0.43ab
	CMV	1.17±1.14a	1.25±0.25a	11.96±0.67ab	8.13±1.21bc	9.60±0.00a	1.08±0.28bc
	BICMV+SBMV	1.33±0.14a	1.67±0.38a	14.35±1.28a	9.43±2.95ab	11.30±1.97a	1.65±0.62abc
	BICMV+CMV	0.83±0.14a	0.83±0.14a	7.26±0.38d	5.53±2.59c	10.83±1.97a	0.84±0.24c
	SBMV+CMV	1.33±0.52a	1.50±0.50a	10.74±0.86bc	8.43±0.61bc	9.93±1.25a	1.45±0.51bc
	BICM+SBMV+CMV	1.17±0.14a	1.25±0.09a	8.36±1.36cd	7.71±1.38bc	9.53±2.39a	1.24±0.36bc
IT98K-1092-1	Healthy control	2.08±0.14a	2.50±0.43a	8.78±0.69bc	8.90±0.87abcd	11.37±1.53a	2.05±0.45a
	BICMV	1.67±0.29a	2.17±0.38a	9.47±0.51ab	7.68±0.61cd	11.20±1.27a	1.68±0.16a
	SBMV	2.00±0.50a	1.42±0.14a	9.23±0.64abc	7.18±1.30d	11.25±2.76a	2.23±0.73a
	CMV	1.33±0.29a	1.42±0.14a	9.20±0.38abc	8.25±1.16bcd	11.15±0.35a	1.15±0.04a
	BICMV+SBMV	2.00±.50a	2.17±0.63a	10.18±0.75a	10.22±0.95ab	11.10±0.14a	2.46±0.72a
	BICMV+CMV	1.58±0.14a	1.75±0.25a	9.64±0.17ab	10.61±0.38a	12.17±0.87a	1.67±0.43a
	SBMV+CMV	1.25±0.00a	1.50±0.25a	9.53±0.63ab	10.96±1.81a	10.77±0.86a	1.55±0.46a
	BICM+SBMV+CMV	1.58±0.58a	1.92±0.72a	8.31±0.47c	9.53±0.92abc	9.60±1.68a	1.69±0.87a
IT97K-1069-6	Healthy control	1.97±0.71a	2.50±0.66a	12.86±0.39a	10.05±1.38a	15.63±0.45a	2.68±0.87a
	BICMV	1.25±0.25a	1.58±0.38b	11.84±1.41a	6.61±3.31a	13.50±0.90ab	1.32±0.58b
	SBMV	1.42±0.14a	1.42±0.38b	12.89±0.89a	6.43±1.50a	13.57±0.99ab	1.58±0.41b
	CMV	1.41±0.38a	1.42±0.38b	11.40±0.73a	6.63±1.33a	12.10±1.99bc	1.19±0.43b
	BICMV+SBMV	1.25±0.25a	1.42±0.14b	10.03±1.63a	6.92±1.47a	11.15±0.35bc	1.41±0.22b
	BICMV+CMV	1.00±0.00a	1.00±0.00b	6.43±2.15b	5.08±1.70a	10.35±0.21c	0.68±0.19b
	SBMV+CMV	1.08±0.63a	1.17±0.76b	9.95±1.74a	5.26±1.21a	11.10±1.13bc	0.83±0.54b
	BICM+SBMV+CMV	1.97±0.71a	1.42±0.58b	12.86±0.39a	7.85±1.40a	15.63±0.45a	1.43±0.43b
IT98K-503-1	Healthy control	1.75±0.25a	2.08±0.14a	11.28±0.28a	9.05±0.69a	14.03±1.97a	1.71±0.23a
	BICMV	0.50±0.25b	0.92±0.52b	3.81±1.91b	3.03±1.29b	4.90±0.00c	0.37±0.24b
	SBMV	0.83±0.39b	0.42±0.52b	3.28±1.79b	1.38±0.59b	10.40±0.00b	0.37±0.16b
	CMV	0.42±0.52b	0.42±0.52b	2.08±2.43b	0.97±1.68b	4.60±0.00c	0.17±0.25b
	BICMV+SBMV	0.50±0.43b	0.50±0.43b	2.09±1.84b	1.33±1.21b	9.20±0.00b	0.51±0.14b
	BICMV+CMV	0.58±0.28b	0.75±0.43b	3.61±1.11b	2.75±1.81b	7.97±3.25b	0.35±0.24b
	SBMV+CMV	0.75±0.43b	0.75±0.43b	3.83±1.11b	2.67±1.23b	9.30±0.00b	0.38±0.24b
	BICM+SBMV+CMV	0.58±0.14b	0.75±0.25b	2.50±0.56b	1.93±0.37b	10.20±0.00b	0.40±0.19b
IT97K-1042-3	Healthy control	2.17±0.38a	2.67±0.14a	12.94±0.36a	8.22±0.81ab	11.97±1.17a	1.98±0.14a
	BICMV	1.58±0.14bc	2.25±o.50a	11.37±1.69ab	6.73±1.69abcd	11.80±0.00a	1.28±0.22c
	SBMV	1.67±0.14b	1.58±0.2ab	11.23±1.10ab	6.58±1.95abcd	10.10±1.00b	1.50±0.09bc
	CMV	1.33±0.14bc	1.58±0.2ab	9.53±2.20bc	5.60±1.21bcd	9.45±0.05b	0.95±0.14d
	BICMV+SBMV	1.58±0.29bc	2.33±0.29a	11.03±0.59ab	8.83±0.15a	10.47±0.31ab	1.68±0.21ab

Table 4.13 Continued

Genotype	Virus isolates	No productive Peduncles	Pod no	Pod length (cm)	Seed no / pod	100 seed wgt (g)	Total seed wgt (g)
	BICMV+CMV	0.58±0.14e	0.67±0.29c	6.28±0.57d	4.08±1.38d	10.85±1.85ab	0.45±0.13e
	SBMV+CMV	1.17±0.29de	1.17±0.29bc	10.50±1.59ab	7.79±1.09abc	9.40±0.00b	0.86±0.25d
	BICMV+SBMV+CMV	0.83±0.29de	1.08±0.29bc	6.99±3.42cd	5.04±2.62cd	9.90±0.050b	0.51±0.13e
IT04K-405-5	Healthy control	1.67±0.14ab	2.00±0.25a	15.73±1.41a	13.38±3.67a	20.23±1.02a	3.68±0.59a
	BICMV	1.75±0.25a	2.08±0.38a	13.40±2.41abc	8.34±3.28bc	14.83±0.65b	2.30±0.23bc
	SBMV	1.67±0.14ab	1.58±0.14ab	14.51±2.90ab	10.75±2.47abc	18.60±0.35a	2.50±0.27b
	CMV	1.33±0.14bc	1.58±0.14ab	11.03±1.28c	7.17±0.42c	10.93±0.81d	1.58±0.39bcd
	BICMV+SBMV	1.33±0.14bc	1.58±0.38ab	13.06±0.87abc	11.79±2.19ab	13.57±2.00bc	1.87±0.13bcd
	BICMV+CMV	1.08±0.29c	1.67±0.38b	10.66±1.66c	9.21±2.32bc	10.50±0.14d	1.03±0.45d
	SBMV+CMV	1.17±0.14c	1.42±0.29b	12.97±2.00abc	10.65±2.20abc	12.33±0.82cd	1.39±0.25cd
	BICMV+SBMV+CMV	1.17±0.14c	1.42±0.29b	11.23±0.52bc	8.25±1.63bc	13.30±0.69bc	1.65±1.09bcd
IT99K-1060	Healthy control	2.17±0.63a	2.33±0.88a	10.90±0.57a	8.18±1.53a	13.17±1.05a	1.95±0.37a
	BICMV	0.50±0.43b	1.00±0.25b	1.85±1.22b	0.36±0.61b	5.10±1.00cd	0.16±0.13b
	SBMV	0.92±0.29b	0.75±0.25b	5.51±1.19b	3.10±0.95b	9.75±0.45ab	0.42±0.11b
	CMV	0.75±0.25b	0.75±0.25b	3.39±1.73b	2.21±0.91b	9.20±0.00b	0.32±0.09b
	BICMV+SBMV	0.58±0.52b	0.58±0.52b	3.48±3.03b	2.62±2.29b	5.40±4.68cd	0.37±0.33b
	BICMV+CMV	0.42±0.38b	0.42±0.38b	3.31±2.92b	2.41±2.18b	7.20±0.0bc	0.18±o.15b
	SBMV+CMV	0.25±0.25b	0.25±0.25b	1.78±1.59b	1.50±1.30b	9.60±0.00ab	0.17±0.15b
	BICMV+SBMV+CMV	0.25±0.25b	0.25±0.25b	1.83±3.17b	1.67±2.89b	3.27±0.00d	0.17±0.29b
IT99K-573-1-1	Healthy control	2.00±0.25a	2.67±0.58a	14.34±1.67a	9.82±2.64a	20.77±2.48a	3.12±0.58a
	BICMV	0.70±0.90bc	1.67±0.29b	5.59±5.70bc	2.89±3.72b	15.20±0.00b	0.95±1.11bc
	SBMV	1.58±0.38ab	0.92±0.29bc	8.61±2.88b	4.50±2.47b	15.07±1.20b	1.37±0.13b
	CMV	0.83±0.14bc	0.92±0.29bc	6.05±2.53bc	3.78±1.48b	11.40±0.00c	0.75±0.16bc
	BICMV+SBMV	1.00±0.50bc	1.25±0.90bc	8.34±1.98b	4.58±1.00b	15.53±1.00b	1.18±0.50bc
	BICMV+CMV	0.33±0.38c	0.42±0.38c	1.05±1.31c	0.83±1.04b	10.80±0.00c	0.22±0.19c
	SBMV+CMV	0.92±o.29bc	1.50±0.90b	5.82±1.06bc	4.00±0.33b	13.83±2.20b	1.03±73b
	BICMV+SBMV+CMV	0.67±0.29c	0.92±0.38bc	3.64±2.62bc	2.75±1.84b	14.10±0.00b	0.73±0.48bc
Ife brown[b]	Healthy control	1.92±0.38a	2.17±0.63a	9.69±0.62a	8.50±0.33a	14.60±1.10a	2.09±0.47a
	BICMV	0.25±0.25b	1.83±0.80ab	1.13±1.00c	0.67±1.15d	11.60±0.00b	0.13±0.12c
	SBMV	1.17±0.29b	0.83±0.63bc	4.97±1.87b	3.55±1.10bc	10.10±0.00bc	0.88±0.20b
	CMV	0.75±0.50bc	0.83±0.63bc	2.80±2.20bc	2.11±1.20bcd	6.50±0.00d	0.44±0.42bc
	BICMV+SBMV	0.25±0.25c	0.25±0.30c	2.31±2.20bc	1.75±1.56cd	8.80±0.00cd	0.16±0.14c
	BICMV+CMV	0.50±0.43bc	0.58±0.57c	3.16±2.19bc	2.50±1.95bcd	9.10±0.00cb	0.47±0.46bc
	SBMV+CMV	1.00±0.43b	1.25±0.66abc	4.78±0.61b	4.38±0.33b	9.47±0.50cb	1.09±0.46b
	BICMV+SBMV+CMV	0.58±0.38bc	1.00±0.75abc	3.58±2.12bc	3.21±1.70bc	9.56±0.64cb	0.60±0.43bc

*BCMV - BlCM, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; b, susceptible. Means followed by the same letter in each column for each cowpea genotype are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

4.1.1.10 Correlation coefficients (r) among disease incidence, severity and yield parameters of BCMV - BlCM, SBMV and CMV infected cowpea genotypes under screen house conditions in 2011

Correlation coefficients between disease incidence, severity and some yield parameters of cowpea lines following infections with BCMV - BlCM, SBMV and CMV in screen house experiments are shown in Tables 4.14 – 4.16. Generally, disease severity was highly positively correlated with incidence in BCMV - BlCM, SBMV and CMV infections. A significant and positive relationship existed between pod length and number of productive peduncles as well as between number of seeds and that of pods while a high negative correlation was observed between symptom severity and number of productive peduncles as well as number of seeds per pod in the three single viral infections.

Infection severity correlated negatively with all the seed yield parameters evaluated except 100-seed weight under BCMV - BlCM inoculated plants. Hundred seed weight was only significantly ($p \leq 0.05$) and positively correlated with pod length ($r = 0.71$) in SBMV infected plants and also with number of productive peduncles ($r = 0.76$) and number of pods per plant ($r = 0.71$) in CMV inoculated cowpea lines. Total seed weight was highly positively correlated with all the seeds yield parameters studied in the three viral inoculations.

In BCMV - BlCM inoculated cowpea genotypes, relationship between disease incidence and all the yield parameters evaluated was not significant. A highly positive ($r = 0.92$) and significant ($p \leq 0.001$) correlation was observed between seed number per pod and pod length. Seed number per pod also correlated positively with the number of pods and of productive peduncles and negatively ($r = -0.75$) with the severity of BCMV - BlCM infections (Table 4. 14). High negative correlations between both virus incidence and severity and all the yield parameters studied were observed, with the exception of 100-seed weight when plants were infected with SBMV. All the yield parameters were positively correlated except between number of seeds per pod and number of productive peduncles per plant. The correlation among them was significant except for 100-seed weight where significant relationship existed only with pod length (Table 4.15). Relationships between incidence and symptom severity in CMV infected plants in many of the yield parameters were not significant. However, significant ($p \leq 0.05$) negative correlations were observed only between CMV severity and number of productive

peduncles, number of seeds per pod and total seed weight. Although negative correlation (r = -0.71) was observed between the total seed weight and infection severity in CMV infected plants, total seed weight correlated positively and significantly with all the yield parameters evaluated (Table 4.16).

4.1.2 Screening of cowpea for resistance to viruses under natural field infection in 2010

4.1.2.1 Virus symptomatology

No visible virus symptom was observed on all the cowpea plants on the field in 2010 trial. Also, few aphids (*Aphis spp*) and other cowpea insect pests especially the pod sucking bugs such as *Anoplocnemis spp* and *Clavigralla spp*. were observed on the field despite weekly chemical sprays. Latent infection was however observed in Ife brown and IT99K-1060.

4.1.2.2 Virus detection

Results of the asymptomatic samples subjected to ACP-ELISA 8 WAP showed detection of CMV and CPMoV in only Ife brown and IT99K-1060. Single infection of CPMoV was observed in Ife brown samples while co-infection of CMV and CPMoV was detected in other Ife brown and IT99K-1060 samples. Thus, serological test for the presence of eight cowpea viruses found in Nigeria (BCMV - BlCM, SBMV, CMV, CABMV, CPMoV, CYMV, CPMMV and BPMV) showed very low natural field viral infection in 2010.

4.1.2.3 Effect of natural field viral infections on yield parameters of cowpea genotypes during 2010

Yield parameter data were significantly different among the cowpea lines (Table 4.17). Lines IT98K-133-1-1, IT98K-1092-1 and IT04K-405-5 produced significantly ($p < 0.05$) higher number of productive peduncles, number of seeds per pod and weight of 100 seed than other genotypes. Lines IT98K-133-1-1 and IT98K-1092-1 produced the highest seed weight per hectare. Line IT98K-1092-1, a more resistant genotype, had the highest values of yield components in five of the six parameters measured but had a shorter pod length while another resistant line IT97K-1042-3 had significantly lower yield trait values than others except in weight of 100 seed. IT99K-1060 and Ife brown, which are highly

Table 4.14 Correlation coefficients (r) among virus disease incidence, severity and yield parameters of cowpea genotypes infected with BCMV - BlCM under screen house conditions in 2011

	Incidence (%)	Severity (1-5)	No prod peduncle[a]	Pod no / plant	Pod length (cm)	seed no / pod	100-seed weight (g)	Total seed weight / plant (g)
Incidence	-							
Severity	0.85**	-						
No prod Peduncles	-0.61ns	-0.86**	-					
Pod no / Plant	-0.61ns	-0.77**	0.72**	-				
Pod length (cm)	-0.38ns	-0.78*	0.93***	0.63*	-			
Seed no / Pod	-0.36ns	-0.75*	0.85**	0.60*	0.92***	-		
100-seed weight (g)	-0.19ns	-0.43ns	0.50ns	0.74*	0.59*	0.45ns	-	
Total seed Weight /plant (g)	-0.32ns	-0.69*	0.92***	0.69*	0.94***	0.93***	0.64*	-

[a]No prod peduncles, number of productive peduncles

*, **, *** significant at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001 respectively; ns, not significant

Table 4.15 Correlation coefficients (r) among virus disease incidence, severity and yield Parameters of cowpea genotypes infected with SBMV under screen house conditions in 2011

	Incidence (%)	severity (1-5)	No prod peduncle[a]	Pod no / plant	Pod length (cm)	seed no / pod	100-seed weight (g)	Total seed weight / plant (g)
Incidence	-							
Severity	0.96***	-						
No prod Peduncles	-0.88**	-0.91**	-					
Pod no / Plant	-0.96***	-0.92***	0.82**	-				
Pod length (cm)	-0.88**	-0.90**	0.68*	0.89**	-			
Seed no / Pod	-0.88**	-0.85**	-0.72*	0.86**	0.93**	-		
100-seed weight (g)	-0.51ns	-0.64ns	0.57ns	0.61ns	0.71*	0.67ns	-	
Total seed Weight/plant (g)	-0.92***	-0.91**	0.89**	0.86**	0.85**	0.93***	0.71*	-

[a]No prod peduncles, number of productive peduncles.

*, **, *** significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively; ns, not significant

Table 4.16 Correlation coefficients (r) among virus disease incidence, severity and yield parameters of cowpea genotypes infected with CMV under screen house conditions in 2011

	Incidence (%)	Severity (1-5)	No prod peduncle[a]	Pod no / plant	Pod length (cm)	seed no / pod	100-seed weight (g)	Total seed weight/ plant (g)
Incidence	-							
Severity	0.75*	-						
No prod Peduncles	-0.50ns	-0.69*	-					
Pod no / Plant	-0.56ns	-0.68ns	0.98***	-				
Pod length (cm)	-0.36ns	-0.65ns	0.92***	0.90***	-			
Seed no / Pod	-0.50ns	-0.81*	0.90**	0.87**	0.94***	-		
100-seed weight (g)	-0.30ns	-0.68ns	0.76*	0.71*	0.68ns	0.68ns	-	
Total seed weight (g)	-0.38ns	-0.71*	0.90**	0.90**	0.92***	0.90**	0.73*	-

[a]No prod peduncles, number of productive peduncles,

*, **, *** significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively; ns, not significant

Table 4.17 Yield parameters of cowpea genotypes under natural field virus infections in 2010

Genotype	No productive peduncle/ plant	Pod no / Plant	Pod length /plant (cm)	seed no /pod	100 seed wgt (g)	Total Seed wgt (Kg/ha)
IT98K-133-1-1	11.88abc	18.31bcd	16.89ab	13.23a	7.32abc	1358.20a
IT98K-1092-1	12.63ab	24.19a	11.29f	12.58ab	6.93abc	1305.00a
IT97K-1069-6	9.13d	14.69d	17.10a	11.73b	6.51abc	557.50bc
IT98K-503-1	11.94abc	21.50ab	14.88d	9.89c	7.66ab	604.80bc
IT97K-1042-3	9.88cd	16.75cd	15.98bc	9.18cd	7.15abc	472.11c
IT04K-405-5	13.00a	20.00abc	15.46cd	13.17a	7.86a	729.76b
IT99K-1060	9.81cd	16.94cd	12.58e	8.35de	6.53abc	524.00bc
IT99K-573-1-1	10.75bcd	22.25ab	15.96bc	7.22e	6.27bc	266.87d
Ife brown*	10.75bcd	20.31abc	12.31e	8.44de	5.80c	508.01c
±S.E.	0.68	1.36	0.35	0.48	0.48	220.32

*Susceptible control, Means followed by the same letter in each column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

susceptible lines, showed significantly lower yield parameters than all others while IT98K-503-1 (another susceptible line) produced low to moderately high yield data.

4.1.3 Screening of cowpea for resistance to viruses under natural field infection in 2011

4.1.3.1 Virus symptomatology, incidence and severity

Infestation of insect vectors was observed on cowpea plant test lines in the field. Aphids (*Aphis spp*), different types of leaf beetles, large population of white flies (*Bemisia tabaci*) and some other cowpea pests were observed on the field. Also, natural field transmission of viruses occurred and infection symptoms were observed (Plates 4.7 and 4.8). Mild to very severe symptoms of virus infections were observed on the field in some of the more susceptible genotypes notably IT98K-133-1-1, IT98K-503-1, IT99K-1060, IT99K-573-1-1and Ife brown.

Although, most of the more resistant genotypes (IT98K-1092-1, IT97K-1042-3 and IT04K-405-5) were symptomless, mild to moderate infection symptoms were observed on some of them. Despite the fact that only mild to moderate symptoms were noticed on some genotypes, none of the nine genotypes was symptomless and very severe symptoms were observed on Ife brown and IT99K-1060. The symptoms observed ranged from mild mottling, mosaic, puckering, vein banding and midrib to veinal chlorosis (Plate 4.7). These progressed and resulted in reduced leaf area, stunted growth and necrosis in the highly susceptible lines. Unlike in the artificially inoculated screen house plants, wilting or death of leaves or whole plants was not observed on the field.

Incidence of virus infection differed significantly among the cowpea lines (Figure 4.1). Incidence was significantly ($p < 0.01$) higher in the more susceptible lines such as Ife brown (91.7%), IT98K-503-1 (89.6%) and IT99K-573-1-1 (84.6%) than in the less susceptible lines. Between 42.4% and 65.0% incidence of viral infections were observed among five genotypes namely; IT97K-1069-6, IT97K-1042-3, IT98K-133-1-1, IT04K-405-5 and IT99K-1060. However, incidence of viruses was significantly lower (33.0%) in IT98K-1092-1, a more resistant cowpea line.

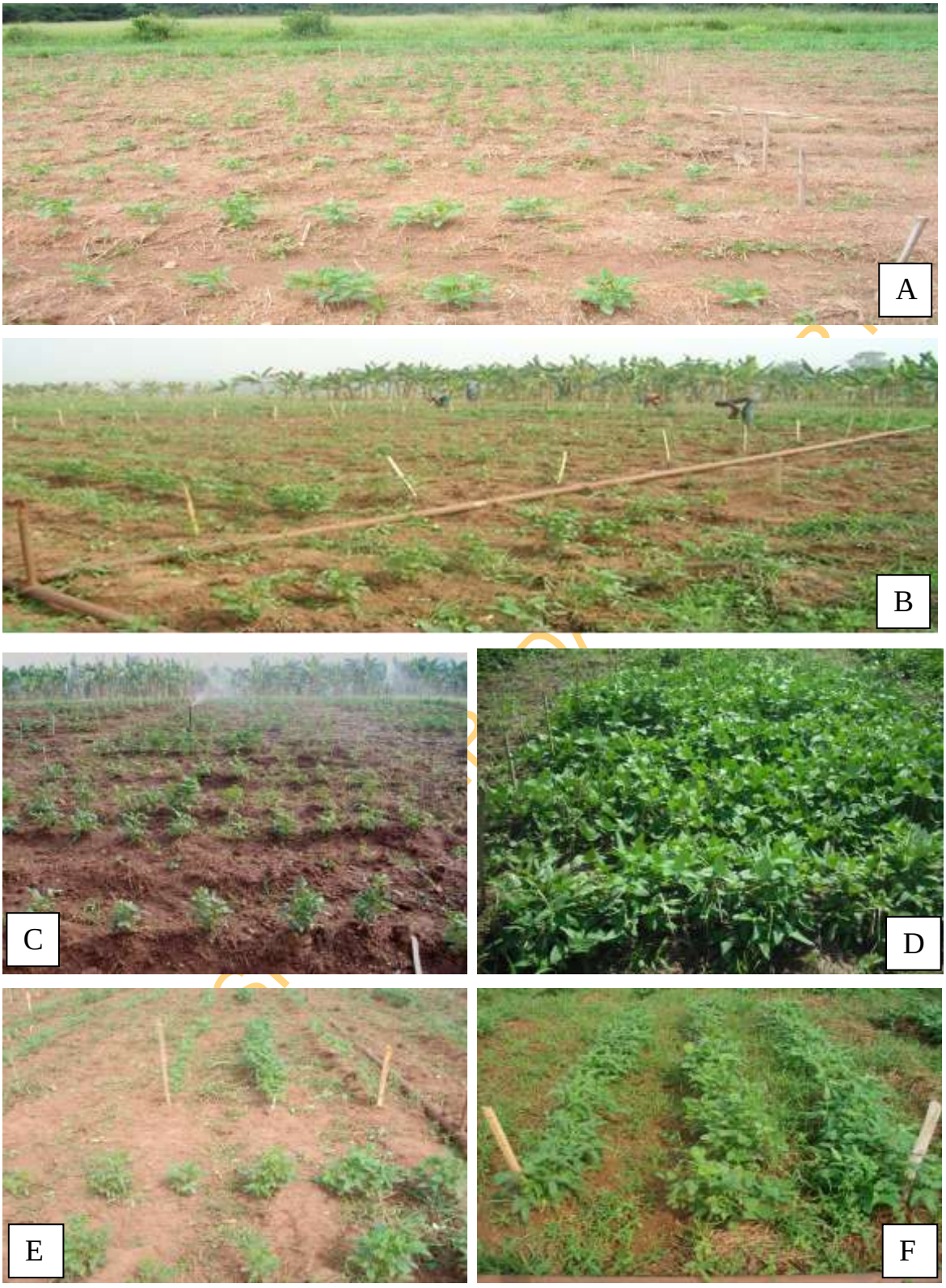

Plate 4.7 Cowpea plants under natural field virus infections. A= First field trial (2010), 3 Weeks After Planting (WAP), B and C = Irrigated cowpea fields of 2nd trial 3 WAP, D = First field trial 8 WAP, E = 2nd field trial showing Ife brown infector rows (middle) and border crops 3 WAP, F = 2nd field trial showing (infector) row in between rows of cowpea line IT97K-1042-3 5 WAP

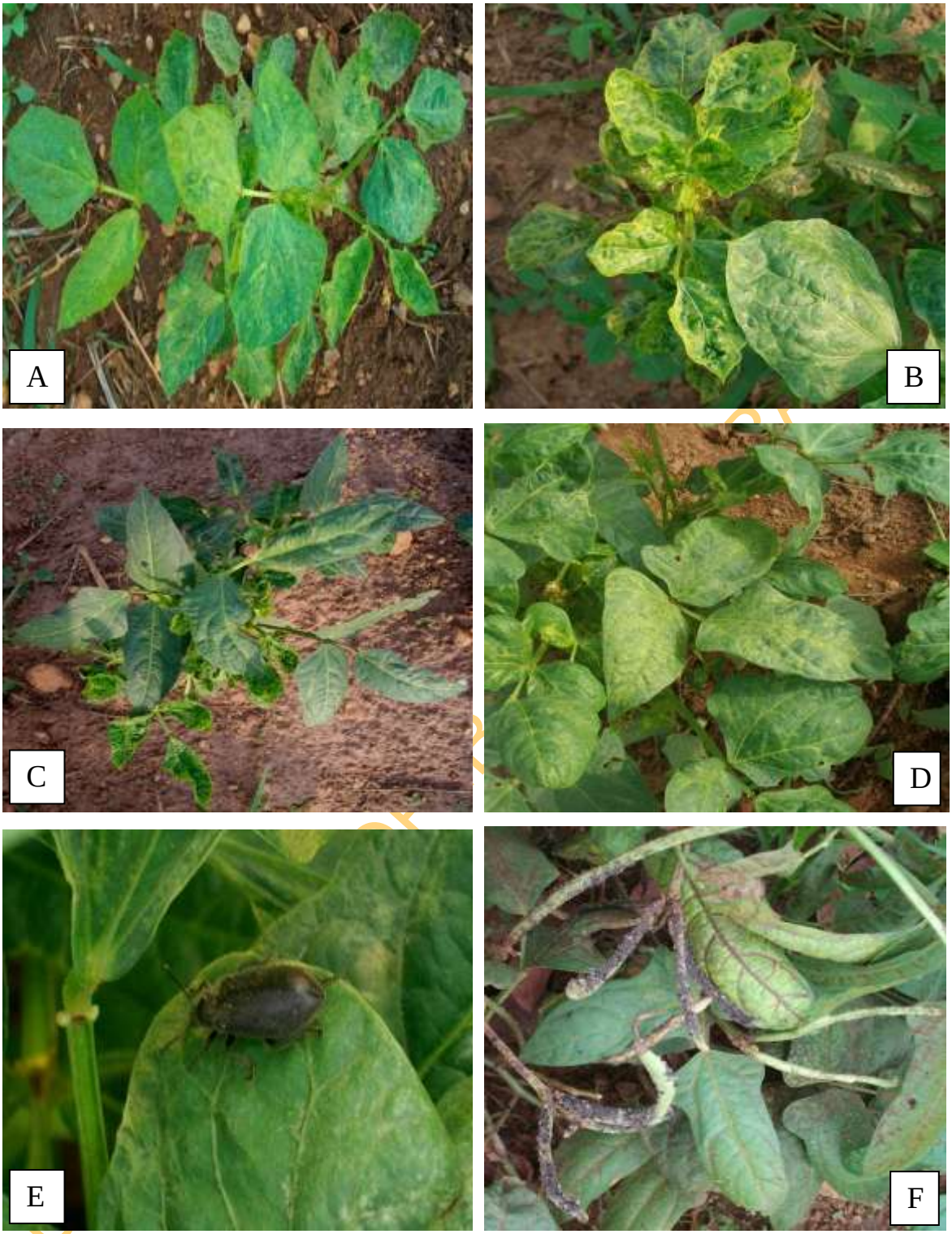

Plate 4.8 Symptoms induced by viruses on cowpea under natural field infections and insect vectors infestation. A = Ife brown showing symptoms of mixed infections of *Cowpea aphid-born mosaic* (CABMV), Bean common mosaic (BCMV-BlCM), *Cucumber mosaic and Cowpea mild mottle* (CPMMV) *viruses*, B = Ife brown with symptoms induced by CABMV, BCMV-BlCM, *Cowpea mottle virus* (CPMoV), *Southern bean mosaic virus* (SBMV) and CPMMV, C = Cowpea line IT99K-1060 infected with CPMoV, SBMV and CPMMV, D = Ife brown with CABMV and BCMV-BlCM, E = Foliage beetle on Ife brown and F = Aphids on cowpea line IT97K-1042-3

Severity of virus infections was also significantly ($p < 0.01$) different among the evaluated cowpea genotypes (Figure 4.1). Among the susceptible genotypes, similar infection severity was observed between IT98K-503-1 (3.9±0.1) and Ife brown (3.6±0.1). Infection severities were higher in the two lines than that of IT99K-1060 (3.3±0.3) and IT99K-573-1-1 (2.9±0.4). Five genotypes showed lower symptom severity that ranged of between 2.0±0.2 and 2.2±0.1 while in IT98K-1092-1, a more resistant genotype, symptom severity was significantly lower (1.8±0.2).

4.1.3.2 Virus detection and resistance evaluation

From the serologically tested symptomatic and asymptomatic samples assayed for eight viruses associated with cowpea in Nigeria (BCMV - BlCM, SBMV, CMV, CABMV, CPMoV, CYMV, CPMMV and BPMV), only CYMV was not detected on the cowpea lines on the field (Table 4.18). None of the genotypes was singly infected and the mixed viral infections observed naturally from field ranged from 2 to 7 viruses per cowpea genotype. BCMV - BlCM was detected on five genotypes (IT98K-133-1-1, IT97K-1069-6, IT04K-405-5, IT99K-1060 and Ife brown) in 3.6 % of the total (222) samples tested, SBMV in four (IT98K-503-1, IT99K-1060, IT99K-573-1-1 and Ife brown) in 5 % of the tested samples and CMV in two (IT97K-1069-6 and Ife brown) lines in 1.4 % of the tested samples (Tables 4.18).

However, high (44.6 %) occurrence of CPMMV was observed on the field. CPPMV was detected in all the genotypes except IT98K-133-1-1, but highest occurrence was found in highly susceptible genotypes (IT99k- 1060, IT98K-503-1 and Ife brown) and also in IT97K-1042-3 and IT04K-405-5. Occurrences of CABMV, CPMoV and BPMV were observed in four genotypes each. From the eight viruses tested for, highest number (7) of viruses was detected in Ife brown which was followed by IT98K-503-1 (5), IT04K-405-5 (4), IT99K-1060 (4), IT97K-1069-6 (3), IT99K-573-1-1 (3), IT98K-133-1-1, IT98K-1092-1, (2) and IT97K-1042-3 (2).

4.1.3.3 Detection of latent infections

ACP-ELISA results were positive in all symptomatic plants including Ife brown. However, some latent infections were observed. For instance, most of the CPMMV infections observed, especially those detected in the genotypes resistant to most of the

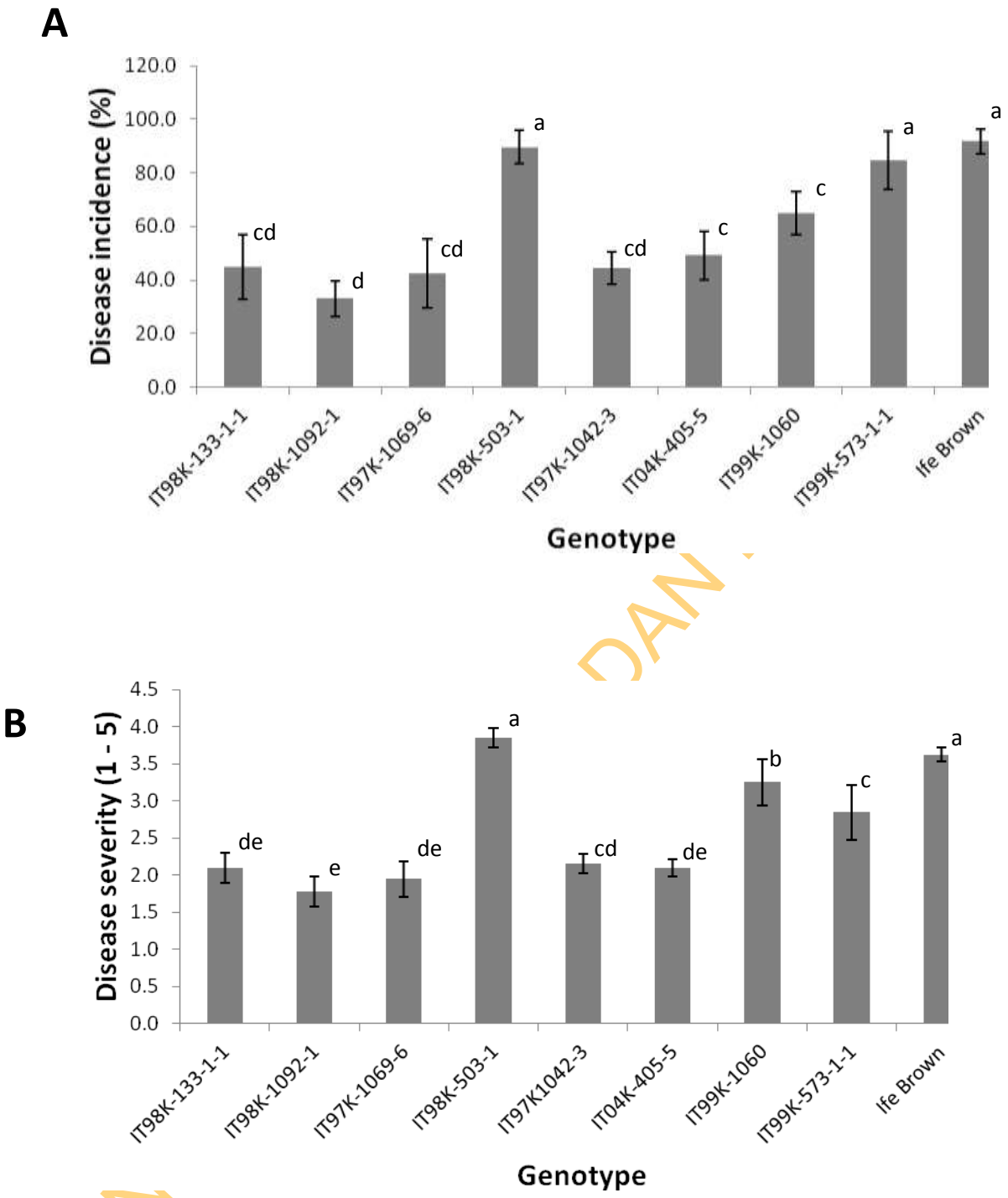

Figure 4.1 Incidence and severity of virus diseases in cowpea genotypes under natural field viral infections in 2011: A = disease incidence; B = disease severity

4.18 Virus detection in cowpea genotypes under natural field infections Using enzyme -linked immunosorbent assay (ELISA) in 2011

Genotype	N	No of ELISA positive plants per virus								No virus
		BICMV	SBMV	CMV	CABMV	CPMoV	CYMV	CPMMV	BPMV	detected
IT98K-133-1-1	24	1	0	0	0	0	0	0	2	2
IT98K-1092-1	21	0	0	0	1	0	0	1	0	2
IT97K-1069-6	22	2	0	0	1	0	0	5	0	3
IT98K-503-1	28	0	3	2	0	4	0	22	3	5
IT97K-1042-3	19	0	0	0	0	3	0	18	0	2
IT04K-405-5	21	2	0	0	2	0	0	15	1	4
IT99K-1060	30	1	4	0	0	4	0	24	0	4
IT99K-573-1-1	28	0	2	0	0	2	0	2	0	3
Ife brown cv.	29	2	2	1	2	2	0	12	2	7
Total	222	8	11	3	6	15	0	99	8	32
%		3.6	5.0	1.4	2.7	6.8	0	44.6	3.6	44.4

BICMV Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*, CAbMV, *Cowpea aphid-borne mosaic virus*; CPMoV, *Cowpea mottle virus*; CYMV, *Cowpea yellow mosaic virus*; CPMMV, *Cowpea mild mottle virus*; and BPMV ,*Bean pod mottle virus;* N = total number of plant samples assayed by ELISA

CPMMV infections observed, especially those detected in the genotypes resistant to most of the viruses, were latent. Similarly, latent infection of CMV was observed in IT98K-503-1 and that of BPMV in IT04K-405-5 and Ife brown

4.1.3.4 Effect of natural field viral infections on yield parameters of cowpea genotypes during 2011

Line IT98K-133-1-1 produced significantly ($p < 0.01$) higher number of productive peduncles, pods per plant, pod length, seed number per pod and seed yield per hectare than most of the other genotypes. Similar pod length was produced by IT97K-1069-6 as well as number of seeds per pod by IT04K-405-5. Line IT04K-405-5 had higher ($p \leq 0.01$) weight of 100 seed than other lines with the exception of IT99K-573-1-1 (Table 4.19). The three more susceptible genotypes namely Ife brown, IT99K-1060 and IT 98K 503-1 produced lower yield than others. IT98K-1092-1, a more resistant line, produced moderate number of productive peduncle, pod number per plant and seed number per pod which were significantly lower than that from IT98K-133-1-1. However, low to moderate value of parameters measured characterized IT97K-1042-3 despite its resistance to BCMV - BlCM and SBMV.

4.1.3.5 Correlation coefficients (r) among disease incidence, severity and yield parameters of cowpea genotypes under natural field infections in 2011

Table 4.20 shows the correlations among disease incidence, disease severity and the yield parameters of cowpea genotypes under natural field conditions in 2011. Disease severity of the viruses was observed to be highly (0.93) correlated with incidence of viral diseases. There were significantly negative correlations between disease incidence, with number of productive peduncles and number of pods per plant. Similar trends were observed between disease severity and number of productive peduncles, number of pods per plant and total seed weight. Weight of 100 seeds did not correlate significantly with all other parameters measured. Similarly, number of seeds per pod showed no significant relationship with other parameters except in number of productive peduncles (0.72).

Table 4.19 Yield parameters of cowpea genotypes under natural field virus infections in 2011

Genotype	No productive Peduncles/ plant	Pod No / Plant	Pod length (cm)	Seed No/ Plant	100 seed wgt (g)	Total seed wgt (kg/ha)
IT98K-133-1-1	22.56a	34.00a	17.67a	16.03a	13.77cd	1529.30a
IT98K-1092-1	16.38b	29.44b	10.82f	12.96b	14.41cd	1104.40d
IT97K-1069-6	17.63b	29.13b	17.36a	13.68b	13.68cd	1354.70b
IT98K-503-1	12.00c	21.13d	12.35e	11.12de	13.01d	931.40e
IT97K-1042-3	16.81b	27.00bc	13.63d	11.89cd	15.29bc	1141.70cd
IT04K-405-5	16.25b	27.00bc	15.87c	15.38a	17.09a	1238.10c
IT99K-1060	13.81c	23.75cd	12.12e	11.45cd	12.82d	770.80f
IT99K-573-1-1	16.25b	29.63b	16.51b	10.47e	16.26ab	1362.70b
Ife brown*	13.44c	21.56d	11.37f	12.04c	13.56d	820.40f
±S.E.	0.75	1.23	0.22	0.29	0.55	73.50

*Susceptible control; Means followed by the same letter in each column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

Table 4.20 Correlation coefficients (r) among virus disease incidence, severity and yield parameters of cowpea genotypes under natural field infections in 2011

	Incidence (%)	severity (1-5)	No prod peduncle[a]	Pod no / plant	Pod length (cm)	seed no / pod	100-seed Weight (g)	Total seed Weight (Kg / ha)
Incidence	-							
Severity	0.93***	-						
No prod Peduncles	-0.65*	-0.75*	-					
Pod no / Plant	-0.66*	-0.81**	0.95***	-				
Pod length (cm)	-0.25ns	-0.45ns	0.71*	0.69*	-			
Seed no / Pod	-0.63ns	-0.63ns	0.72*	0.58ns	0.51ns	-		
100-seed weight (g)	-0.19ns	-0.45ns	0.21ns	0.3ns	0.38ns	0.19ns	-	
Total seed weight (Kg/ha)	-0.48ns	-0.69*	0.86**	0.90***	0.88**	0.57ns	0.46ns	-

[a]No prod peduncles, number of productive peduncles

*, **, *** significant at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001 respectively; ns, not significant

4.1.3.6 Resistance classes of cowpea genotypes to field infections of BCMV - BlCM, SBMV and CMV determined by disease severity and enzyme-linked immunosorbent assay

Although, viruses other than BCMV - BlCM, SBMV and CMV were detected on the field, resistances to the three viruses by the test cowpea lines were investigated. Cowpea genotypes were classified into their resistance status to BCMV - BlCM, SBMV and CMV using the combination of viral disease severity scores and serological detection of proxy concentrations of the viruses (Table 4.21). Results of cowpea screening for resistance to BCMV - BlCM, SBMV and CMV under natural field infections showed a host response nearly similar to that observed in the screenhouse. Lines IT98K-1092-1 and IT97K-1042 were observed to have resistance to BCMV - BlCM, SBMV and CMV. Both IT98K-133-1-1and IT97K-1069-6 displayed resistance to SBMV and CMV, the former showing susceptibility to field infection of BCMV - BlCM while the latter showed tolerance. Susceptibility to BCMV - BlCM was observed in IT04K-405-5, which also showed resistance to the remaining two viruses. Resistance to BCMV - BlCM and CMV and susceptibility to SBMV was displayed in IT99K-573-1-1. Ife brown was observed to be susceptible to the three viruses, highly susceptible to BCMV - BlCM and CMV and susceptible to SBMV. Line IT99K-1060 showed susceptibility to BCMV - BlCM and SBMV and IT98K-503-1 was susceptible to SBMV and CMV, the former showing resistance to CMV and the latter to BCMV - BlCM.

4.1.4 Nucleic acid sequencing for confirmation of virus identity

RT–PCR on total RNA extracts from the inoculated cowpea plants that served as maintenance hosts for virus culture produced amplicons of the expected sizes (BCMV - BlCM, ~ 700 bp; SBMV 500 bp and CMV, 500 bp: Plates 4.09 – 4.11). RNA sequences of the three isolates further confirmed the identity of the viruses used in this study when subjected to similarity search from GenBank databases using BLAST program (NCBI). Edited cDNA sequences used comprised of 683, 503 and 489 bp of BCMV - BlCM, SBMV and CMV respectively. The sequenced RNAs of the three viruses showed high levels of similarity to the viruses registered in the GenBank. For SBMV, 92% similarity to SBMV sequence of accession number DQ481604 was obtained. Also, 95 similarity to BCMV - BlCM sequence of accession number FJ653926.1 was obtained for BCMV - BlCM while for CMV, 98% similarity was obtained with CMV sequence of accession number D49496.1 (Table 4.22; Appendix 1).

Table 4.21 Resistance classes of cowpea genotypes under natural field infections by BICMV, SBMV and CMV based on disease severity and enzyme-linked immunosorbent assay (ELISA) in 2011*

Genotype	DS	N	BCMV - BlCM		SBMV		CMV	
	(1 – 5)		ELISA	Class	ELISA	Class	ELISA	Class
IT98K-133-1-1	2.1±0.2de	24	++	S	-	R	-	R
IT98K-1092-1	1.8±0.2e	21	-	R	-	R	-	R
IT97K-1069-6	2.0±0.2de	22	+++	T	-	R	-	R
IT98K-503-1	3.9±0.1a	28	-	R	+++	HS	++	S
IT97K-1042-3	2.2±0.1d	19	-	R	-	R	-	R
IT04K-405-5	2.1±0.1de	21	++	S	-	R	-	R
IT99K-1060	3.3±0.3b	30	++	S	+++	HS	-	R
IT99K-573-1-1	2.9±0.4c	28	-	R	+	S	-	R
Ife brownb	3.6±0.1a	29	+++	HS	++	S	+++	HS

*DS, disease severity; BCMV -BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; [b]susceptible check; - = ELISA Negative (overnight ELISA reading at 405nm Absorbance): + = ELISA positive (+, ≥ 2 x H; H represents absorbance value of healthy control); ++ = moderately positive (≥ 3 x H); +++ = highly positive (≥4 x H)HS, highly susceptible; S = susceptible; T = tolerant; R = resistant. N = Sum of sixteen each of asymptomatic plants plus varying numbers of symptomatic plant samples tested. Means followed by the same letter in each column are not significantly different (P < 0.01) according to Duncan's multiple range test.

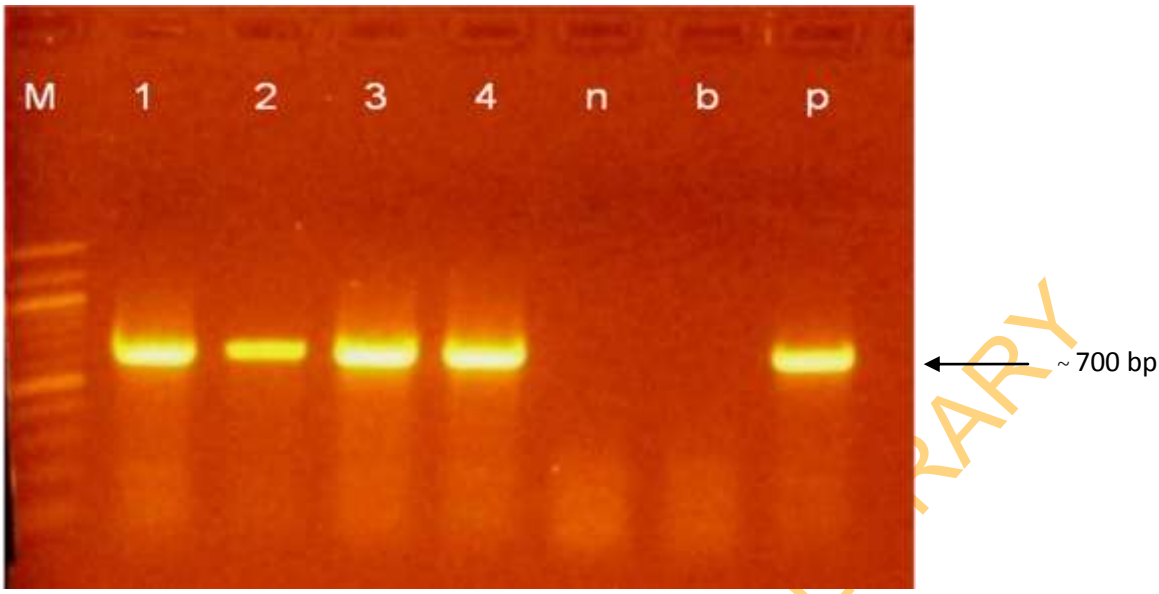

Plate 4.9 Detection of BCMV - BlCM infection in cowpea by RT-PCR with CIF/CIR primers; Electrophoresis with Ethidium-bromide stained 1.5 % agarose gel; M, DNA size marker (100 bp; Promega, USA); lanes 1 - 4, extracts from test samples; n = negative control consisting of extract from healthy cowpea; b = buffer control; p = positive control consisting of extract from BCMV - BlCM infected susceptible cowpea

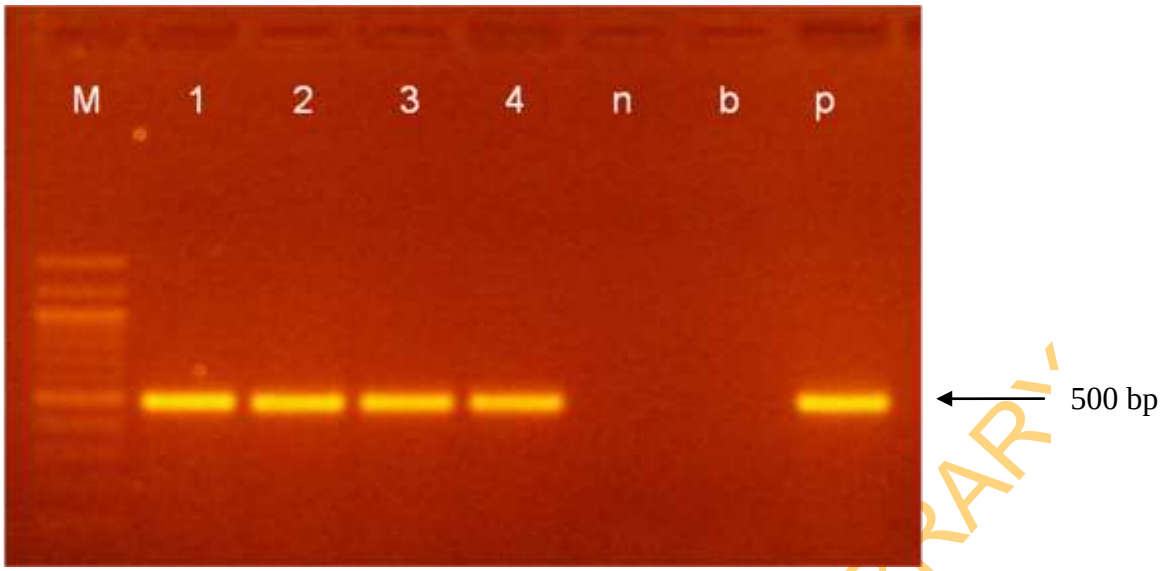

Plate 4.10 Detection of SBMV infection in cowpea by RT-PCR with SBMVF / SBMVR primers; Electrophoresis with Ethidium-bromide stained 1.5 % agarose gel; M, DNA size marker (100 bp; Promega, USA); lanes 1 - 4, extracts from test samples; n = negative control consisting of extract from healthy cowpea; b = buffer control; p = positive control consisting of extract from SBMV infected susceptible cowpea

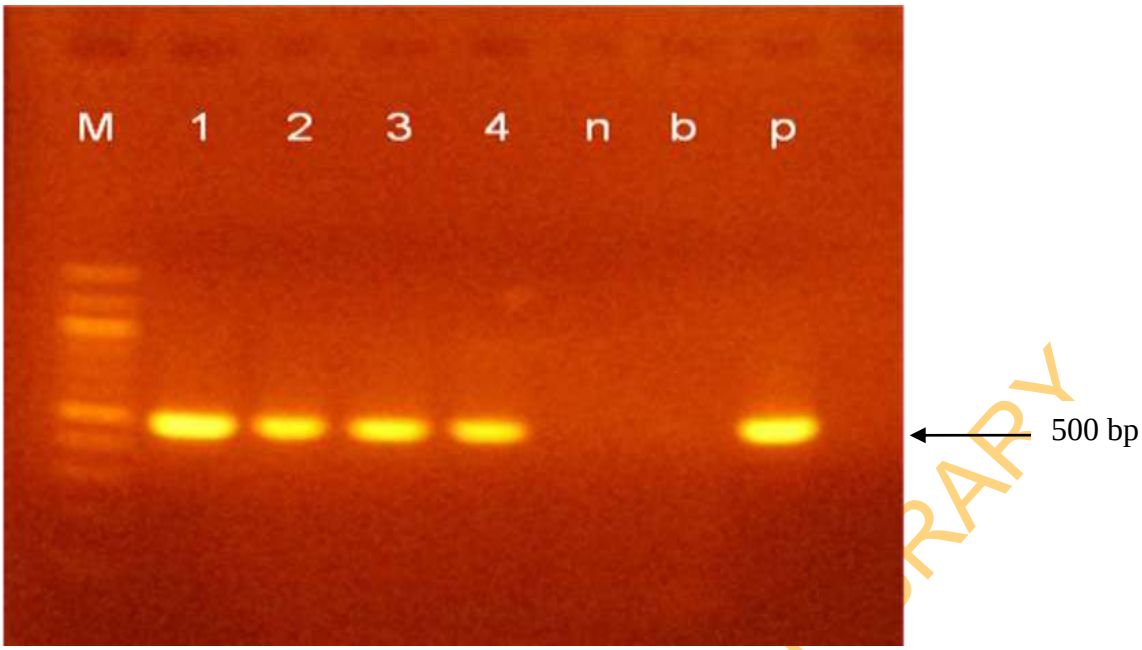

Plate 4.11 Detection of CMV infection in cowpea by RT-PCR with CMV1 / CMV2 primers; Electrophoresis with Ethidium-bromide stained 1.5 % agarose gel; M, DNA size marker (100 bp; Promega, USA); lanes 1 - 4, extracts from test samples; n = negative control consisting of extract from healthy cowpea; b = buffer control; p = positive control consisting of extract from CMV infected susceptible cowpea

Table 4.22 Percentage sequence identity of BCMV - BlCM, SBMV and CMV isolates

Isolate Sequenced	Homology (%)	Virus Confirmed
BCMV – BlCM	95	BCMV - BlCM *strain of Bean common mosaic virus*
SBMV	92	*Southern cowpea mosaic virus* strain of SBMV
CMV	98	CMV

BICMV, *Bean common mosaic virus - blackeye cowpea mosaic strain*; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*

4.2 Genetic studies for determination of mode of inheritance of resistance to BCMV - BlCM, SBMV and CMV diseases in cowpea

4.2.1 Inheritance of resistance to *Bean common mosaic virus - blackeye cowpea mosaic strain* in cowpea

4.2.1.1 Evaluation of parental lines, F_1, F_2, BCP_1 and BCP_2 generations for resistance to BCMV - BlCM

Data on the inheritance of resistance to Bean common mosaic virus - blackeye cowpea mosaic strain in cowpea are presented in Table 4.23 and 4.24. The parental lines, F_1, F_2, BCP_1 and BCP_2 generations evaluated for resistance to BCMV - BlCM showed that plants of resistant parent IT97K-1042-3 were all symptomless and negative to ACP-ELISA which were confirmed negative by RT-PCR. The susceptible parent plants developed systemic infection symptoms characteristic of BCMV – BlCM indicating true breeding of the parental lines. Symptom expression, which began from between eight and twelve days post inoculation (DPI), was as described in section 4.1.1.1, started with mild mottling which later progressed into mosaic and vein banding with aging. The F_1 generation plants developed infection symptoms similar to that of the susceptible parent showing that resistance to BCMV - BlCM was inherited recessively. Plants of the F_2 generation responded to artificial inoculation by either being symptomless or showing mild or severe symptoms. Evaluation of these plants for resistance to BCMV - BlCM showed 72 resistant and 251 susceptible plants (Table 4.23).

The segregation pattern, subjected to a chi-square ($\chi 2$) test gave a goodness-of-fit to 1 resistant: 3 susceptible which indicated that resistance of the cowpea line IT97K-1042-3 to BCMV - BlCM is conditioned by a single homozygous recessive gene pair. Symptoms observed on most of the symptomatic backcross generation plants were not severe. While all plants of backcross to susceptible parent (BCP_2) were symptomatic. Backcrossed plants to resistant parent (BC_1) segregated into 18: 22 resistant: susceptible respectively which fitted a ratio of 1 resistant: 1 susceptible plant ($p > 0.05$). Thus, the backcross generations confirmed the monogenic inheritance of resistance to the virus. The F_2 generation that resulted from a reciprocal cross between the same parents gave 157 susceptible to 69 resistant plants which fitted into a segregation ratio of 3 susceptible to 1 resistant plants (Table 4.24). Evaluation of the backcross to resistant parent also resulted in the ratio of 1 susceptible: to 1 resistant plant. The results obtained from reciprocal cross indicated the

absence of maternal or cytoplasmic inheritance and confirmed the monogenic recessive mode of inheritance of BCMV - BlCM.

4.2.2 Inheritance of resistance to *Southern bean mosaic virus* in cowpea

4.2.2.1 Evaluation of the parental lines, F_1, F_2, BCP1 and BCP_2 generations for resistance to SBMV

The parental plants, F_1, F_2, BC_1 and BC_2 generations inoculated with the SBMV showed that the parental lines IT99K-1060 and IT98K-1092-1 were susceptible and resistant to SBMV respectively. Following SBMV inoculation, plants of the susceptible parent (P_1) showed symptoms which were confirmed by serological examinations using ACP-ELISA, whereas P_2 plants were asymptomatic and negative to ELISA as well as RT-PCR. All the F_1 plants evaluated showed resistance to the SBMV infection which indicated dominance of the gene for resistance to SBMV in IT98K-1092-1. Evaluation of F_2 generation revealed 209 resistant and 16 susceptible plants, which when subjected to Chi square analysis ($p > 0.05$) fitted into a segregation ratio of 15 resistant: 1 susceptible plants. This showed an epistatic interaction of two dominant genes in duplicate gene actions (Table 4.25).

The backcross to the susceptible parent showed a segregation of 26 resistant: 9 susceptible plants which fitted into 3 resistant: 1 susceptible ratio ($p > 0.05$). This further confirmed the result from F_1 and F_2 evaluation indicating that inheritance of resistance to SBMV in IT99K 1092-1 is conditioned by duplicate dominant genes.

4.2.3 Inheritance of tolerance to *Cucumber mosaic virus* in cowpea

4.2.3.1 Evaluation of the parental lines, F_1, F_2, BCP_1 and BCP_2 generations for tolerance to CMV

The rate of success achieved in the crosses and backcrosses of CMV tolerant line IT98K-1092-1 and susceptible line IT99K-573-1-1 were lower compared with other which necessitated more hybridization to achieve adequate number of F_1, F_2, BC_1 and BC_2 generations for inheritance studies. The parental lines bred true. When inoculated with CMV, most of the tolerant parent plants (IT98K-1092-1) did not produce any visible symptom while few of the plants produced mild symptoms (severity 1 – 2) of mottling and inter-venal chlorosis but without puckering.

Table 4.23 Inheritance of resistance to *Bean common mosaic virus - blackeye cowpea mosaic strain in cowpea* from a direct cross and backcross generations of resistant (IT97K-1042-3) and Susceptible (IT99K-1060) lines

Generations*	No of plants			Expected	$\chi 2$	Probability
	R	S	Total	Ratio		
Resistant parent						
IT97K-1042-3 (R)	30	-	30			
Susceptible parent						
IT99K-1060 (S)	-	35	35			
IT97K-1042-3 X S (F1)	-	33	33			
IT97K-1042-3 X S (F2)	72	251	323	3 : 1	1.278	0.30 - 0 .20
Backcross to R						
IT97K-1042-3 X (RXS)	18	20	38	1:1	0.106	0.80 - 0.70
Backcross to S						
IT99K-1060 X (RXS)	-	30	30	-	-	-

*R, resistant; S, susceptible

Table 4.24 Reciprocal cross and backcross generations of resistant (IT97K-1042-3) and susceptible (IT99K-1060) cowpea lines for inheritance studies of resistance to *Bean common mosaic virus - blackeye cowpea mosaic strain*

Generations*	No of plants			Expected Ratio	$\chi 2$	Probability
	S	R	Total			
Susceptible parent IT99K-1060 (S)	33	-	33			
Resistant parent IT97K-1042-3 (R)	-	42	42			
IT99K-1060 X R (F_1)	28	-	28			
IT99K-1060 X R (F_2)	157	69	226	3:1	3.687	0.10 – 0.05
Backcross to S IT99K-1060 X (S X R)	41	-	41	-	-	-
Backcross to R IT97K-1042-3 X (S X R)	22	12	34	1 : 1	2.942	0.10 – 0.05

*R, resistant; S, susceptible

Table 4.25 Inheritance of resistance to *Southern bean mosaic virus* in cowpea using a one-way cross and backcross generations of susceptible (IT99K-1060) and resistant (IT98K-1092-1) Lines

Generations*	No of plants			Expected	$\chi 2$	Probability
	R	S	Total	Ratio		
Susceptible parent IT99K-1060 (S)	-	28	28			
Resistant parent IT98K-1092-1 (R)	40	-	40			
IT99K-1060 X R (F_1)	45	-	45			
IT99K-1060 X R (F_2)	209	16	225	15. 1	0.301	0.70 - 0.60
Backcross to S IT99K-1060 X (SXR)	26	9	35	3. 1	0.006	0.95 - 0.90
Backcross to R IT98K-1092-1 X (SXR)	36		36	-	-	-

*R, resistant; S, susceptible

The CMV inoculated susceptible parent plants (IT99K-573-1-1) produced visible symptoms of mottling, mosaic, inter-venal chlorosis and puckering which manifested at about 8 DPI. Symptom expression was obvious in all the susceptible plants (severity scores 3 - 4) and both tolerant and susceptible parental lines and the F_1, F_2, BCP_1 and BCP_2 generations tested positive to CMV using ACP-ELISA. Meanwhile, symptom expression began to fade in most of the CMV symptomatic plants starting from three or four WPI whereas the plants remained ELISA positive to the virus.

The CMV inoculated F_1 plants of the cross between the tolerant and susceptible lines showed reactions similar to that of the tolerant parent, in which most plants were symptomless with few showing mild symptoms. This indicated a dominant mode of inheritance of tolerance to CMV in IT99K-1092-1. The F_2 generations segregated into 307 tolerant: 26 susceptible plants which gave a goodness-of-fit to 15 tolerant: 1 susceptible segregation ratio, giving an indication that inheritance of tolerance to CMV in the cowpea line is controlled by duplicate dominant genes (Table 4.26). The segregation ratio of 3:1 tolerant: susceptible plants obtained from plants of the backcross to the susceptible parent confirmed the digenic inheritance of the tolerance to CMV. Reciprocal cross between the same parents gave similar segregation ratio of 15 tolerant: 1 susceptible plants in the F_2 generations (Table 4.27), indicating the absence of maternal or cytoplasmic inheritance and confirming the digenic nature of the inheritance.

4.3 Seed transmission of single and mixed viruses in cowpea

4.3.1 Symptom assessment of seed transmitted BCMV - BlCM, SBMV and CMV in singly and mixed infected cowpea genotypes

Seeds harvested from the six susceptible cowpea test lines infected singly or mixed were sown and plants were assessed for transmission of viruses. Percentage germination of the seeds ranged from 80 to 100 under single infection and 77 to 100 under mixed infections (Table 4.28 and 4.29). Symptom appearance was observed in some of the cowpea genotypes from 7 to 8 days after plating (DAP) while others were symptomless. Symptoms observed on each cowpea line were similar to that observed on artificially inoculated plants but generally less severe. However, severe symptoms were observed on plants that developed from seeds harvested from those infected with BCMV - BlCM and co-infected with CMV. The severity of symptom expression depends on genotype and mode of infections, either single or mixed and this seems to be higher in seeds from mixed infected plant than in singly (Plates 4.12 g - h). Cowpea lines

Table 4.26 Inheritance of tolerance to *Cucumber mosaic virus* in cowpea using a direct cross and backcross generations of tolerant (IT98K-1092-1) and susceptible (IT99K-573-1-1) lines

Generations*	No of plants			Expected Ratio	$\chi 2$	Probability
	T	S	Total			
Tolerant parent						
IT98K-1092-1 (T)	42	-	42			
Susceptible parent						
IT99K-573-1-1 (S)	-	36	36			
IT98K-1092-1 X S (F_1)	28	-	28			
IT98K-1092-1 X S (F_2)	307	26	333	15. 1	1.387	0.30 - 0.20
Backcross to T						
IT98K-1092-1 X (TXS)	22	-	22	-	-	-
Backcross to S						
IT99K-573-1-1 X (TXS)	29	8	37	3. 1	0.243	0.70 - 0.60

T, tolerant: S, susceptible

Table 4. 27 Reciprocal cross and backcross generations of tolerant (IT97K-1042-3) and susceptible (IT99K-1060) cowpea lines for inheritance studies of tolerance to *Cucumber mosaic virus* in cowpea

Generations*	No of plants			Expected	$\chi 2$	Probability
	S	T	Total	Ratio		
Susceptible parent						
IT99K-573-1-1 (S)	12	-	12			
Tolerant parent						
IT98K-1092-1 (T)	-	49	49			
IT99K-573-1-1 X T (F1)	-	47	47			
IT99K-573-1-1 X T (F2)	16	299	315	15. 1	0.741	0.50 - 0.30
Backcross to S						
IT99K-573-1-1 (SXT)	5	17	22	3. 1	0.061	0.95 - 0.90
Backcross to T						
IT98K-1092-1 X (SXT)	-	42	42	-	-	-

T, tolerant: S,susceptible

with seed transmission of BCMV - BlCM produced mosaic, mottling and vein banding (Plate 4.12a). Seed transmission of SBMV showed mosaic, inter-venal chlorosis while that of CMV displayed mosaic, mottling, necrotic lesion and mild puckering (Plate 4.12 b - c). Visual assessment of symptoms under single infection showed that 7 out of 32 plants (21.9%) were symptomatic for BCMV - BlCM transmission in IT98K-503-1 and 2 plants out of 42 germinated seeds produced BCMV - BlCM symptom in Ife brown. Only one from the forty six (2.2%) germinated IT99K-1060 seed showed symptoms of SBMV transmission, while plants from seeds of four lines (IT98K-133-1-1, IT98K-503-1, IT99K-1060 and IT99K-573-1-1) from CMV inoculated plants produced symptoms in 1 or 2 of the germinated plants (Table 4.28).

Under mixed infections, severity of symptom expression of seed transmitted viruses was higher in cowpea genotypes infected with BCMV - BlCM + CMV than with BCMV - BlCM + SBMV and SBMV + CMV (Plates 4.12). While only one cowpea genotype each produced symptom in BCMV - BlCM + SBMV and SBMV + CMV infections, five out of the six cowpea genotypes were symptomatic under triple infections (Table 4. 29).

4.3.2 Seed transmission of BCMV - BlCM, SBMV and CMV in singly and mixed infected cowpea genotypes determined by enzyme linked immunosorbent assay

The three viruses were seed transmitted. However, their transmission rates varied with cowpea genotype, virus type and infection either single or mixed. For single infections, BCMV- BlCM was seed transmitted only in IT98K-503-1 and Ife brown, SBMV in IT99K-1060 while CMV was transmitted in all the lines with the exception of IT97K-1069-6 and Ife brown. Generally, higher transmission was observed from serological tested than through symptomatology (Tables 4.30 and 4.31). For instance, under single infections, 2 out of 50 (4 %) seedlings of IT98K-133-1-1 showed symptoms of CMV transmission, while 13 out of the same plants (26 %) tested ELISA positive to CMV transmission. In IT98K-503-1, 7 out of 32 (21.9%) plants were symptomatic for BCMV - BlCM infection whereas 8 of these plants (25%) were positive to ELISA. BCMV - BlCM and SBMV were not seed transmitted in most of the cowpea genotypes even under mixed infections. Higher transmission rates were observed in seeds from singly infected plants than from mixed infected. Seed from the singly infected plants showed that IT98K-503-1 and Ife brown had seed infections (25 % and 4.8 %) of BCMV - BlCM, only IT99K-1060 showed SBMV (2.2 %) infection while IT98K-133-1-1 (26 %), IT98K-503-1 (8.3 %),

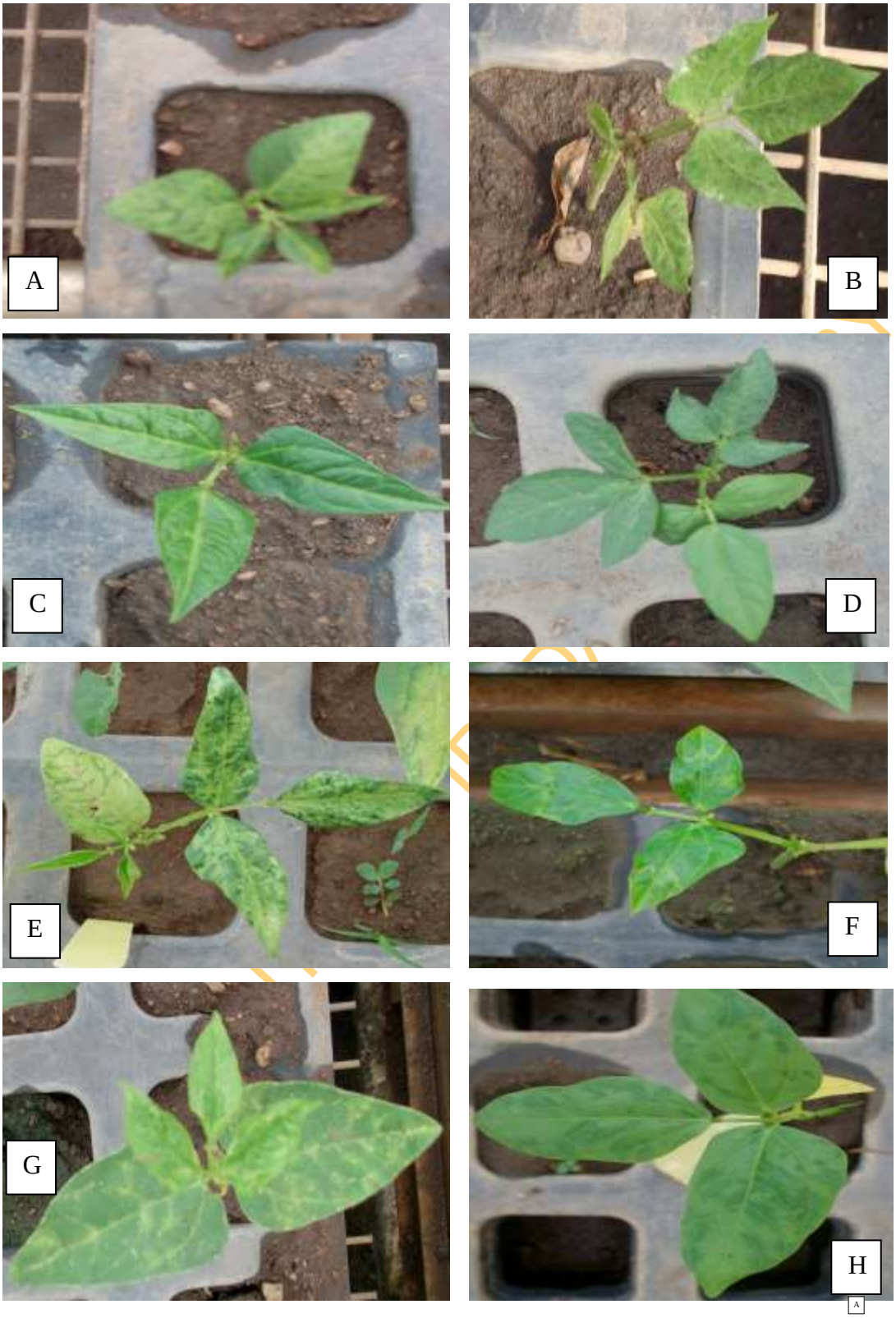

Plate 4.12 Symptoms of seed transmitted *Bean common mosaic virus - blackeye cowpea mosaic strain* (BCMV-BlCM); *Southern bean mosaic virus* (SBMV) and *Cucumber mosaic virus* (CMV) on cowpea genotypes under single and mixed infections: A = BCMV - BlCM on IT98K-503-1, B = SBMV on IT99K-1060, C = CMV on IT99K-1060, D = BCMV-BlCM + SBMV on IT98K-503-1, E = BCMV-BlCM + CMV on IT98K-133-1-1, F = SBMV + CMV on IT98K-133-1-1, G = BCMV -BlCM + SBMV + CMV on Ife brown and H = BCMV-BlCM + SBMV on IT98K-503-1.

Table 4.28 Symptom assessment of seed transmitted BCMV - BlCM, SBMV and CMV in singly infected cowpea genotypes*

Genotype	BCMV – BlCM			SBMV			CMV		
	Germ (%)	No symp	Sym	Germ (%)	No symp	Sym	Germ %	No Symp	Sym
IT98K-133-1-1	100.0	0/50	-	96.0	0/48	-	100.0	2/50	M, nl, de
IT97K-1069-6	96.0	0/48	-	94.0	0/47	-	94.0	0/47	-
IT98K-503-1	80.0	7/32	M, vb	100.0	0/37	-	80.0	1/36	M, nl
IT99K-1060	98.0	0/49	-	92.0	1/46	M, ic	90.0	1/37	nl, p
IT99K-573-1-1	92.5	0/37	-	98.o	0/49	-	100.0	1/50	M
Ife brown	84.0	2/42	mo, vb	96.o	0/48	-	80.0	0/40	-

*BCMV - BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*; Germ (%) = percentage seed germination; No symp = number of symptomatic plants / germinated seeds; sym = symptom; M = mosaic; mo = mottling; nl = necrotic lesion; p = puckering; de = defoliation; ic = intervainal chlorosis; vb = vein banding

Table 4.29 Symptom assessments of seed transmitted BCMV - BlCM, SBMV and CMV in mixed infected cowpea genotypes

Genotype	BCMV-BlCM + SBMV			BCMV-BlCM + CMV			SBMV+CMV			BCMV-BlCM + SBMV+CMV		
	Germ %	No Symp	sym	Germ %	No Symp	Sym	Germ %	No Symp	sym	Germ %	No Symp	sym
IT98K-133-1-1	96.0	0/48	-	96.0	3/48	M, mo, vb, p	100.0	2/50	M, mo	100.0	4/50	M, mo
IT97K-1069-6	98.0	0/49	-	88.0	0/44	-	96.0	0/48	-	96.0	2/48	M, p
IT98K-503-1	98.0	2/49	Ic	88.0	1/44	M, ic	77.0	0/23	-	96.3	2/26	M, ic
IT99K-1060	92.0	0/46	-	80.0	2/40	M, vb	94.0	0/30	-	88.4	0/38	-
IT99K-573-1-1	86.0	0/43	-	85.0	0/34	-	100.0	0/50	-	96.0	1/48	M
Ife brown	98.0	0/49	-	90.0	0/45	-	92.0	0/46	-	94.0	2/47	M, P, ic

*BCMV - BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber Mosaic virus*; Germ % = percentage seed germination; No symp = number of symptomatic plant / Germinated seed; sym = symptoms; M = mosaic; mo = mottling; p = puckering; ic = interveinal chlorosis; vb = vein banding

IT99K-1060 (2.8 %) and IT99K-573-1-1 (2.0 %) showed seed transmission of CMV (Table 4.30).

Mixed infection affected transmission of BCMV – BlCM under BCMV – BlCM + SBMV infection where BCMV – BlCM was not transmitted in the six tested genotypes whereas, SBMV was transmitted in two genotypes. Similar occurrence of zero transmission was observed for SBMV under SBMV + CMV and triple infection. Multiple virus transmissions was observed in IT98K133-1-1 (4 % BCMV - BlCM, 12 % CMV), IT97K-1069-6 (2.0 % BCMV + BlCM and 2.0 % CMV) and Ife brown (2.2 % BCMV + BlCM and 2.2 % CMV) all under triple infections.

Variability was observed in seed transmission rates in each of the viruses among the cowpea genotypes under mixed infections. For instance, in IT98-133-1-1, lower CMV transmission rates were observed in seed from plants with double or triple CMV infections than from singly infected plants. In this line, seed transmission rate of CMV reduced from singly infected plant (26 %) to doubly (17.4 % and 12.3 %) and to that of seed from triply infected plants (12 %) (Table 4.30 to 4.31). Seed transmissibility of viruses was hindered with co-infection in some genotypes and enhanced in others. For instance, in IT98K-503-1, while 25 % BCMV - BlCM transmission was observed in seed from singly infected plant, the virus was not transmitted in seed with BCMV - BlCM + SBMV co-infection. On the contrary, the 4.0 % and 2.0 % BCMV - BlCM transmissions were observed in lines IT98K-133-1-1 and IT97K-1069-6 respectively under triple inoculation whereas there was no BCMV - BlCM transmission in these lines under single infection.

SBMV was not seed transmitted in most of the seeds from mixed infected plants except under BCMV - BlCM + SBMV in IT98K-503-1 (2.1 %) and Ife brown (2.0 %). Low virus transmission was observed in cowpea line IT97K-1069-6 which only transmitted BCMV - BlCM (2.0 %) and CMV (2 %) under triple infection. Among the three viruses, highest transmission rate of CMV was observed, followed by BCMV - BlCM with very low transmission of SBMV.

Table 4.30 Seed transmission of BCMV - BlCM, SBMV and CMV in singly infected cowpea genotypes determined by enzyme linked immunosorbent assay (ELISA)

Genotype	BCMV – BlCM		SBMV		CMV	
	ELISA Positive Plant	Seed Trans %	ELISA positive plant	Seed trans %	ELISA positive Plant	Seed Trans %
IT98K-133-1-1	0/50	0.0	0/48	0.0	13/50	26.0
IT97K-1069-6	0/48	0.0	0/47	0.0	0/49	0.0
IT98K-503-1	8/32	25.0	0/31	0.0	3/36	8.3
IT99K-1060	0/49	0.0	1/46	2.2	1/36	2.8
IT99K-573-1-1	0/37	0.0	0/49	0.0	1/50	2.0
Ife brown	2/42	4.8	0/48	0.0	0/40	0.0

BICMV, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, *Southern bean mosaic virus*; CMV, *Cucumber mosaic virus*, seed trans % = percentage seed transmission

Table 4.31 Seed transmission of BCMV - BlCM, SBMV and CMV in cowpea genotypes under multiple infections determined by enzyme linked immunosorbent assay (ELISA)

Genotype	BCMV - BlCM + SBMV				BCMV – BlCM + CMV				SBMV + CMV				BCMV-BlCM + SBMV+CMV					
	BCMV - BlCM		SBMV		BCMV- BlCM		CMV		SBMV		CMV		BCMV-BlCM		SBMV		CMV	
	ELISA +ve plant	Seed trans %	ELISA +ve Plant	Seed Trans %	ELISA +ve plant	Seed trans %	ELISA +ve plant	Seed trans %	ELISA +ve plant	Seed trans %	ELISA +ve Plant	Seed trans %	ELISA +ve plant	Seed trans %	ELISA +ve plant	Seed trans %	ELISA +ve plant	Seed trans %
IT98K-133-1-1	0/43	0.0	0/49	0.0	0/46	0.0	8/46	17.4	0/49	0.0	6/49	12.3	2/50	4.0	0/50	0.0	6/50	12.0
IT97K-1069-6	0/46	0.0	0/49	0.0	0/45	0.0	0/45	0.0	0/50	0.0	0/50	0.0	1/49	2.0	0/49	0.0	1/50	2.0
IT98K-503-1	0/42	0.0	1/48	2.1	0/37	0.0	1/37	2.7	0/23	0.0	0/23	0.0	0/25	0.0	0/25	0.0	2/25	8.0
IT99K-1060	0/44	0.0	0/44	0.0	3/40	7.5	0/40	0.0	0/28	0.0	0/28	0.0	0/38	0.0	0/38	0.0	0/38	0.0
IT99K-573-1-1	0/33	0.0	0/35	0.0	0/27	0.0	0/27	0.0	0/50	0.0	1/50	2.0	1/48	2.1	0/46	0.0	0/46	0.0
Ife brown	0/49	0.0	1/49	2.0	2/44	4.6	0/4	0.0	0/44	0.0	2/44	4.5	1/46	2.2	0/46	0.0	1/46	2.2

BCMV - BlCM, Bean common mosaic virus - blackeye cowpea mosaic strain; SBMV, Southern bean mosaic virus; CMV, Cucumber mosaic virus; seed trans % = percentage seed transmission, ELISA +ve plant = ELISA positive plants

CHAPTER FIVE

DISCUSSION

It is essential to understand the genetics of a desirable character in order to choose the appropriate breeding procedure for incorporating it into an improved variety. Genes for resistance and mode of inheritance of the resistance, either as dominant or recessive are very important in breeding programmes aimed at developing disease resistant varieties. Cowpea is susceptible to a number of viruses that limit its productivity. Viral diseases therefore, remain a major constraint to large scale production of cowpea and other major legumes in Nigeria (Shoyinka *et al.*, 1988; Taiwo and Shoyinka, 1988; Thottappilly and Rossel 1992). Unlike for other pathogens, chemical agents such as fungicides and bactericides are not effective in controlling virus diseases (Kumar, 2009). Thus, planting resistant crop varieties has been reported to be the most economical, practical and effective method of controlling viral diseases in plants (Taiwo, 2003).

Evaluation of eight improved cowpea breeding lines newly developed by IITA for resistance to single and mixed infections of three economically important viruses namely; BCMV - BlCM, SBMV and CMV showed variation of symptoms depending on cowpea genotype, virus and whether single or mixed infections. Similar variation in symptoms has been reported in cowpea (Collins *et al.*, 1985). Virus identities authenticated by RNA sequence similarity search from GenBank databases revealed 92%, 95% and 98% homologies to registered SBMV, BCMV-BlCM and CMV respectively. The three viruses generally incited systemic foliar symptoms of mosaic and mottling on susceptible plants. BCMV - BlCM induced inter-veinal chlorosis with the characteristic symptom of vein banding. SBMV produced chlorotic local lesions with systemic inter-veinal chlorosis, mild puckering and leaf deformation, while CMV incited chlorotic local lesion, abscission of inoculated leaves, veinal and mid-rib chlorosis, puckering and leaf deformation. They also caused stunted growth while BCMV - BlCM and CMV incited poor or no pod formation and plant death in highly susceptible genotypes. These observed symptoms of cowpea viruses are similar to those reported by other workers (Shoyinka *et al.*, 1978; Thottappilly and Rossel 1992; Thottappilly and Rossel 1996; and Hampton *et al.*, 1997). High virus incidence and symptom severity following single infections of BCMV - BlCM, SBMV and CMV in cowpea lines IT99K-1060 and IT98K-503-1 implies their high susceptibility

to these viruses whereas low incidence and severity of BCMV - BlCM and SBMV infections in IT98K-1092-1 and IT97K-1042-3 denotes their ability to withstand either the movement or replication of the two viruses. Although, incidence of CMV disease was observed in all the cowpea genotypes evaluated, its symptom severity was very low in IT98K-1092-1, indicating low susceptibility of the line to CMV. No hypersensitivity response was observed in the resistant lines. The latent infections of CMV observed in IT98K-1092-1and in other cowpea lines have been reported in cowpea varieties (Abdullahi *et al.*, 2001) and in some other crops such as Bell pepper (*Capsicum annuum* cv. Early calwonder) (Garcia-Riuz and Murphy, 2001), Spinach (Bos *et al.*, 1980) and Alfalfa (Veerisetty and Brakke, 1977).

Categorizing infection severity into AUDPC has been employed and proved effective in classifying plants virus resistance status (Ariyo *et al.*, 2001). In this study, AUDPC confirmed the susceptibility of IT99K-1060 and IT98K-503-1 to BICMV, SBMV and CMV and supported the high resistance against the three viruses observed in IT98K-1092-1 under single and mixed infections. This result was in agreement with the classification using infection severity combined with ELISA and RT-PCR techniques. However, AUDPC could not effectively distinguish between tolerant and susceptible cowpea lines. Also, the IT97K-1042-3 resistance to SBMV, susceptibility by all except one of the evaluated cowpea lines to CMV and that IT98K-133-1-1 and IT97K-1069-6 to BCMV - BlCM were not showed by AUDPC analysis. This is because the disease severity data alone, as used in AUDPC analysis, could only indicate the presence of viruses in the plants and gives the rate of infections through symptomatology over a period of time. However, it could not indicate the type and concentrations of the viruses especially under co-infections and the interactions between the mixed viruses. Determination of resistance status of plant to diseases from symptomatology alone has been discouraged (Odedara *et al.*, 2009; Hobbs *et al.*, 1987). Kumar (2009) reported that disease diagnosis based on symptoms is unreliable because different viruses may cause similar symptoms and different symptoms may be induced by one virus. Many abiotic stresses and other pathogens such as phytoplasma may cause symptoms characteristic of virus infections. Also, detection of a virus in a plant does not necessarily prove that the virus causes the disease but constant association of a virus with a set of symptoms is often used as the '*proof*' that the virus detected causes the disease (Kumar, 2009).

Cowpea viruses have been reported in Nigeria to occur naturally in mixtures, causing mixed-infection (Shoyinka, *et al.* 1997). This study revealed that mixed infections produced more severe symptoms on the susceptible lines than single infections resulting in abscission of some inoculated leaves, reduced leaf areas, stunted growth, few or no pod formation and premature death in highly susceptible lines. The combination of BCMV-BICM + CMV produced more severe symptoms than BCMV-BICM + SBMV and SBMV + CMV. Triple infections incited more severe symptoms in most of the plants than the single or double and caused defoliation of the first trifoliate leaves in IT99K-1060 and Ife brown 7 to 9 DPI some of which resulted in plant death. Virus titres also increased with co-infection in most of the cowpea lines.

Occurrence of more severe symptoms under mixed viral infections has been reported. Rentería-Canett *et al*, (2011) reported similar occurrence in *Pepper huasteco yellow vein virus* (PHYVV) and *Pepper golden mosaic virus* (PepGMV). Balogun *et al.*, (2002) observed synergistic increase of disease severity from mixed infection of PVX and *Tobacco mosaic virus* with more growth reduction in simultaneous inoculation than in sequential inoculation. The synergy has been reported to be due to enhancement in either or both of the viruses involved in the co-infections (Balogun *et al.*, 2002; Syller, 2011). This enhancement is, according to reports, based on different mechanisms. It may result from enhanced concentration and increased in virus synthesis per infected cell (Goodman and Ros, 1974), increase in the number of infected cells (Ishimoto *et al.*, 1990) and enhanced transport of the virus in the host (Baker 1987).

Analysis of seed yield parameters from the screen house experiments showed that most of the susceptible cowpea lines produced lower seed yield parameters than the resistant ones. Mixed infections also resulted in a significant reduction in most of the yield parameters than the single infections. Hughes and Shoyinka (2003) have indicated that yield losses due to viral infection in sub-Saharan Africa depends on the time of infection, virus strain, possible virus mixtures, cultivars and environmental interactions especially climate. Also, triple virus infections, as reported by Taiwo and Akinjogunola (2006), caused greater reductions in growth and yield parameters than single and double viral infections. Lack of significant differences in the rates of reduction in some yield parameters observed between the double infections BCMV - BlCM + CMV and the triple infections (BCMV - BlCM + SBMV + CMV) indicated strong synergy between BCMV - BlCM and CMV on cowpea which is not influenced by SBMV.

Coefficient of correlation results showed a highly positive correlation between viral disease severity and incidence from screen-house resistance evaluations. Total seed weight was highly positively correlated with the number of pods per plant, length of pods and number of seeds per pod under the three viral inoculations. However, a high negative correlation was observed between the symptom severity and the entire seed yield parameters studied in the three single viral inoculations.

The absence of symptom of viral infection in the cowpea lines in the first field trial could be attributed to lack of adequate virus isolates for infection and disease establishment and also to the low population of insect vectors for effective transmission. The early weekly insecticide spray at the vegetative stage (4 WAP) might have perhaps reduced the insect population for transmission of viruses. However, latent infections of CMV and CPMoV observed in two susceptible lines indicates the ability of the viruses to move and or multiply in the two lines under low infection. Availability of adequate virus isolates through the use of infector plants and presence of insect population to vector the viruses resulted in virus infections in the second field trial. Mild to severe symptoms observed and positive serological test confirmed the presence of natural infections on the field.

The field screening results supported the screen-house evaluations. Also, negative correlation existed between the virus incidence or severity and most of the yield parameters indicating reduction in yield components due to the natural field viral infections. Multiple viral infections that occurred naturally on the field were as reported in Nigeria by Shoyinka, *et al.*, (1997) while the field detection of seven viruses: BCMV - BlCM, SBMV, CMV, CABMV, CPMoV, CPMMV and BPMV is in line with Taiwo (2003) and Shoyinka *et al.*, (1997) reports of these cowpea viruses in Nigeria. Latent infections observed under screen house screening also occurred on the field on CPMMV and CMV. Also, high incidence of CPMMV observed can be attributed to the high incidence of white fly (*Bemisia tabaci*) noticed in the first few weeks of the plant before insecticide spray which started from 6 WAP.

However, unlike in screen house experiments, screening under natural field infection did not provide detailed information on the host response to specific virus and thus could not provide adequate data for the final classification of cowpea lines into resistance status. This was due to the mixed infections with other cowpea viruses beyond BCMV - BlCM,

SBMV and CMV obtained naturally on the field which obscured effective ranking of the cowpea lines into resistance to specific viruses. This limitation is peculiar to field screening of insect transmittable diseases. Meanwhile, combination of severity scores and serological results of field data supported the classification of the most resistance and the highly susceptible lines as observed in the screen house.

In this study, combination of symptomatology, AUDPC analysis, virus detection by ACP-ELISA and RT-PCR was effectively used in classifying the evaluated cowpea genotypes to their resistance classes. Screen-house evaluations and field trials showed different sources of single and multiple resistances to BCMV - BLCM and SBMV and tolerance to CMV in the evaluated cowpea lines. Although, fragments of ~700 bp and 500 bp were amplified from BCMV-BlCM and SBMV susceptible infected lines respectively, no amplified fragments were detected in PCR products of the BCMV-BlCM and SBMV inoculated resistant lines. The same virus detection methods have been reported effective (Lima *et al.,* 2011) in grouping cowpea lines into resistance classes. Evaluation of cowpea lines for virus resistance revealed line IT98K-1092-1 as source of multiple resistances to BCMV-BlCM and SBMV and tolerance to CMV while IT97K-1042-3 was resistant to BCMV-BlCM and SBMV. None of the cowpea lines was resistant to the three viruses. Lines IT98K-133-1-1, IT97K-1069-6 and IT04K-405-5 were resistant to SBMV and susceptible to other two viruses. High susceptibility to the three seed borne viruses obtained in IT99K-1060 and IT98K-503-1 makes them unsuitable for propagation in the viruses' endemic areas.

Though cowpea genotypes with single and multiple resistances to viruses especially BCMV-BlCM, CABMV and CPMV have been reported, there are limited reports on resistance to SBMV, CMV and multiple resistances to BCMV-BlCM and SBMV co-infection. A previous study (Bashir *et al*., 1995) on screening cowpea varieties from IITA showed that lines IT86F 2089-5, IT86D-880, IT90K-284-2, IT90K-76, IT86D-611-3 were highly resistant (immune) to BCMV-BlCM. Multiple resistance to BlCMV-BlCM, CPMV and CABMV have been reported in cowpea breeding lines IT96D-659, IT96D-660, IT97K-1068-7 and IT95K-52-34 (Singh and Hughes, 1999). It has also been reported by VanBoxtel *et al.,* (2000) that cowpea breeding lines IT86D-880 and IT86D-1010 were resistant to three isolates of BlCMV-BlCM and five strains of CABMV while IT82D-889, IT90K-277-2 and TVu201 showed resistance to one or more isolates of CABMV. Also,

combined resistance and immunity to CABMV and CMV have been reported in cowpea lines TE87-98-8G, TE87-98-13G and TE87-108-6G and in IT84S-2135, IT84S-1627 (Singh et al., 2002) while multiple resistance to the two viruses was reported in TVu 15656 and single resistance to CMV in TVu 410 (Mih *et al*., 1991). The development of resistant cultivars has been universally considered the most effective method to control viral diseases in cowpea, indicating that an increase in the number of virus resistant genotypes will generate more alternatives for breeders to produce resistant cultivars (Hampton *et al*., 1997). This is more important since many of the commercial cowpea cultivars are still susceptible to viral diseases. Thus, single and multiple resistance to BCMV – BlCM, SBMV and tolerance to CMV from the new improved cowpea breeding lines produced from this study will be useful in developing virus resistant varieties. The R genes for BCMV - BlCM and SBMV can be incorporated into high yielding cowpea varieties for improved quality and yield.

The synergy produced by co-infection of BCMV – BlCM and CMV in cowpea plants has been reported to have cause "Cowpea stunt" disease in USA which resulted in a nearly complete yield loss (Pio-Ribeiro *et al.*, 1978; Kuhn, 1990). This co-infection has the potential to reach an epidemic situation in Nigeria since the same insect vector transmits the two viruses. Thus, the cowpea line IT98K-1092-1 with resistance to BCMV - BlCM and tolerance to CMV will be of great importance in a proactive management of this disease. Since multiple sources of resistance provide a broader genetic background and probably a more stable resistance than could be expected from single resistance sources, lines IT98K-1092-1 and IT97K-1042-3 with multiple resistances to BCMV-BlCM and SBMV will be of great use in transferring multiple virus resistance to susceptible higher yielding cowpea varieties. This will generate virus resistant cultivars which proffers a lasting solution to yield losses to viral diseases.

Host responses observed included tolerance where virus was detected without symptoms, which merely delayed symptom expression after inoculation, followed by mild symptoms (Lima *et al.*, 2011). Tolerance response to CMV and SBMV observed in IT98K-1092-1 and IT99K-573-1-1 respectively makes the plants suitable for propagation as farmer can achieve good yield even in virus endemic areas. This host response is very prevalent in nature and has been used to considerable benefit in some crops e.g. the control of CMV in cucumber (Roger, 2002). Tolerance to CMV has also been reported in pepper accession "Perennial" (Nono-Wondim *et al.*, 1993).

Genetics of resistance especially the mode of inheritance of resistance to the virus in the cowpea line will help in developing an effective breeding programme for resistant cultivar with durable characters (Arshad, *et al.*, 1998). Investigation of the mode of inheritance of resistance to BCMV - BlCM, SBMV and tolerance to CMV in the resistant and tolerant cowpea lines obtained showed that the lines were true breeding. In inheritance studies of resistance to BCMV - BlCM, all the F_1 generation plants obtained from a cross between a resistant parent IT97K-1042-3 and susceptible parent IT99K-1060 were susceptible, indicating recessive genes for resistance to BCMV - BlCM in IT97K-1042-1. The segregation pattern of the F_2 generation subjected to a chi-square ($\chi 2$) test (Gomez and Gomez, 1984) gave a good fit to 1 resistant: 3 susceptible ratios, which suggested that the resistance is conditioned by a single gene pair.

Results of F_1, F_2 and the backcross generations of the direct and reciprocal crosses indicated a qualitative inheritance of resistance to BCMV - BlCM in cowpea line IT97K-1042-3 which is controlled by a single recessive gene pair, with neither maternal nor cytoplasmic factors. The same mode of inheritance of resistance to BCMV - BlCM have been reported by Arshad, *et al.*, (1998) in six cowpea genotypes (IT86F-2089-5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2062-5 and BP1CP3) and by Walker and Chambliss (1981) in cowpea cultivar "Worthmore". Taiwo *et al.*, (1981) also reported that a single recessive gene was responsible for high level of BCMV - BlCM resistance in cowpea lines TVu-2740, TVu-3273, TVu-2657 and TVu-2845. In contrast, a single dominant gene for resistance to BCMV - BlCM has been reported in cowpea cultivars "White Acre-BVR" (Quatara and Chambliss, 1991), "Pinkeye Purple Hull BVR" (Strniste, 1987) and in bean (Phaseolus vulgaris) cultivar "Black Turtle Soup" (Provvidenti *et al.*, 1983). Thus, resistance to BCMV – BlCM in cowpea is conditioned by single recessive gene which mode of inheritance seems to depend on variety. It has also been reported that more than half of the recessive R genes identified confer resistance to potyviruses where BCMV – BlCM belongs (Shukla *et al.*, 1994). Knowledge of mode of inheritance of the new improved cowpea line IT97K-1042-3 will be useful in breeding programmes.

Successful crosses could not be made between the SBMV resistant line IT98K-1092-1 and susceptible line IT99K-1060 when the former was used as female parent and the latter as source of pollen. This was perhaps due to incompatibility between the two lines which

might be a homomorphic incompatibility, depending on the type and number of alleles controlling a compatible pollination between the pollen and the pistil (Acquaah, 2007). As a result, reciprocal crosses could not be generated to investigate cytoplasmic inheritance of SBMV resistance. However, evaluating the crosses between these parental plants with the resistant line used as the pollen source, the F_1 generation plants were all resistant, indicating a dominant nature of inheritance of SBMV resistance in IT98K-1092-1.

Segregation pattern of F_2 generation (15 resistant: 1 susceptible) was in line with the hypothesis of digenic dominant non-allelic model. Backcross to the susceptible parent confirmed the above result by segregating into 3 resistant to 1 susceptible plant. The segregation pattern and Chi square analysis of the F_1, F_2, BC_1 and BC_2 populations showed that duplicate dominant genes with epistatic interaction conditioned the inheritance of SBMV resistance in cowpea line IT98K-1092-1. Brantley and Kuhn (1970) and Fery (1980) also reported similar dominant inheritance of SBMV but under the control of a single dominant gene. Inheritance of non-necrotic resistance to SBMV in cowpea, according to Hobbs *et al.*, (1987), is dependent upon cowpea cultivar. Using a cross between a SBMV susceptible line "California Blackeye" and three resistant lines, the moderate resistant of "Early Pinkeye" was conferred by a single gene with partial dominance, that of "Iron" appeared to be controlled by multiple genes with incomplete dominance while the resistance of "PI 186465" was largely controlled by one gene with partial dominance for resistance. Apart from varietal dependence, variations observed in the modes of inheritance of the same virus in different cultivars may also result from variation in virus isolates or strains. For instance, Lima *et al.*, (2011) reported that the genes for immunity to CPSMV isolates of the cowpea genotypes CNC 0434 and 'Macaibo' are likely different. This is because while the former is immune to all CPSMV isolates tested, the latter is immune to all except one of the isolates. CNC 0434 is immune to all CPSMV isolates, including $CPSMV_{MC,}$ a CPSMV isolate capable of infecting cowpea cultivar "Macaibo", while 'Macaibo' is infected only by $CPSMV_{MC}$.

Source of resistance to CMV was not achieved from the eight improved lines evaluated but a tolerant line was obtained. However, evaluation of the genetic basis of the tolerant line obtained using a CMV tolerant IT98K-1092-1 and susceptible parental line IT99K-573-1-1 showed that F_1 generation plants were tolerant, indicating a dominant mode of inheritance of tolerance to CMV. The Chi square analysis of the segregation pattern gave 15 tolerant to 1 susceptible ratio of the F_2 generation which indicated the presence of two

duplicate dominant genes. This result was confirmed by the 3 tolerant to 1 susceptible segregation ratio of Backcross generation to the susceptible parent. Absence of maternal or extra-chromosomal factor in the inheritance of tolerance to CMV was produced by the absence of reciprocal difference obtained in the reciprocal cross between the susceptible and tolerant parental lines. Thus, the evaluation indicated that inheritance of tolerance to CMV in the cowpea line IT98K-1092-1 is governed by duplicate dominant non-allelic genes. Many workers have reported one dominant gene for the control of resistance of CMV (Dezeeuw and Crum, 1963, Khalf-Allah *et al.*, 1973; Fery, 1980). However, Report on inheritance of tolerance to CMV is limited in cowpea. Tolerance to CMV in pepper has been found to be incompletely dominant and quantitatively inherited (Lapidot *et al.*, 1997). Also, the inter-allelic relationship of the resistance genes observed for both SBMV resistance and tolerance to CMV has been described (Kang *et al.*, 2005) where resistance alleles at two or more loci are required to observe a virus resistance response.

Knowledge of genetic inheritance of resistance in cowpea lines together with information on genetic variability in the viruses should help plant breeders and virologists develop breeding strategies that will provide effective and stable disease management. The information on genetics of resistance in edible legumes reveals that most resistance is inherited in an oligonenic manner (Meiners, 1981). Though, there is more likelihood of breakdown of monogenic resistance with evolution of new virulent strain with the passage of time than the resistance conditioned by polygenes (Arshad, *et al.*, 1998), it appears that monogenic resistance is not always unstable in edible legumes. In fact, monogenic resistance has held up for extended periods even with some variable pathogens, such as *Bean common mosaic virus* for nearly half a century and for bean anthracnose for nearly twenty years. Only few diseases of legumes like bean rust and lima bean downy mildew has monogenic resistance being of short duration (Meiners, 1981). It is more convenient to transfer monogenic than multigenic resistance to develop improved cultivars (Arshad, *et al.*, 1998) since the use of monogenic resistance requires fewer resources. Monogenic and digenic nature of inheritance observed in this study will enhance easier transfer of the viral R genes than in quantitative inheritance in developing virus resistant cowpea varieties.

Seed transmission of viruses seriously limits crop potential yield because seed-borne viruses can reduce the quality of seeds and cultivation of infected stock may bring about onset of disease epidemics (Jones and Coutts, 1996). Mild to severe foliar symptoms of seed transmitted BCMV - BlCM, SBMV and CMV were observed on the cowpea plants

especially on IT98K-133-1-1, IT98K-503-1, IT99K-1060 and Ife brown although not all the six susceptible genotypes tested showed transmission of the three viruses. The symptoms ranged from mottling, mild mosaic and vein banding in BCMV - BlCM transmission to mottling, mosaic, veinal and inter-veinal chlorosis and mild puckering in SBMV and CMV transmission. Similar symptoms of cowpea seed transmission have been reported in BCMV - BlCM (Udaya shankar *et al.*, 2009), CMV (Abdullahi *et al.*, 2001) and SBMV (Thottappilly and Rossel, 1988). Symptomatology alone was not adequate in seed transmission study especially due to the occurrence of latent infection. Thus, the use of serology for virus detection produced a higher transmission result than symptomatology. Abdullahi *et al.*, 2001 observed similar result in CMV where symptom assessment gave transmission rate of 6% while ELISA produced 30%. Higher transmission rate was observed in singly infected seed than in mixed infected ones. This might be due to virus-virus interactions resulting in cross protection or antagonism or mutual exclusion among the viral partners.

Seed Transmission rates of BCMV - BlCM ranged from 4.8 % to 25.0 % in singly infected seeds and 2.0 % to 7.5 % from mixed infected seed. SBMV transmission rate ranged from 0.0 % to 2.2 % in singly infected and 2.0 % to 2.1 % in mixed infected seed, while CMV seed transmission ranged from 2.0 % to 26.0 % in singly infected seed and 2.0 % to 17.4 % under mixed infected seeds. These results are close to the seed transmission rates earlier reported in cowpea. Thirty percent seed transmission of BCMV - BlCM has been reported (Frison *et al.*, 1990) while incidence of seed-borne as high as 50 % BCMV - BlCM was observed by Gillaspie *et al.*, (1993). SBMV has been reported to be seed borne at rates of 3 – 4 % (Thottappilly and Rossel, 1988) and 30 % seed transmission rate has been reported in CMV (Abdullahi *et al.*, 2001).

Multiple virus transmissions, which have not been adequately reported, was observed in this study. Multiple transmission was observed from seeds of IT98K133-1-1 (4 % BCMC-BlCM, 12 % CMV), IT97K-1069-6 (2.0 % BCMV + BlCM and 2.0 % CMV) and Ife brown (2.2 % BCMV + BlCM and 2.2 % CMV) under triple infections.

Mixed infection hindered transmission of BCMV - BlCM under BCMV - BlCM + SBMV infection where BCMV - BlCM was not transmitted in the six tested genotypes whereas, SBMV was transmitted in two genotypes. Similar hindrance to seed transmission was observed of SBMV under SBMV + CMV and triple infections. The difference in seed

transmissibility of viruses under mixed infection compared with single infections might be due to interaction between or among the viruses in the hosts. Virus-virus interaction was also observed in the resistance evaluations in screen-house experiments where different results were obtained under mixed infections compared with that of single infections in few cowpea genotypes. For instance, in IT98K-133-1-1 susceptibility to BCMV - BlCM observed under single infection could not be detected in double infection with SBMV. Variation in the rate of seed transmission of each virus under mixed infected seed where co-infection resulted in reduced CMV transmission in cowpea line IT98-133-1-1 but to enhancement of BCMV - BlCM transmission in IT98K-133-1-1 and IT97K-1069-6 might also be due to the type of virus-virus and virus-host interactions occurring in mixed infections. Some of the mechanisms involved under virus-virus interaction, as reported by many workers, include, cross protection, mutual exclusion, recombination, gene silencing and some of which usually result in development of a new variant (DaPalma *et al.*, 2010; Sherwood and Fulton, 1982; Rentería-Canett *et al.* 2011; Fagoaga *et al.*, 2006; Syller, 2011). In this study, highest rate of seed transmission was observed for CMV, followed by BCMV - BlCM with low transmission of SBMV.

CHAPTER SIX

SUMMARY AND CONCLUSION

This study was conducted to investigate the mode of inheritance of resistance to three economically important virus diseases of cowpea. Fifty improved cowpea breeding lines developed by IITA were initially screened for resistance to five cowpea viruses in a screen house experiment. Then, eight cowpea lines were selected out of the lines based on resistance status and evaluated for single and multiple resistance to BCMV – BlCM, SBMV and CMV, under both screen house conditions and natural field viral infections. Two resistant/tolerant (IT98K1092-1 and IT97K-1042-3) and two highly susceptible lines (IT99K-1060 and IT99K-573-1-1) to the viral diseases were selected from these evaluations and used in generating crosses and backcrosses of resistant x susceptible/ tolerant x susceptible plants for each of the viruses.

Six cowpea generations comprising of P_1 and P_2, F_1, F_2, BCP_1 and BCP_2 of each of the viruses were evaluated to determine their reactions to the viruses and segregation patterns into two classes of resistant and susceptible plants to each virus. The objectives of this study were to evaluate the eight improved cowpea breeding lines for single and multiple resistances against BCMV - BlCM, SBMV and CMV, investigate the effects of the viruses on their seed yield parameters, carry out genetic studies to determine the mode of inheritance of resistance to the three virus diseases and determine seed transmission of the viruses under single and mixed infections.

The conclusions and recommendations were as follows:

1. Combination of virus disease severity with serology strengthened with nucleic acid detection tools give a better evaluation and classification of cowpea genotypes into resistance status than symptomatology alone.

2. This study established new sources of single and multiple resistances to BCMV - BlCM, SBMV and tolerance to CMV in new improved cowpea breeding lines. Cowpea line IT99K-1092-1 is a source of multiple resistances to BCMV - BlCM and SBMV and tolerance to CMV, IT97K-1042-3 has multiple resistance to BCMV - BlCM and SBMV while IT98K-133-1-1, IT97K1069 and IT04K-405-5 are sources of single resistance to SBMV.

3. The study provided information on genetic studies on patterns of inheritance of BCMV - BlCM, SBMV and CMV resistance required in breeding for cowpea varieties that are resistant/tolerant to these viruses. Inheritance of resistance to BCMV - BlCM was found to be conditioned by single recessive gene pair in cowpea line IT97K-1042-3 while duplicate dominant genes controlled both resistance to SBMV and tolerance to CMV in IT98K-1092-1.

4. The genetic potential of cowpea breeding line IT98K-1092-1 was unveiled which indicated that the line, after further trials, can be released as new variety or used as a source of virus resistance genes that can be introgressed into susceptible higher yielding varieties.

5. Since mixed infection involving BCMV - BlCM + CMV has been reported to cause a devastating "cowpea stunt" disease, cowpea line IT98K-1092-1 with resistance to BCMV - BlCM and tolerance to CMV can serve as a parent line for introgression of disease resistance genes into higher yielding susceptible varieties to control this disease.

6. High susceptibility to BCMV - BlCM, SBMV and CMV was found in both IT99K-1060 and IT98K-503-1, making them unsuitable for planting in viruses' endemic areas.

7. Seed transmission rates of viruses in the cowpea lines were found to be high for CMV and BCMV - BlCM but low for SBMV. This shows the importance of planting virus-free cowpea seed especially in CMV and BCMV - BlCM diseases management

8. Further studies are required on genotyping of the virus resistance genes, gene mapping to determine the locations of the genes in the genome and development of DNA markers for the resistance genes.

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APPENDIX

>A *Bean common mosaic virus* strain *Bean common mosaic virus - blackeye cowpea mosaic strain*; Primer CIF/CIR; 683 bp, 95% similarity to BCMV - BlCM of accession No. FJ653926.1

TTCCGCGGTGGGGTCGGGGAAATCGACAGGTCTCCCACACAATTTGGCCAAGAAAGGAAAAGTTTT
ACTTTGTGAACCAACAAGGCCACTTGCTGAAAACGTTAGCAAACAACTGAGTAAAGATCCCTTTTA
TCAACATGTCACACTCAGAATGCGGGGAATGAACAAGTTTGGATCCAGCAATATCACTGTGATGAC
AAGTGGATTTGCTTTTCACTATTATGTGAACAACCCGCAGCAATTAAGTGATTTTGATTACATCAT
TTTCGATGAGTGTCATGTAATGGATAGCTCAGCCATTGCTTTCAACTGTGCTTTAAAGGAGTTTGA
GTTTGCAGGTAAGTTAATCAAGGTTTCAGCAACACCACCAGGACGTGAATGTGAGTTCACAACTCA
ACACCCTGTGAAGTTAAAAGTAGAGGAACAATTGTCTTTCGCTAATTTTGTACAAGCTCAAGGTAC
AGGTTCTAATGCTGACATGATACAGCATGGAGCTAACTTACTGGTGTATGTAGCGAGTTACAACGA
AGTTGATCAATTATCAAGATTGTTAATTGAGAAGAACTTCAAAGTGACAAAGGTGGATGGAAGAAC
AATGCAGATGGGGAATGTTGAAATCACAACAATGGGATCTGAGGGTAAACCACACTTTGTTGTTGC
CACCAATATATGAAATGGCGTAA

>B *Southern cowpea mosaic virus*; Primer SBMV F/R; 503 bp; 92% similarity to SBMV of accession No. DQ481604.1

TTGGTCCTTTCGACGCAATCTTCGAGCAAGTTGTCTCGAAAGCAACGTCGGCGGAGATTAACCAAA
AGGCCAGCCCAGGGGTCCCCCTCTCCCGCCTCGCCACCACCAACAAGGACTTAATGGCGCAACACT
TGCAGTTCGTTGCTGCTTGTGTAACTGAAAGGTTGCTACTCCTAGCCTCCTTCGAGAACATACATG
ATCTTTCTCCCACTGAGATGGTAGAGATGGGTTTGTGTGATCCGGTGCGACTCTTCGTTAAGCAAG
AACCGCATCCATCTCGTAAGTTGAAGGAAGGGAGGTATCGGCTTATCTCCTCGGTCTCGATAGTCG
ACCAATTGGTTGAAAGGATGCTCTTCGGAGCTCAGAACGAGTTGGAGATTGCTGAGTGGCAATCTA
TCCCTTCAAAACCCGGTATGGGACTTTCCGTCACCCACCAAGCTGACGTGATATTCCGTGACTTGC
GAGTCAAACATACCGTGTGTCCTGCAGCTGAAAGCAGACAA

>C *Cucumber mosaic virus*; Primer CMVI/CMV2; 489 bp; 98% similarity to CMV of accession No. D49496.1

TGCCGTAAGCTGGATGGACAACCCGTTCACCGCAAAGCGTTTAGTGACTTCAGGCAGTTTATAGCG
ATACTGCCAACTCAGCTCCCGCCTCAGAAAATGGAGGGAGGGTCCTGGGAAACACGGATTCAAACT
GGGAGCACTCCAGATGTGGGAATACGTTGGTGCTCAATGTCGACATGAAGCACTAGCTCATCCGAC
TCAAGTGTATCGTCTTTTGAATACACGAGTACGGCGTACTTACGCATGTCGCCAATATCAGCGCGC
ATCGGCGAAAGATCATACAACAATTTATTGTTGGCTTGGACTCCGGATGCAGCATACTGATAAACC
AGTACCGGTGAGGCTCCGTCCGCAAACATAGTAGAAATGGCGGCGACGGACAAGTCCGAAGAGGCA
GGAACTTTACGGACTGTCACCCACACGGTAGAATCAAATTTCGGCAAAGGATTAACTCGAATTTGA
ATGCGCGAAACAAGCTTCTTATCATAA

Appendix 1: Virus RNA sequences used in similarity search from GenBank databases using BLASTN and percentage similarity to submitted viruses A = BCMV - BlCM B = SBMV and C = CMV.