JOURNAL OF CROP IMPROVEMENT
https://doi.org/10.1080/15427528.2022.2090476

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Inheritance of resistance to three endemic viral diseases of cowpea in Nigeria

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ABSTRACT

Mosaic diseases, caused by bean common mosaic virus-blackeye cowpea mosaic strain (BCMV-BlCM), southern bean mosaic virus (SBMV), and cucumber mosaic virus (CMV), hamper the productivity of cowpea (*Vigna unguiculata* (L.) Walp.). Under single or mixed infections, these endemic viruses significantly reduce cowpea yield in sub-Saharan Africa. Planting resistant varieties is the most effective control method. Knowledge of the mode of inheritance of viral resistance is crucial in developing resistant varieties. Inheritance of resistance to BCMV-BlCM, SBMV, and CMV was investigated in two improved cowpea breeding lines. For BCMV-BlCM, crosses were made between resistant IT97K-1042-3 (female) and susceptible IT99K-1060 (male); for SBMV, between resistant IT98K-1092-1 (male) and susceptible IT99K-1060 (female); and for CMV, between tolerant IT98K-1092-1 (female) and susceptible IT99K-573-1-1 (male). The F_1 progenies were advanced to F_2, and some F_1 plants were backcrossed to the two parental lines. Reciprocal crosses were made and the 7-day-old seedlings of P_1, P_2, F_1, F_2, BCP_1, and BCP_2 were phenotyped by mechanical inoculation with BCMV-BlCM, SBMV, and CMV under screenhouse conditions. Data on disease incidence and severity were taken at weekly intervals for 5-week post-inoculation. Virus infections were confirmed via antigen-coated plate enzyme-linked immunosorbent assay or reverse transcription-polymerase chain reaction. Chi-square analysis of the genetic segregation indicated that a recessive gene pair in IT97K-1042-3 controlled the inheritance of resistance to BCMV-BlCM. Duplicate dominant genes conditioned the resistance to SBMV and tolerance to CMV in IT98K-1092-1. The backcrosses confirmed the monogenic and digenic inheritance patterns, whereas reciprocal crosses indicated absence of cytoplasmic effects.

ARTICLE HISTORY
Received 01 April 2022
Accepted 13 June 2022

KEYWORDS
ACP-ELISA; cowpea viruses; epistatic effect; inheritance pattern; RT-PCR

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most economically and nutritionally important indigenous African grain legumes. It is a diploid species (2 n = 2x = 22), often self-pollinated, and belongs to the family *Fabaceae* (Boukar et al. 2019). It is the most widely grown and consumed legume in Western Africa

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(Boukar et al. 2013). Nigeria is the largest producer of cowpea grain, accounting for about 40.9% of the 8.9 million tons of annual global production (FAO 2020). Viral diseases remain a significant constraint to cowpea productivity in sub-Saharan Africa (SSA) (Thottappilly and Rossel 1992; Legg et al. 2019). Three of the most important seed-transmitted viruses causing significant yield losses in cowpea in SSA are bean common mosaic virus-blackeye cowpea mosaic strain (BCMV–BlCM, genus *Potyvirus* family *Potyviridae*); southern bean mosaic virus (SBMV, genus *Sobemovirus*); and cucumber mosaic virus (CMV, genus *Cucumovirus*, family *Bromoviridae*) (Taiwo 2003; Boukar et al. 2019) (Table 1). More serious cowpea grain yield reductions were observed when mixed infections with more than one of these viruses occurred (Boukar et al. 2013). Co-infection of CMV and BCMV-BlCM, both transmitted by the same vector, has been reported to produce a synergistic interaction, leading to severe stunting and significant losses in cowpea productivity (Gillaspie, Hajimorad, and Ghabrial 1998; Ogunsola et al. 2021).

The use of host-plant resistance is the most economical and environment-friendly option for managing virus diseases (Orawu et al. 2013). Resistance mechanisms in the plant can either block virus replication, interfere with local (cell-to-cell) or vascular (leaf-to-leaf) movement, or/and induce a hypersensitive response (HR) related to programmed cell death (Jones and Dangl 1996). Knowledge of the inheritance pattern of resistance to the virus responsible for causing the disease symptom is essential for the success of a breeding program (Kang, Yeam, and Jahn 2005). Previous studies have identified several resistance (R) genes (Bashir and Hampton 1996; Umaharan, Ariyanayagam, and Haque 1997) and sources of resistance to single and dual viral infections in some cowpea germplasm (Boukar et al. 2013). Although, there are reports on the mode of inheritance of resistance to some viruses infecting cowpea (Taiwo, Provvidenti, and Gonsalves 1981; Orawu et al. 2013), information is still sparse for many of the important

Table 1. Properties of bean common mosaic virus-blackeye cowpea mosaic strain (BCMV-BlCM), southern bean mosaic virus (SBMV), and cucumber mosaic virus (CMV) infecting cowpea.

Virus	Genus	Insect transmission		Symptom	Yield Loss (%)	Reference
		Vector	Mode			
BCMV-BlCM	Potyvirus	Aphid	Non-persistent	M, Mo, Vb, Vc	54–74	Udayashankar et al. (2010); Jordan and Hammond (2008)
SBMV	Sobemovirus	Leaf beetles	Non-persistent	M, Vc, Nl, Ld	11–59	Givord (1981); Karim (2016)
CMV	Cucumovirus	Aphid	Non-persistent	M, Mo, Cl, Ld	14–20	Zitter and Murphy (2009)

M: mosaic; Mo: mottling; Vb: vein banding; Vc: vein clearing; Nl: necrotic lesion; Ld: leaf distortion; Cl: chlorotic lesion.

cowpea viruses. Moreover, most of the evaluation of sources of virus resistance and inheritance of resistance in cowpea genotypes were limited to field evaluations or virus detection by ELISA, both of which have limitations. For instance, results from field screening under natural infections usually vary because of a lack of control on variables, such as, inoculum source, time of infection and vector activity necessary to ensure uniform infection (Ogunsola et al. 2021). Virus negative results by enzyme-linked immunosorbent assay (ELISA), without verification by polymerase chain reaction (PCR)-based diagnostics methods, may be inaccurate due to serologically variable strains of viruses or low virus titer undetectable in ELISA but detectable by PCR (Aliyu, Balogun, and Kumar 2012). This information on the inheritance of virus resistance in cowpea is required in resistance breeding programs because most of the available landraces and commercial cowpea varieties lack durable resistance to the most frequently reported viruses in West Africa (Legg et al. 2019).

The genetic bases for resistance to many viral diseases in cowpea are still largely unknown. Understanding the inheritance of resistance to viral diseases of legumes, especially those caused by BCMV-BlCM, SBMV, and CMV, is of prime importance for the selection of effective breeding strategy, mating design, and molecular breeding techniques for improved virus-resistant crop varieties (Akbar et al. 2018). A recent study has identified resistance to BCMV-BlCM in cowpea line IT97K-1042-3 and both resistance to SBMV and tolerance to CMV in cowpea line IT98K-1092-1 (Table 2) (Ogunsola et al. 2021). The line IT90K-284-2, a progenitor of IT97K-1042-3 (Table 2), had also been reported earlier to have a high level of resistance to BCMV-BlCM (Bashir et al. 1995). However, the patterns of inheritance to these viruses have not been characterized. This study investigated the inheritance of resistance to mosaic disease caused by BCMV-BlCM, SBMV, and CMV in some improved cowpea breeding lines (Table 2). Understanding the genetics of cowpea resistance to viral diseases contributes to developing genomic markers for accelerating the development of improved virus-resistant cowpea varieties.

Materials and methods

Plant materials

Isolates of BCMV-BlCM, SBMV, and CMV used were available in the Virology and Molecular Diagnostic (VMD) Unit of IITA, Ibadan. The pure isolates of these viruses were established and maintained by mechanical inoculation onto healthy plants of susceptible cowpea genotypes (Ife Brown, TVu 2657 and TVu 76) in an insect-proof screenhouse. Four improved cowpea breeding lines were developed at IITA, namely, IT97K-

Table 2. Characteristics of cowpea genotypes evaluated.

Genotypes	Response[a]	Seed coat color	Growth habit	Maturity	Pedigree
IT98K-1092-1	Resistant to BCMV-BlCM, and SBMV but tolerant to CMV	Black	S.E	Medium	IT93K-596 × TVu 12349
IT97K-1042-3	Resistant to BCMV-BlCM and SBMV but susceptible to CMV	Brown	E	Early	IT90K-284 × Achishiru-2
IT99K-573-1-1	Susceptible to BCMV-BlCM, CMV but tolerant to SBMV	White	P	Medium	IT93K-596-6-12 × IT86D-880
IT99K-1060	Susceptible to BCMV-BlCM, SBMV and CMV	Brown	E	Early	Unknown

Source: Cowpea Breeding Unit, IITA, Ibadan, Nigeria.
[a]Cowpea response to viral infections (Ogunsola et al. 2021).
S.E: semi-erect; E: erect; P: prostrate.

1042-3 (resistant to BCMV-BlCM and SBMV), IT98K-1092-1 (resistant to BCMV-BlCM and SBMV and tolerant to CMV), IT99K-573-1-1 (susceptible to BCMV-BlCM and CMV and tolerant to SBMV), and IT99K-1060 (susceptible to all three viruses), were used as parental lines (Table 2). Seeds were treated with Benlate fungicide at 1.0 g per 40 seeds. The seeds were sown in 10 in. (25 cm) plastic pots filled with sterilized sandy loam soil and placed in an insect-proof screenhouse. Prostrate and semi-erect plants were staked to enhance crossing.

Crossing procedures

The hybridizations were carried out in an insect-proof screenhouse and followed the emasculation and hand-pollination procedure for cowpeas described by Myers (1991). Crosses were made between the parental lines resistant (P_1) and susceptible (P_2) to each virus (Tables 3, 4, and 5). Floral buds of the female parents that had reached their maximum unopened size (a day prior to opening) were carefully emasculated by making approximately 4.0 mm cut on the concave part of the unopened flower. The upper part of the cut segment was lifted with forceps, exposing the style and stamens, and anthers were carefully removed. Flowers that opened on male parents were plucked and their pollen dusted by rubbing the anthers on the stigma and hairy segment of the style of emasculated flowers of the female parent plants. Labeled tags were carefully fixed to the base of the pollinated female flowers to develop to pod maturity.

Table 3. Inheritance of resistance to bean common mosaic virus (BCMV-BlCM) in cowpea.

Generations and crosses	No. of plants R	S	Total	Expected ratio	χ^2	Probability	BCMV-BlCM resistance gene action
Resistant parent (R) IT97K-1042-3 (aa)	30	-	30				Recessive
Susceptible parent (S) IT99K-1060 (AA)	-	35	35				Dominant
F_1 (R × S) (aa × AA)	-	33	33				Recessive resistance
F_2	72	251	323	3:1	1.278	0.30–0.20	Monogenic recessive
Backcrosses							
BCP_1 (R × F_1)	18	20	38	1:1	0.106	0.80–0.70	Monogenic recessive
BCP_2 (S × F_1)	-	26	26	-	-	-	
Reciprocal cross							Monogenic recessive
F_1 (S × R) (AA × aa)	-	28	28				
F_2	69	157	226	3:1	3.687	0.10–0.05	
Backcrosses							
BCP_1 (S × F_1)	-	38	38	-	-	-	
BCP_2 (R × F_1)	19	15	34	1:1	0.471	0.50–0.40	

aa: line with recessive trait.
AA: line with dominant trait.
R: resistant plants; S : susceptible plants.

Table 4. Inheritance of resistance to southern bean mosaic virus (SBMV) in cowpea.

Generations and crosses	No. of plants R	S	Total	Expected ratio	χ^2	Probability	SBMV resistance gene action
Susceptible parent (S) IT99K-1060 (aabb)	-	28	28				Recessive
Resistant parent (R) IT98K-1092-1 (AABB)	40	-	40				Dominant
F_1 (S × R) (aabb × AABB)	45		45				Dominant resistance
F_2	207	18	225	15. 1	1.178	0.30–0.20	Duplicate dominant
Backcrosses							
BCP_1 (S × F_1)	26	9	35	3. 1	0.009	0.95–0.90	Duplicate dominant
BCP_2 (R × F_1)	36		36	-	-	-	

aabb: Line with recessive trait.
AABB: Line with dominant trait.
R: resistant plants; S : susceptible plants.

The BCMV-BlCM resistant line IT97K-1042-3 (female parent) was crossed with the susceptible IT99K-1060. The F_1 plants were advanced to F_2, whereas the former was backcrossed to each of the two parents to generate BCP_1 and BCP_2 (Table 3). An SBMV-resistant line IT98K-1092-1 was crossed to the susceptible line IT99K-1060 (Table 4). However, in this case, the resistant line was used as a male parent because of the very low rate of successful crosses when the resistant line was used as a female parent. Some of the F_1 plants were advanced to F_2 and also backcrossed to the two parents to produce BCP_1 and BCP_2 generations (Table 4). The CMV-tolerant cowpea

Table 5. Inheritance of tolerance to cucumber mosaic virus (CMV) in cowpea.

Generations and crosses	No. of plants T	S	Total	Expected ratio	χ^2	Probability	CMV tolerance gene action
Tolerant parent (T) IT98K-1092-1 (AABB)	23	-	23				Dominant
Susceptible parent (S) IT99K-573-1-1 (aabb)	-	36	36				Recessive
F_1 (T × S) (AABB × aabb)	23	-	23				Dominant tolerance
F_2	264	25	289	15. 1	2.845	0.10–0.05	Duplicate dominant
Backcrosses							
BCP_1 (T × F_1)	16	-	16	-	-	-	
BCP_2 (S × F_1)	25	7	32	3. 1	0.167	0.70–0.60	Duplicate dominant
Reciprocal crosses							Duplicate dominant
F_1 (S × T) (aabb × AABB)	13	-	13				
F_2	166	16	182	15. 1	2.005	0.20–0.10	
Backcrosses							
BCP_1 (S × F_1)	13	5	18	3. 1	0.075	0.80–0.70	
BCP_2 (T × F_1)	18	-	18	-	-	-	

AABB : Line with dominant trait.
aabb : Line with recessive trait.
T : tolerant plants; S : susceptible plants.

line IT98K-1092-1 (female parent) and susceptible line IT99K-573-1-1 were crossed reciprocally, and the F_1 progeny was advanced to F_2, using some of them to generate backcrosses BCP_1 and BCP_2 (Table 5). Mature pods of the generations P_1, P_2, F_1, F_2, BCP_1, and BCP_2 from each cross were harvested, dried in the screenhouse, and shelled, and seeds were stored at 4°C until planting for virus resistance screening.

Evaluation of cowpea generations for reactions to viruses

Seeds of P_1, P_2, F_1, F_2, BCP_1, and BCP_2 were planted at a rate of two seeds per pot in an insect-proof screenhouse. Seedlings from the crosses were tested to confirm successful hybridization based on traits like shape, length, and width of the terminal leaflets, length of internodes, pod length, and shape of the plants compared to the parental lines. Seedlings were mechanically inoculated with BCMV-BlCM, SBMV, or CMV according to the parents crossed. Virus inoculum was prepared by grinding virus-infected cowpea leaves in a pre-chilled sterilized mortar in 1:10 (w/v) ratio in 0.05 M phosphate buffer, pH 7.5, containing 0.04% (v/v) β-mercaptoethanol. Inoculation was carried out by dusting carborundum (600 mesh) on the primary leaves of six- to eight-day-old seedlings to create micro-wounds, and freshly prepared inoculum of each virus was applied on respective test plants. Each population was screened on an individual plant basis, with each pot well labeled to identify

each plant. Sixteen Ife brown plants were raised and inoculated as a positive control, whereas 10 plants of each parental line were used as healthy control.

Virus detection using ACP-ELISA

Five weeks after inoculation (WAI), all plants were tested for BCMV-BlCM, SBMV, or CMV based on the type of virus under study. Antigen Coated Plate-Enzyme-linked Immunosorbent Assay (ACP-ELISA) containing homologous anti-rabbit antibodies for each virus available at the Virology and Molecular Diagnostics Unit of IITA, Ibadan, Nigeria, was used (Kumar et al. 2001). About 100 mg of tissue from the leaf apex of each plant was used for virus testing in a 96-well NUNC MaxiSorb (Nunc, Denmark) ELISA plate. Alkaline phosphatase (ALP)-labeled anti-rabbit antibodies were used to detect the immobilized antigen–antibody complex, and p-nitrophenyl phosphate (Sigma, Gillingham, UK) was used as substrate. After 1 h of incubation, absorbance readings were taken at 405 nm (A405 nm) in a Multiscan Plus ELISA plate reader (Labsystems, Helsinki, Finland). Sample with A405 nm value of at least twice (2x and above) that of the healthy control was considered positive for the virus. Samples that tested negative with ELISA were verified by retesting samples with Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using the primer pair corresponding to the respective viruses, according to Kumar (2009).

Virus detection by RT-PCR

Total RNA was isolated from the apical leaf tissues (100 mg) according to a modified cetyltrimethyl ammonium bromide (CTAB) method (Abarshi et al. 2010) and used for detection of BCMV-BlCM, SBMV, and CMV by RT-PCR according to the procedure described by Kumar (2009). BCMV-BlCM amplification was performed using the primer pair, CI-F, CGIVIGTIGGIWSIGGIAA RTCIAC and CI-R, ACICCRTTYTCDATDATRTTIGTIGC, which amplifies 700 bp segment (Kumar 2009). SBMV was detected using the primer pair, SBMV-F, TGGTCCTTCGACGCAATCT and SBMV-R, GTCTGCTTCAGCT GCAGGACA, which amplifies 500 bp segment (Salem et al. 2010) and CMV was detected using the primer pair CMV-F, GCCGTAAGCTGGATGGACAA and CMV–R, TATGATAAGAAGCTTGTT TCGCG, which amplifies 500 bp segment (Wylie et al. 1993). PCR amplification was performed in 12.5-µl reaction mixture comprising 10x PCR reaction buffer (supplied with Taq enzyme), 0.75 µl of 25 mM $MgCl_2$, 0.25 µl mixture of 10 mM deoxynucleotide triphosphates, 0.25 µl of respective primers, 12 units of Moloney-murine leukemia virus (M-MLV) reverse transcriptase (RT) (Promega Corporation, USA), 0.3 units of Taq DNA polymerase (Promega Corporation, Madison, Wisconsin, USA), and 2.0 µl of 10 ng/µl total RNA and sterile distilled water. Virus RNAs were amplified with

Applied Biosystems (GeneAmp® PCR System 9700) Cycler machine. Amplification of BCMV-BlCM RNA was done as follows: one cycle for 30 min at 42°C and 35 cycles of denaturation at 94°C for 30 sec, primer annealing at 40° C for 30 sec, extension at 68°C for 1 min and final extension at 72°C for 10 min. Similar thermal cycler conditions were used to detect SBMV and CMV except that annealing temperatures were 54°C and 50°C, respectively, for SBMV and CMV. Amplified RT-PCR products were separated in 1% agarose gel electrophoresis in 0.5 × TBE buffer and visualized under a UV transilluminator (BioRad) after staining in ethidium bromide (0.5 μg/ml).

Classification of test plants based on visual rating, serology, and molecular diagnostics

Viral disease incidence was determined using the ratio of the number of symptomatic to the total number of inoculated plants. Disease severity was determined by taking weekly symptom severity scores from the period of one-week after inoculation (WAI) to 5 WAI. Severity scale 1–5 was used, where 1 = no visible symptom, 2 = very mild mosaic or mottling on few leaves, 3 = mosaic or mottling on many leaves, 4 = severe mosaic, severe mottling, and mild stunting and 5 = severe mosaic, severe mottling, severe stunting with necrosis or death of leaves and/or plants (Ogunsola et al. 2021). Inoculated test plants in the different populations were classified as resistant, tolerant, or susceptible based on symptom severity scores, and diagnostic confirmation for the presence or absence of viruses was done via ACP-ELISA and/or RT-PCR. Plants without symptoms (severity score 1) and virus-negative in diagnostic test were classified as resistant (R). Plants with a severity score between >1 and 2 (mild mosaic or mottling without reduction in growth and vigor) and test positive for virus were classified as tolerant (T). Plants with a severity score of 3, 4, and 5 and positive to virus in the diagnostic test were classified as susceptible (Ogunsola et al. 2021).

Data analysis

Plants of P_1, P_2, F_1, F_2, BCP_1, and BCP_2 generations of each cross were classified into resistant or susceptible to BCMV-BlCM, SBMV, and tolerant or susceptible to CMV. Data of the F_2, BCP_1, and BCP_2 individuals in the direct and reciprocal crosses, scored as resistant/tolerant or susceptible, were subjected to Chi-square (χ^2) analysis for goodness-of-fit to test the deviation of the observed segregation data from the theoretically expected Mendelian segregation ratio. The comparison between the observed and expected frequencies was used to determine the patterns of inheritance and estimate the number of genes controlling resistance/tolerance, using χ^2 analysis as per the

formula of Gomez and Gomez (1984) given as follows: $\chi^2 = \sum (O - E)^2/E$ where O = observed number of individuals and E = expected number of individuals

Results

Inheritance of resistance to BCMV–BlCM disease in cowpea

Evaluation of the parental lines and F_1, F_2, BCP_1, and BCP_2 generations for resistance to BCMV–BlCM showed that all inoculated plants of line IT97K-1042-3 were symptomless (Figure 1) (a1), negative to ACP-ELISA, and confirmed negative by RT-PCR (Figure 2 (a)). The susceptible parental plants (IT99K-1060) developed systemic symptoms characteristic of BCMV–BlCM (Figure 1 (a3)) and tested positive for BCMV–BlCM via ELISA and RT-PCR (Figure 2 (a2)). Symptom expression started with mild mosaic and mottling, which progressed into mosaic and vein banding with the aging of plants. The F_1 plants developed symptoms similar to that of the susceptible parent (Figure 1 (a2)), suggesting that resistance to BCMV–BlCM was recessive. Plants of F_2 generation responded to virus inoculation with some symptomless plants that tested negative in ELISA and RT-PCR, whereas others showed mild or severe symptoms (Figure 1 (a4)). Evaluation of the F_2 plants for resistance to BCMV–BlCM showed 72 resistant and 251 susceptible plants (Table 3). The segregation pattern by Chi-square (χ^2) test gave a goodness-of-fit of 1 resistant: 3 susceptible, which indicates that a single recessive gene pair conditions the resistance to BCMV–BlCM in IT97K-1042-3. Symptoms observed on most of the symptomatic backcross generation plants were not severe (Figure 1 (a5 and a6)). Plants resulting from backcross to the susceptible parent (BCP_2) were all symptomatic, indicating that they were susceptible, whereas those from backcross to the resistant parent (BCP_1) segregated into 18 resistant: 20 susceptible. This fitted a ratio of 1 resistant: 1 susceptible ($p > 0.05$). These results of the backcross generations confirmed the monogenic inheritance of resistance to BCMV-BlCM (Table 3) in cowpea line IT97K-1042-3. The F_2 generation that resulted from a reciprocal cross between the same parents gave 157 susceptible to 69 resistant plants, which fitted a segregation ratio of 3 susceptible to 1 resistant plant (Table 3). Evaluation of the backcross to resistant parent also resulted in 15 susceptible to 19 resistant plants, which gave a goodness-of-fit to 1 susceptible: 1 resistant segregation ratio, indicating the absence of maternal or cytoplasmic inheritance.

Inheritance of resistance to SBMV disease in cowpea

Symptoms developed on susceptible parental plants of line IT99K-1060 (P_1) following inoculation with SBMV (Figure 1 (b1)). Serological analysis using

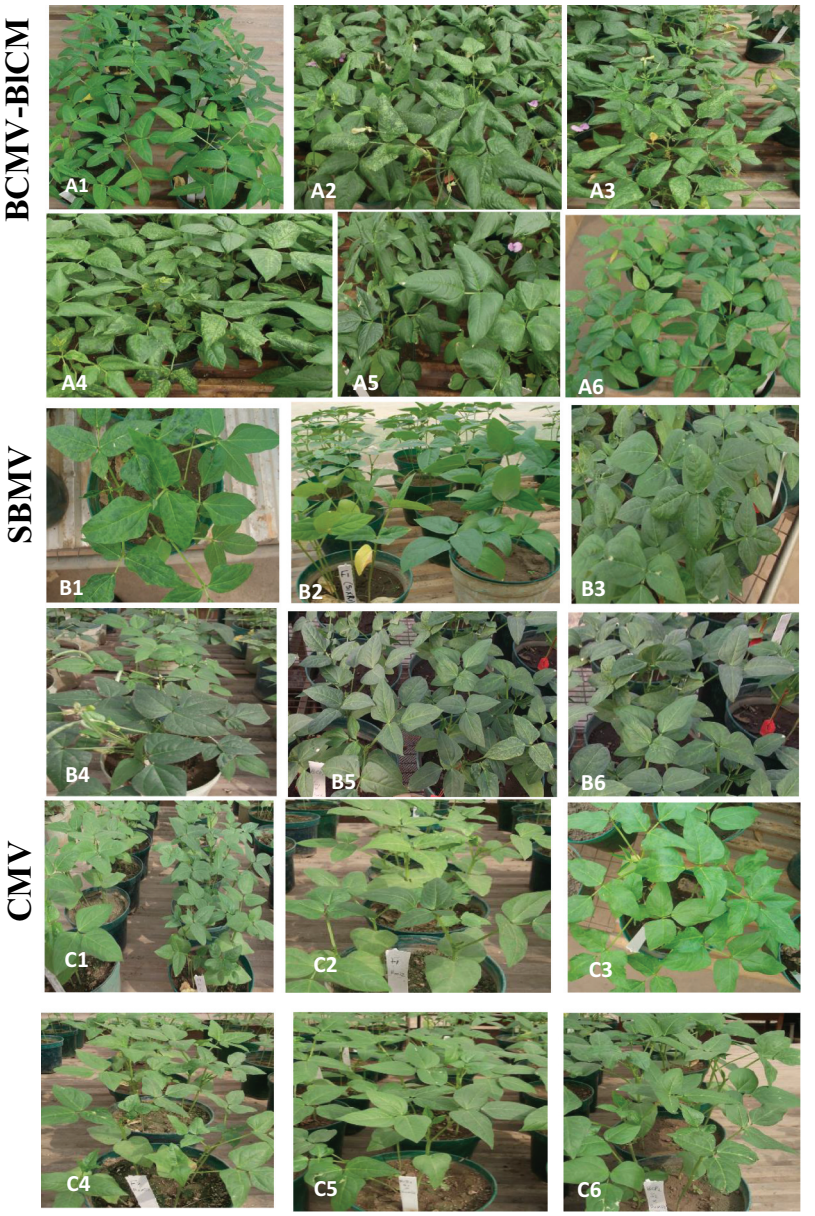

Figure 1. (a – c). P_1, F_1, P_2, F_2, BCP_1 and BCP_2 generations of direct crosses between (a) BCMV-BICM (b) SBMA and (c) CMV resistant (R), tolerant (T) or susceptible (S) cowpea lines. (a1) P_1, BCMV-BICM resistant male parent: IT97K-1042-3; (a2) F_1 (R x S); (a3) P_2, BCMV-BICM susceptible female parent: IT99K-1060; (a4) F_2; (a5) BCP_1 (R x F1); (a6) BCP_2 (S x F1); (b1) P_1, SBMV susceptible female parent: IT99K-1060; (b2) F_1 (S x R); (b3) P_2, SBMV resistant male parent: IT98K-1092-1; (b4) F_2; (b5) BCP_1 (S x F1); (b6) BCP_2 (R x F1); (c1) P_1, CMV tolerant: IT98K-1092-12; (c2) F_1 (T x S); (c3) CMV P_2, CMV susceptible: IT99K-573-1-1; (c4) F_2; (c5) BCP_1 (T x F1); (c6) BCP_2 (S x F1). A = BCMV-BICM, B = SBMV and C = CMV.

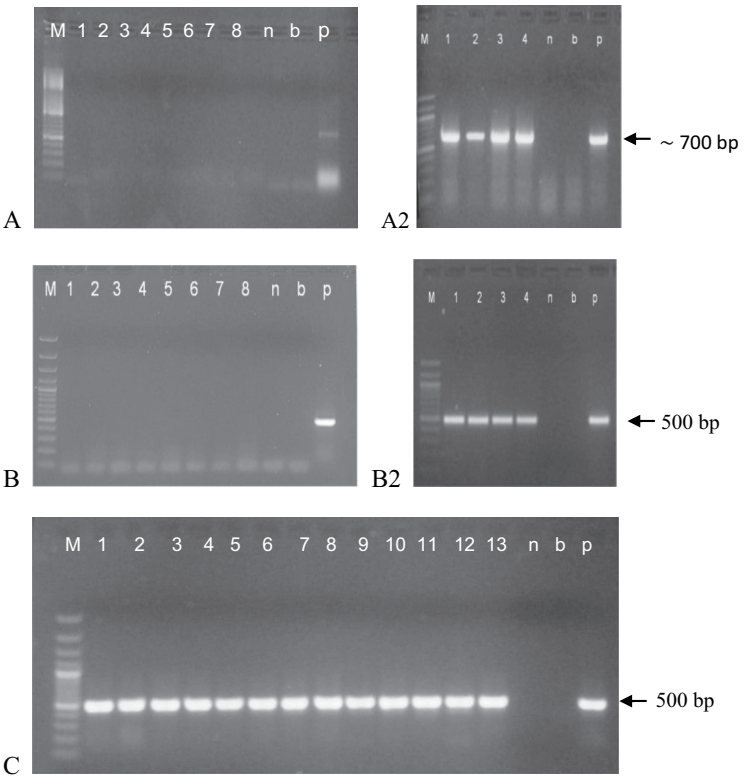

Figure 2. (a – c) Detection or lack of detection of (a) BCMV-BlCM, (b) SBMV and (c) CMV in cowpea by RT-PCR; M = DNA size marker (100 bp ladder; Promega); lanes 1–4, 1–8 and 1–13 = extracts of plant samples (A = samples with no detection of BCMV-BlCM, A2 = BCMV-BlCM infected samples; B = samples with no detection of SBMV and B2 = SBMV infected samples; C = CMV infected samples); n = uninfected cowpea sample; b = no template control; p = virus positive control.

ACP-ELISA confirmed the presence of the virus in the plants, whereas P_2 plants were asymptomatic (Figure 1 (b3)) and negative when tested by ELISA and RT-PCR (Figure 2 (b)). All the F_1 plants evaluated showed resistance to SBMV infection, suggesting the dominance of resistance to SBMV in parent IT98K-1092-1. Visual evaluation and diagnostic test of F_2 generation plants revealed 207 resistant and 18 susceptible, which when subjected to Chi-square analysis ($p > 0.05$) fitted a segregation ratio of 15 resistant:1 susceptible (Table 4). This indicated an epistatic effect of two dominant genes in duplicate gene action. The backcross to the susceptible parent showed segregation of 26 resistant: 9 susceptible plants, which fitted the 3 resistant: 1 susceptible ratio. This suggested that duplicate dominant genes conditioned the resistance of IT99K 1092–1 to SBMV.

Inheritance of tolerance to CMV disease in cowpea

When inoculated with CMV, some of the tolerant parent plants (IT98K-1092-1) did not produce visible symptoms, whereas others showed mild symptoms (severity scores >1 to 2) of mottling and inter-veinal chlorosis but without puckering (Figure 1 (c1)). The susceptible parent plants (IT99K-573-1-1) inoculated with CMV produced visible symptoms of mottling, mosaic, inter-veinal chlorosis, and puckering, which began to appear 8 days after inoculation (Figure 1 (c3)). Symptom expression was obvious in all susceptible plants (severity scores 3 to 4). Both tolerant and susceptible parental lines and F_1, F_2, BCP_1, and BCP_2 generations tested positive for CMV using ACP-ELISA and RT-PCR (Figure 2 (c)). Meanwhile, symptoms faded in some CMV symptomatic plants, starting from 4 weeks after inoculation, although such plants remained positive for CMV via the ELISA test. The F_1 plants derived from the cross between tolerant and susceptible lines when inoculated with CMV showed reactions similar to that of the tolerant parent, in which some plants were symptomless, whereas others showed mild symptoms (Figure 1 (c2)). This suggested that the tolerance of line IT99K-1092-1 to CMV was a dominant trait. Following the visual observation, the F_2 generation segregated 264 tolerant: 25 susceptible plants, which gave a goodness-of-fit to 15 tolerant: 1 susceptible segregation ratio (Table 5). The segregation ratio of 3:1 tolerant: susceptible plants (25 tolerant: 7 susceptible plants) obtained from the backcross to the susceptible parent supported the digenic inheritance of tolerance to CMV (the expected backcross ratio 1:1:1:1 of a dihybrid cross modified into 3:1 tolerant to susceptible plants due to the duplicate dominant epistatic gene action). Reciprocal crosses between the same parental lines gave similar segregation ratios of 15 tolerant: 1 susceptible in the F_2 generation, indicating the absence of extrachromosomal (maternal) inheritance (Table 5).

Discussion

In this study, analyses of F_1, $F_{2,}$ backcrosses, and reciprocal crosses between resistant and susceptible lines indicated that resistance to BCMV-BlCM in cowpea line IT97K-1042-3 was controlled by a recessive gene pair, with no maternal effects. Previous studies on BCMV-BlCM resistance in cowpea suggested diverse mechanisms with respect to the mode of inheritance. Single recessive genes were reported to be responsible for resistance to the virus by Arshad et al. (1998) and Taiwo, Provvidenti, and Gonsalves (1981). In contrast, Quatara and Chambliss (1991) observed a single dominant gene-controlled resistance to this virus in cowpea. Similar results of a single dominant gene conditioning the resistance to BCMV-BICM were reported

in common bean (*Phaseolus vulgaris*) and cowpea (Provvidenti, Gonslaves, and Taiwo 1983; Sharma et al. 2008).

Unlike the previous studies, which were based on the identification of resistant plants using only symptom expression and serological detection, the PCR-based detection method used in this study increased the accuracy of the classification of infected cowpea populations into resistant and susceptible plants. Meanwhile, the single dominant resistance gene reported in cowpea cultivar "White Acre-BVR" by Quatara and Chambliss (1991) is different from that found in the present study. A combination of the two genes in a cowpea variety should confer more durable resistance to BCMV-BICM than if either gene is used as a source of resistance. Should any of the two genes break down due to the development of a new mutant virus, the other gene would still confer resistance to the virus. The possibility of a breakdown of resistance gene in cowpea has been observed with the single dominant gene (*Rac*) (Bata et al. 1987) in germplasm line TVu3000 (Boukar et al. 2020) that was used to confer resistance to aphids in the seedling stage of plant development.

Crosses between the SBMV-resistant line IT98K-1092-1 and susceptible line IT99K-1060 could not be made when the former was used as a female parent. The F_1 and segregating populations (F_2, BCP_1, and BCP_2) derived from the cross between IT98K-1092-1 and IT99K-1060 were evaluated for their reactions to the virus. Chi-square analysis of the F_1, F_2, BCP_1, and BCP_2 populations showed that duplicate dominant genes with an epistatic interaction conditioned the inheritance of resistance to SBMV in the cowpea line IT98K-1092-1. Brantley and Kuhn (1970) and Fery (1980) have earlier reported dominance of resistance to SBMV in cowpea controlled by a single gene. Furthermore, the inheritance pattern of non-necrotic resistance to SBMV in cowpea was cultivar-dependent. A cross between an SBMV-susceptible line "California Blackeye" and three resistant cowpea lines showed that SBMV resistance in cultivar "Early Pinkeye and "PI 18646" was conferred by a single gene with partial dominance, whereas that in "Iron," the third variety, was attributable to multiple genes with incomplete dominance (Hobbs et al. 1987).

The symptoms induced by SBMV are reported to be variable among cowpea lines, ranging from asymptomatic lines to those showing severe mosaic, with deformed leaves (Kuhn 1990). Being seed-borne, SBMV has become widely distributed across several countries where cowpea is grown (Thottappilly and Rossel 1992). This is a further reason why concerted efforts should be made to develop improved cowpea varieties with resistance to this virus. The detection of the line with resistance to the virus controlled by duplicate dominant genes with epistatic effects should enable the successful development of resistant varieties.

In the case of CMV, only a tolerant cowpea line (IT98K-1092-1) was detected. The absence of symptoms by some of the CMV-tolerant plants might be due to the already reported latency of the virus in IT98K-1092-1 (Ogunsola et al. 2021). The F_1, F_2, BCP1, and BCP2 resulting from the cross between IT98K-1092-1 and susceptible IT99K-573-1-1 showed F_1 plants to be tolerant. This is evidence that tolerance in line IT98K-1092-1 is dominant to susceptibility. The F_2 and backcross generations confirmed that tolerance to CMV in the cowpea line IT98K-1092-1 was governed by duplicate dominant genes, with the absence of maternal or extra-chromosomal factors. Reports on the mode of inheritance of tolerance to CMV are limited in cowpea. The CMV, which is seed-borne and transmitted by aphids, is considered to have mild effects on the crop under a single infection (Pio-Ribeiro, Kuhn, and Brantley 1980) but causes severe symptoms in the case of mixed infection with other viruses (Ogunsola et al. 2021). It is, however, widespread in distribution. In view of the presently available knowledge on the genome of this virus, CMV-mediated transgenic resistance is feasible as a means of controlling the disease in cowpea (Hampton, Thottappilly, and Rossel 1997).

The non-parametric chi-square analysis for testing the goodness-of-fit to simple genetic model has been used in several studies to investigate the mode of inheritance of resistance to viral diseases of cowpea (Quatara and Chambliss 1991; Arshad et al. 1998; Orawu et al. 2013; Silva et al. 2021) and many other legumes (Ogundiwin et al. 2002; Sharma et al. 2008). Monogenic (Quatara and Chambliss 1991; Silva et al. 2021) or digenic inheritance (Barro et al. 2016) of virus resistance were mostly reported in cowpea and other legumes. However, in some cases, viral diseases of cowpea were observed to be quantitatively inherited (Orawu et al. 2013). Investigating quantitative inheritance is usually carried out using combining ability estimates of cowpea lines or a generation mean analysis (Akbar et al. 2018). In addition, studies involving both quantitative and qualitative analyses of resistance to viruses in cowpea have been reported. For instance, cowpea aphid-borne mosaic virus (CABMV) resistance was reported to be conditioned by a single recessive gene in seven cowpea populations and more than one recessive gene in eight other populations, in which both additive and non-additive gene actions were indicated for the virus resistance (Orawu et al. 2013).

Most of the reported studies on the inheritance of resistance to BCMV-BlCM (Taiwo, Provvidenti, and Gonsalves 1981; Quatara and Chambliss 1991; Arshad et al. 1998) and SBMV (Hobbs et al. 1987) in cowpea were based on plant virus assessment by symptomatology and ELISA. When not validated by molecular diagnosis, both methods might impair the accuracy of the observed classes of the segregating plant populations categorized into resistant or susceptible plants.

Recently, BCMV-BlCM, SBMV, and CMV have been reported to be among the most important cowpea-infecting viruses in SSA (Legg et al. 2019; Kumar et al.

2021). Knowledge of patterns of inheritance of resistance and tolerance to the viruses in cowpea varieties found here should help plant breeders and virologists develop breeding strategies that will provide effective and stable disease management. Although there is a higher likelihood of breakdown of monogenic resistance following the evolution of new virulent strains with time than the resistance conditioned by polygenes (Arshad et al. 1998), monogenic resistance is not always unstable in edible legumes. For instance, monogenic resistance to BCMV was observed in common bean (*Phaseolus vulgaris*) for nearly half a century. Resistance to anthracnose in the same crop lasted for almost 20 years. It was only in a few diseases of legumes, such as bean rust and lima bean downy mildew, where monogenic resistance was for a short duration (Meiners 1981). It is easier and faster to transfer monogenic than multigenic resistance to other desired cultivars (Arshad et al. 1998) since the use of monogenic resistance requires fewer resources. The monogenic inheritance of BCMV-BlCM resistance and digenic nature of inheritance of resistance and tolerance to SBMV and CMV, respectively, as observed in this study, can enhance ease of transfer of the viral R genes from the identified resistant lines in developing virus-resistant cowpea varieties. The genomic resources generated in cowpea can be explored to facilitate progress in the development of varieties with resistance to these and other disease-causing organisms.

Acknowledgments

This work was supported by the CGIAR Research Program on Dryland Cereals and Legumes, and the Bill & Melinda Gates Foundation (BMGF) (Grant Number: OPP48014) funded Tropical Legumes – II Project. The first author is grateful to IITA Ibadan for providing a PhD research fellowship and the Nigeria Agricultural Quarantine Service (NAQS) for granting study leave.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Bill and Melinda Gates Foundation [Grant Number: OPP48014].

Author contributions

The study was conceived, designed, and supervised by PL Kumar and CA Fatokun. KE Ogunsola conducted experiments, carried out data collection and data analysis, and prepared the first draft of the manuscript. Funding acquisition was by PL Kumar, CA Fatokun, and O Boukar. All authors reviewed and approved the final manuscript.

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