

Genetic inheritance of resistance to cowpea aphid-borne mosaic virus in cowpea

Martin Orawu · Rob Melis · Mark Laing · John Derera

Received: 24 February 2012 / Accepted: 3 July 2012 / Published online: 15 July 2012
© The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract Cowpea aphid-borne mosaic virus (CABMV) is a major virus disease in Uganda that causes substantial loss of the cowpea crop especially in growth and yield. The mode of gene action conferring resistance to the virus is not well understood. The objective of the study was to determine the genetic inheritance of resistance in cowpea crosses. Three susceptible (S) cowpea landraces that are commonly grown by farmers were crossed with five introduced resistant cowpea varieties in accordance with a North Carolina mating design II scheme. The F_1 , F_2 and BC_1F_1 progenies generated were evaluated in the field together with their parents. They were then infected with two infection methods namely: by spreader-rows of S cultivar (Ebelat) and artificial inoculation of virus extracts. The results obtained showed that general combining ability (GCA) and specific combining ability (SCA) effects were significant, indicating that both additive and non-additive gene effects controlled virus infection. The results further demonstrated that

the GCA effects (59.8 %) were more important than SCA effects (40.2 %) in determining virus resistance in the cowpea varieties. Utilisation of good general combiners of the varieties MU-93, IT82D-516-2, SECOW-2W and IT85F-2841 in hybridisation to improve virus resistance in cowpea crosses would be recommended. The result of this study provided an indication that CABMV resistance was conditioned by more than one recessive gene in eight populations, but also revealed resistance to be conditioned by a single recessive gene in the other seven populations. Observation of continuous distribution of progenies for severity data in the F_2 populations also confirmed significance of quantitative inheritance for CABMV resistance. Therefore, the significance of GCA effects suggests that recurrent selection could be applied to accumulate the additive genes for resistance in F_2 populations.

Keywords Cowpea · Cowpea aphid-borne mosaic virus · Gene action

M. Orawu (✉)
National Semi-Arid Resources Research Institute
(NaSARRI), National Agricultural Research
Organization, Plot 11-13, Lugard Avenue,
P.O. Box 295, Entebbe, Uganda
e-mail: orawum@gmail.com

R. Melis · M. Laing · J. Derera
African Centre for Crop Improvement, Plant Pathology,
University of KwaZulu-Natal, Private Bag X01,
Scottsville, Pietermaritzburg 3209, South Africa

Introduction

Cowpea is one of the major food legumes cultivated commercially in most tropics and sub-tropics and it is known to provide an important source of proteins for the rural poor farmers in developing countries (Bashir et al. 2002). However, sufficient production of cowpea has been dwindled by several factors, but most

importantly is due to prevalence and persistence of virus disease infection. Cowpea aphid-borne mosaic virus (CABMV) is considered an important constraint on cowpea crop in all agro-ecological zones, wherever it is grown (Emechebe and Lagoke 2000; Bashir et al. 2002). The CABMV is economically important virus because it can cause yield losses exceeding 87 % under field conditions (Kaiser and Mossahebi 1975; Mali and Thottappilly 1986; Bashir and Hampton 1996; Shoyinka et al. 1997). The virus is transmitted non-persistently by several aphid species, but *Aphis craccivora* Koch is the major aphid vector (Atiri et al. 1984). The CABMV is a pathogen of many crops, including common beans (*Phaseolus vulgaris* L.) Fabaceae and has a wide host range (Behnckken and Maleevsky 1977). The CABMV, a member of the genus potyvirus belonging to the family potyviridae, is one of the plant viruses that cause the most widespread disease in cowpea in the world (Rybicki and Pietersen 1999). The intriguing characteristics of potyviral diseases are the appearance of mosaic, vein clearing, mottling, deformation and stunting of plants, which are characteristics of CABMV.

In the management of virus diseases, the use of host plant resistance is considered to be the most economical and environmentally friendly. Heritable forms of resistance have been found in certain cultivars or landraces (Fraser 1992). Byoung-Cheorl et al. (2005) noted that the use of resistant (R) varieties is cost-effective for farmers, but considerable time and cost may be involved in developing varieties with appropriate levels of resistance. Although several measures have been examined for control of the virus, host plant resistance is viewed as the most economical, practical and environmentally friendly option (Bashir and Hampton 1996). Breeding for resistance has become increasingly common practice in controlling viral diseases (Arshad et al. 1998). Sources of resistance to CABMV have been identified and used in cowpea improvement (Van Boxtel et al. 2000). However, resistance to CABMV and blackeye cowpea mosaic viruses (BICMV) in *P. vulgaris* (L.) Fabaceae has been reported to be conferred independently by single dominant factors that appear to be closely linked (Provvidenti et al. 1983).

Previous reports indicated that several sources of genetic resistance to viruses in cowpea have been identified (Bashir and Hampton 1996; Umaharan et al. 1997b). The concerted efforts by the IITA research

team have transferred R genes into popular cowpea landraces to boost production for cowpea growers in West and Central Africa (IITA 1998). In Uganda, the major cowpea growing areas are in the eastern and northern regions, where the crop is grown for food security and cash income. Despite the demand by farmers in the cultivated regions for commercialisation of cowpea, it is often hampered by the epidemics of CABMV. The CABMV disease is thus a major hindrance to cowpea production in the regions, sometimes registering zero yields in fields grown with susceptible (S) cowpea cultivars. Indeed, CABMV has been reported to be common in the cowpea growing regions of Uganda (Edema et al. 1997), and is a threat to cowpea production. Besides, there have been no efforts to improve resistance to CABMV in the local S cowpea cultivars in Uganda. This work, therefore, focused on the development of R varieties for future use by breeders and other stakeholders for commercialisation.

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

Materials and methods

Experimental conditions at the research station (NaSARRI)

Experimental trials were established under natural field and greenhouse conditions at the research station. The climatic conditions such as temperatures ranged from 17.5 to 27.5 °C for minimum and maximum, respectively, while rainfall ranged from 1,000 to 1,300 mm. In the greenhouse, physical watering was done to irrigate the plant stands whenever necessary at

the research station. Generally, the soil type for crop growth at the research station constitutes clay-loamy. However, light duration was taken as constant during the time of the study.

Field establishment of cowpea genotypes for screening against CABMV

A total of 54 cowpea genotypes were evaluated and screened for resistance to CABMV in the field conditions at NaSARRI (data not presented). The design was a randomised complete block design with two replications. Two replications were considered because of limited seeds that were available. The replicates were separated by 2 m alleys with 1 m between plots and blocks. There were nine blocks each containing six plots within a replication. Individual genotype was planted at a spacing of 90 cm between rows and 40 cm within rows in a plot size of 4 × 3.6 m.

Field inoculation techniques and screening of cowpea genotypes

Two infection methods were employed namely: spreader-rows of S cultivar and artificial inoculation. The first method was done by planting the individual genotypes in rows in each plot surrounded by a S cultivar (Ebelat). The S cultivar was planted 10 days earlier to provide high pressure of aphids (*A. craccivora* Koch) and CABMV inoculum. In addition, the second method was carried out on the test genotypes by artificial inoculation of fully expanded primary leaves of 14-day-old seedlings with the virus extract. The extract was prepared by detaching and grinding the symptomatic leaves obtained from the insect-proof cage in a 0.01 M phosphate buffer. The insect-proof cage was used to rear aphids and allowed to feed on S cowpea seedlings of Ebelat from which the leaves were obtained to prepare the virus extract. The symptomatic leaf extract was prepared and by using a micropipette, 200 µl of the virus inoculum was measured and used to inoculate the individual test genotype on the young growing plant leaves in the field following carborundum powder (abrasive agent) application to the leaves to be inoculated. The carborundum powder was used to induce wounds on the plants to enhance virus penetration into the plant cells.

As a result of visual observation and ELISA tests, it was showed that out of the total of 54 cowpea genotypes evaluated, 49 genotypes were discarded due to severe infection by CABMV, while 5 genotypes were retained for breeding work because of good resistance to the virus (data not presented).

Plant materials and breeding activities in the greenhouse

Five genotypes that showed R levels to CABMV infection from a total of 54 genotypes were selected and used for breeding work. The R genotypes selected included IT82D-889, IT85F-2841, IT82D-516-2, MU-93 and SECOW-2W, and S landraces included Ebelat, Ecirikukwai and Blackcowpea. The S landraces are the cowpea cultivars commonly grown by farmers in Uganda. The parents were planted in five litre plastic buckets filled with top soil and watered using plastic watering container whenever necessary in the greenhouse. In order to synchronise the flowering periods of male and female parents, staggered planting was done at an interval of 5 days for any of the parents. In generating the crosses, the R parents were used as males, while the S set was used as females; in accordance of a North Carolina mating design II scheme to develop F₁ crosses. The seeds of each F₁ cross were harvested and planted in separate plots in the field for evaluation. The F₂ crosses were generated as result of selfing of F₁ crosses. To obtain the backcrosses, some of the F₁ seeds of each cross were planted separately in plastic buckets in the greenhouse. At flowering, each F₁ cross was backcrossed to the respective recurrent parents to generate BC₁F₁ seeds in the greenhouse using backcross scheme Fig. 1.

Field evaluation and inoculum technique

The 15 crosses generated in respect to F₁, F₂ and BC₁F₁ populations were planted separately in the field. The test populations were subjected to virus infection using two infection methods as mentioned before namely: natural and artificial technique. Using the natural technique as the first method of infection in the field trials, the seeds of the test plant populations (F₁, F₂ and BC₁F₁) were planted 10 days after introducing the spreader-infecter rows of S Ebelat. This was purposely done in order to generate adequate pressure of aphids (*A. craccivora* Koch) to cause

Table 1 Analysis of variance for CABMV assessment of three females, five males and F₁ progenies evaluated at NaSARRI

Source of variation	DF	Sum of square	Mean square Final severity
Replication	2	485.6	242.8
Genotypes	22	5797.1	263.5***
Parents (P)	7	2454.5	350.7***
G.C.A/females	2	1716.8	858.4***
G.C.A/males	4	280.3	70.1 ns
SCA	8	1345.1	168.1**
Crosses (C)	14	3342.2	238.7***
P versus C	1	0.4	0.4 ns
Error	44	1560.0	35.5

Data significant at ** $P \leq 0.01$ and *** $P \leq 0.001$, respectively and *ns* data not significant

Table 2 Estimates of GCA effects for severity and AUDPC of CABMV infection on eight cowpea parents

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

Data significant at ** $P = 0.01$ and *** $P = 0.001$, respectively; *SE* standard error, *AUDPC* area under disease progress curve

uniform infestation and transmission of the CABMV inoculum.

The second method of infection was done using artificial technique on the same test populations on fully expanded primary leaves when they were 14-day-old with the CABMV extract in the field trials. This was done with inoculum extract that were prepared by growing adequate plants of the S cultivar, Ebelat, in plastic buckets. To propagate the CABMV,

Table 3 Estimate of SCA effects for final severity and AUDPC of CABMV infection on F₁ crosses

F ₁ crosses	SCA effects	
	Final severity	AUDPC
Ebelat × IT82D-889	5.8	2.4
Ebelat × IT85F-2841	1.0	1.3
Ebelat × IT82D-516-2	-2.8	-1.5
Ebelat × MU-93	3.1	2.2
Ebelat × SECOW-2W	-7.1**	-4.4**
Ecirikukwai × IT82D-889	4.3	0.7
Ecirikukwai × IT85F-2841	5.1	1.9
Ecirikukwai × IT82D-516-2	-2.7	-0.6
Ecirikukwai × MU-93	-3.6	-1.2
Ecirikukwai × SECOW-2W	-3.1	-0.8
Blackcowpea × IT82D-889	-10.1***	-3.1
Blackcowpea × IT85F-2841	-6.1	-3.2
Blackcowpea × IT82D-516-2	5.5	2.1
Blackcowpea × MU-93	0.5	-1.0
Blackcowpea × SECOW-2W	10.2***	5.2**
SE	3.4	2.0

Data significant at ** $P = 0.01$ and *** $P = 0.001$, respectively

live and young viruliferous aphids at nymph stage were maintained on young growing cowpea seedlings in an insect-proof cage (made of shade net of 5 × 5 × 2.5 m) at the research station (NaSARRI) in eastern Uganda. This was to enhance transmission and increase CABMV inoculum in the healthy growing seedlings. The aphids were allowed to feed on the plants for a period of 2 weeks for proper transmission of virus and aphids were continuously transferred and maintained on new growing cowpea seedlings in plastic buckets in an insect-proof cage. The CABMV extract was prepared by detaching and grinding young symptomatic leaves obtained from the insect-proof cage in a 0.01 M phosphate buffer. The symptomatic leaf extract was prepared and using a micropipette, 200 µl of the inoculum was measured and used to inoculate leaves per plant of the test plant populations together with their parents in the field following carborundum powder (abrasive agent) application. The two inoculum techniques were intended to provide an even distribution of virus disease pressure on the test plant populations so that no population escaped from the CABMV infection. The test

Fig. 1 Backcross scheme showing F₁ backcrossed to the recurrent parent

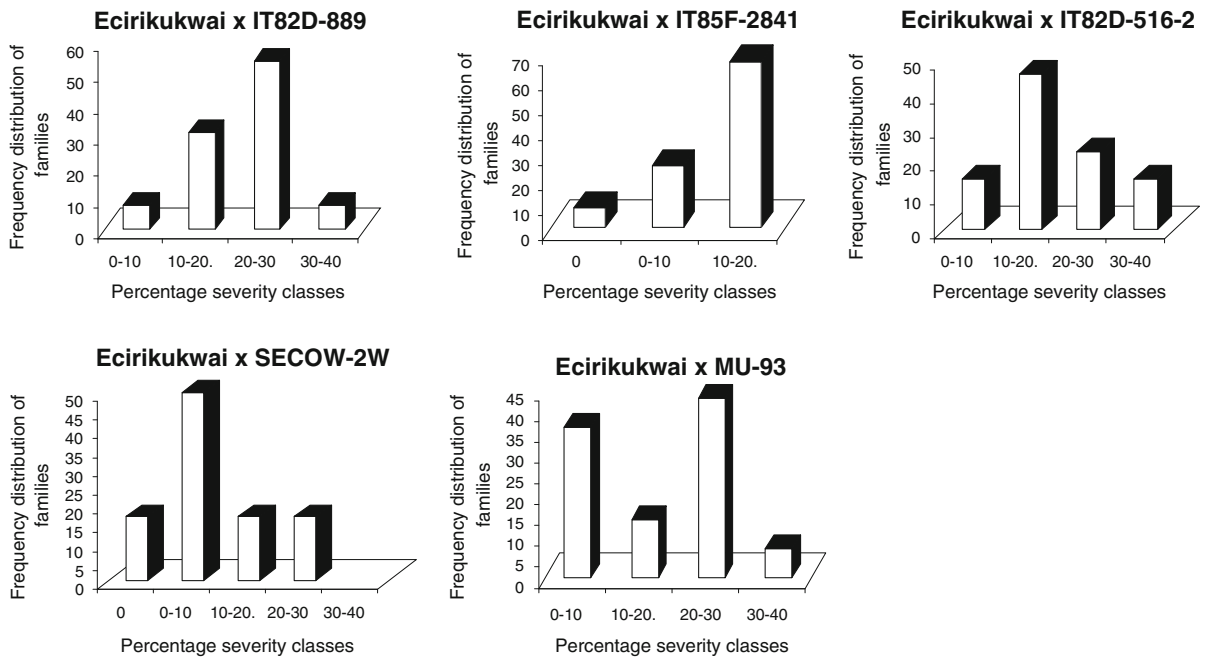
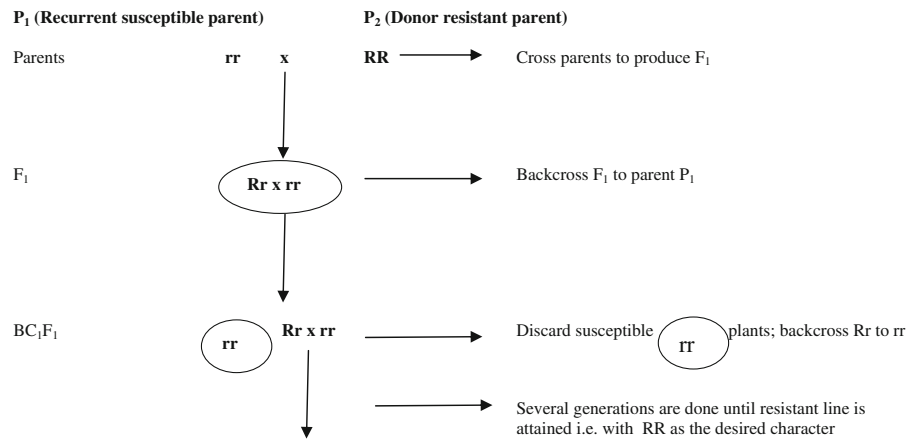


Fig. 2 Frequency distribution of families in percentages for percentage severity of F₂ plant populations involving crosses of the S cultivar Ecirikukwai with the R ones MU-93, SECOW-2W, IT85F-2841, IT82D-516-2 and IT82D-889

populations were monitored visually for any development of symptomatic infection of CABMV on each cross.

Data collection and analysis

For each population, CABMV severity was scored on individual plants and number of observed R and S plants in each population was counted and recorded.

The disease severity was assessed visually as plant leaves with virus symptoms using a rating scale of 1–9: where 1 = 0 % (no virus symptoms) and 9 = >60 % (very severe virus symptoms and death of the plant). Five data sets of severity assessments were used to calculate the area under disease progress curve (AUDPC) for the crosses and their parents as described by Anilkumar et al. (1994). Using the models as indicated below, the following were carried out:

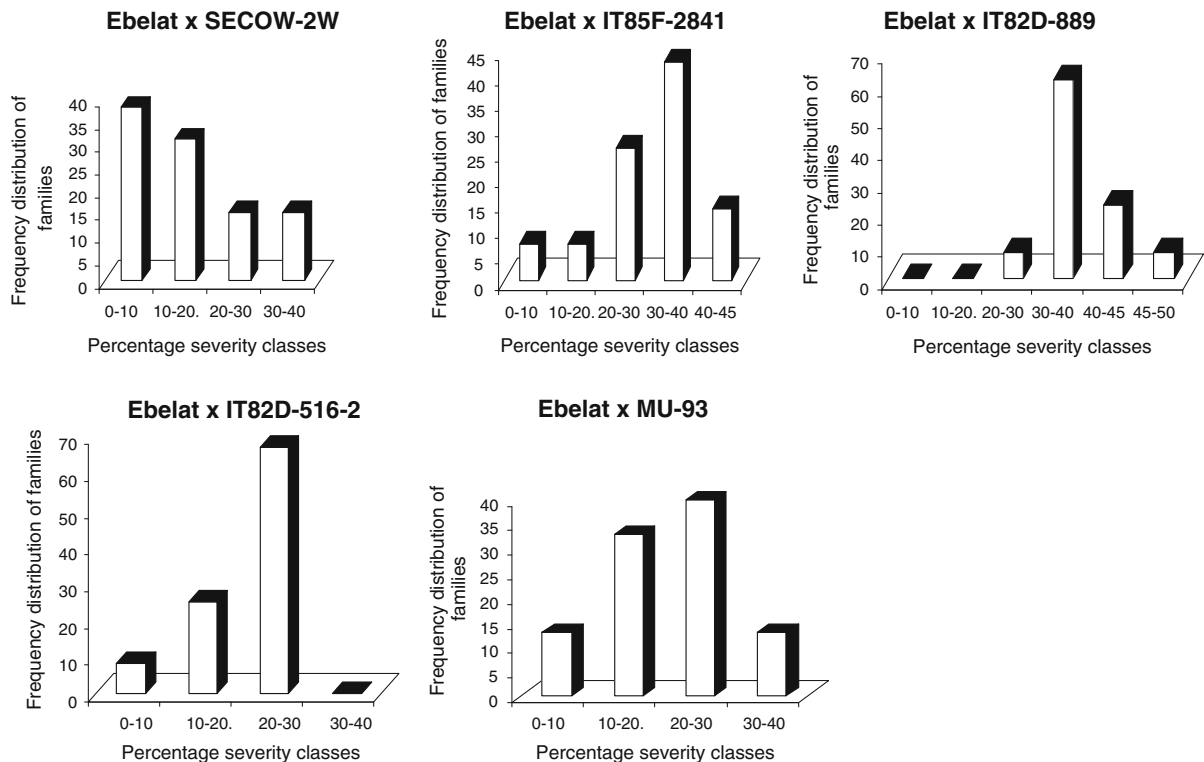


Fig. 3 Frequency distribution of families in percentages for percentage severity of F_2 plant populations involving crosses of the S cultivar Ebelat with the R ones MU-93, SECOW-2W, IT85F-2841, IT82D-516-2 and IT82D-889

Mean disease severity data were analysed using the following fixed effects model

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

where: Y_{ijk} is the k th observation on i th male \times j th female progeny, μ is the general mean, m_i is the general combining ability (GCA) effect of i th male, f_j is the GCA effect of j th female, $(m \times f)_{ij}$ is the interaction effect, equivalent to the specific combining ability (SCA) effect, and ε_{ijk} is the error associated with each observation.

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

Where: n = the total number of observations, X_i = disease severity in percentages at the i th observation, t = time in days after virus inoculation at i th observation, and t_{i+1} = interval between two consecutive observations.

Combining ability estimates were calculated as described by Singh and Chaudhary (1985). Relative

importance of GCA and SCA was determined by calculating the percentage of the sum of squares for the crosses attributable to GCA and SCA effects (Menkir and Ayodele 2005). Observed and expected phenotypic segregation ratios of R to S crosses were tested by using χ^2 for goodness of fit, assuming a monogenic model for inheritance of resistance.

Results and discussion

Combining ability estimates

There were significant ($P \leq 0.001$) differences among the crosses and parents for disease severity (Table 1). The GCA females and the SCA effects were highly significant ($P \leq 0.001$). The significant GCA mean squares due to the female parents and SCA effects indicates that both additive and non-additive gene effects, respectively, were important for determining severity of CABMV resistance. The high magnitude of GCA in comparison to SCA is an indication of the

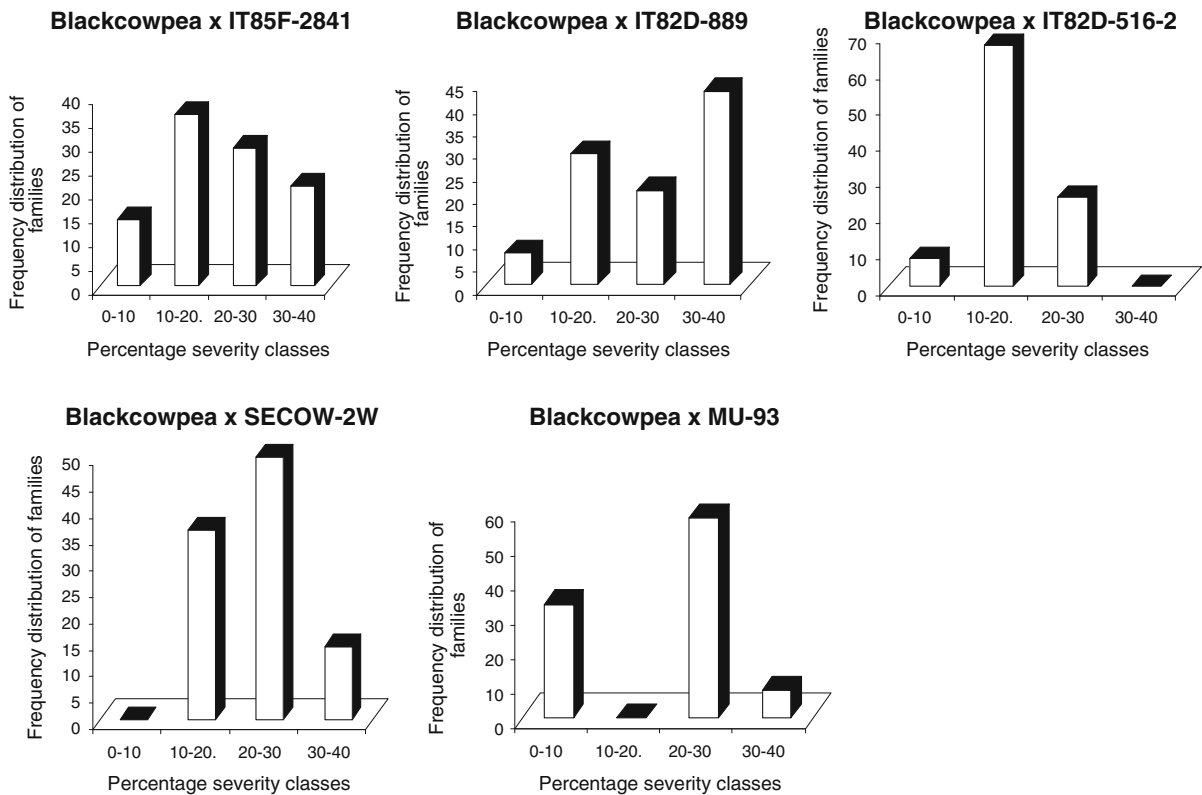


Fig. 4 Frequency distribution of families in percentages for percentage severity of F₂ plant populations involving crosses of the S cultivar Blackcowpea with the R ones MU-93, SECOW-2W, IT85F-2841, IT82D-516-2 and IT82D-889

Table 4 Phenotypic ratios of R:S of seven F₂ populations with single gene effects when fitted on 1:3 genetic model

Cross	Phenotype	Observed	Expected	χ^2
Blackcowpea × MU-93	R (0–10 %)	11	8.25	1.22
	S (>10 %)	22	24.75	
Ecirikukwai × IT85F-2841	R (0–10 %)	11	8.25	1.22
	S (>10 %)	22	24.75	
Ecirikukwai × SECOW-2W	R (0–10 %)	6	7.00	0.19
	S (>10 %)	22	21.00	
Ebelat × SECOW-2W	R (0–10 %)	11	9.00	0.59
	S (>10 %)	25	27.00	
Blackcowpea × IT85F-2841	R (0–10 %)	6	9.75	1.92
	S (>10 %)	33	29.25	
Ecirikukwai × × MU-93	R (0–10 %)	14	10.50	1.56
	S (>10 %)	28	31.50	
Ecirikukwai × IT82D-516-2	R (0–10 %)	6	9.25	1.52
	S (>10 %)	31	27.75	

Critical χ^2 value for one degree of freedom at $P \leq 0.05 = 3.84$

greater contribution of additive gene effects over the non-additive gene effects to CABMV resistance. The proportions (%) of sum of squares for the crosses

attributable to GCA and SCA for CABMV severity were 51.4 % for GCA due to females, 8.4 % for GCA due to males and 40.2 % for the SCA effects.

Table 5 Phenotypic ratios of resistance R:S of eight F₂ populations with no good fit when tested on 1:3 genetic model

Cross	Phenotype	Observed	Expected	χ^2
Blackcowpea × IT82D-889	R (0–10 %)	3	9.75	6.23**
	S (>10 %)	36	29.25	
Ecirikukwai × IT82D-889	R (0–10 %)	3	9.25	5.63*
	S (>10 %)	34	27.75	
Ebelat × IT82D-516-2	R (0–10 %)	3	8.50	4.75*
	S (>10 %)	31	25.50	
Ebelat × IT82D-889	R (0–10 %)	3	9.75	6.23**
	S (>10 %)	36	29.25	
Ebelat × × IT85F-2841	R (0–10 %)	3	9.00	5.33*
	S (>10 %)	33	27.00	
Ebelat × MU-93	R (0–10 %)	6	11.25	3.27
	S (>10 %)	39	33.75	
Blackcowpea × IT82D-516-2	R (0–10 %)	3	8.50	4.75*
	S (>10 %)	31	25.50	
Blackcowpea × SECOW-2W	R (0–10 %)	3	9.75	6.23**
	S (>10 %)	36	29.25	

Critical χ^2 value for one degree of freedom at $P \leq 0.05 = 3.84$; Data significant at ** $P = 0.01$ and *** $P = 0.001$, respectively

However, the GCA mean squares due to male parents was not significant ($P > 0.05$), indicating that female parents contributed more to disease severity than their male counterparts. The cowpea genotypes (male parents) were purposely evaluated to identify the best R genotypes for use in improving CABMV resistance in the local cowpea landraces which are widely grown.

Strong negative values of GCA effects of the parents showed contribution of GCA towards resistance for CABMV disease. It was observed that the female parent (Ecirikukwai) had highly negative GCA effects indicating that it contributed resistance in the crosses. This explains the fact that a cultivar (Ecirikukwai) may become S, but recovers from the infection of the virus. The positive significant values indicate contributions to susceptibility among the parents. The expression of resistance, which is reflected in negative values, is due to high gene frequency for resistance, while the positive values are due to low gene frequency for CABMV resistance. The female parent Ecirikukwai expressed a higher negative GCA effect for virus resistance, while Ebelat had positive GCA and Blackcowpea had non-significant GCA effects (Table 2). This observation indicated that susceptibility levels varied among the female parents, with Ecirikukwai being the least S, while Ebelat contributed more to susceptibility in the crosses. Results suggest that resistance levels also varied among the male parents, where IT82D-516-2 was the

most R as indicated by significant negative GCA effects (Table 2). The male genotype IT82D-889 had the least level of resistance as indicated by significant positive GCA effects, implying little contribution to CABMV resistance in crosses. The other three male parents IT85F-2841, MU-93 and SECOW-2W had non-significant GCA effects, indicating that they had little contribution to additive CABMV resistance exhibited by the crosses. The parental strains with negative GCA effects could be regarded as desirable combiners for resistance. Three superior crosses were observed, with negative SCA effects (Table 3). The cross Blackcowpea × IT82D-889 was the best specific combiner, while Blackcowpea × SECOW-2W was positive and the poorest specific combiner. However, significance of SCA effects would not be useful because cowpea breeding programmes do not aim at producing F₁ hybrids; but the importance of GCA effects suggests that early generation selection in F₂ would be effective in breeding for resistance to CABMV in cowpeas. Utilisation of good general combiners such as MU-93, IT82D-516-2, SECOW-2W and IT85F-2841 in hybridisation work followed by selection in segregating populations would be beneficial in the breeding programme. This could be done by adopting progeny selection techniques for exploiting additive genetic variance to improve inbred progenies with a superior performance than the parents (Jatasra 1980; Hanson et al. 1998) (Fig. 1).

Table 6 Phenotypic ratios of R:S BC₁F₁ populations when fitted on 1:1 genetic model

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

Critical χ^2 value for one degree of freedom at $P \leq 0.05 = 3.84$; Data significant at ** $P = 0.01$ and *** $P = 0.001$, respectively

Segregation analysis to CABMV resistance

The frequency distribution of families in percentages for percentage severity of F₂ plants showed segregation for CABMV severity in all populations, and in general revealed continuous distribution of progenies (Figs. 2, 3, and 4). Large numbers of S plants within the individual populations were observed in the populations Ecirikukwai × IT82D-889, Ecirikukwai × MU-93, Ebelat × IT85F-2841, Ebelat × IT82D-516-2, Ebelat × MU-93, Ebelat × IT82D-889, Blackcowpea × IT82D-889, Blackcowpea × SECOW-2W and Blackcowpea × MU-93. Nevertheless, a few plants within the population were observed with moderate resistance to CABMV infection (Figs. 2, 3, and 4). This

suggests that there is more than one gene controlling resistance to CABMV in the individual parents. It was also observed that when the F₂ populations were subjected to a critical χ^2 test, seven populations showed a good fit to a segregation ratio of 1R:3S suggesting single gene effects (Table 4), while eight populations did not show good fit to a segregation ratio of 1R:3S, suggesting that more than one recessive gene is involved in the inheritance of resistance to CABMV (Table 5).

Similarly, other backcross populations segregated in ratios of 1R:1S (Table 6), while others were skewed towards susceptibility with most progenies fitting in the susceptibility classes (10–40 % disease severity). Observation of individuals with partial resistance to

CABMV, and continuous distribution of severity scores in most populations, suggests that resistance was conditioned by more than one recessive gene, with minor gene at a different locus. The susceptibility to CABMV in F_2 populations showed that susceptibility was dominant to resistance. Frequency distribution of segregating F_2 populations was not normal, but had skewed distributions which may be explained by dominance gene action that exhibited in some populations. Bjarko and Line (1988) reported that lack of discrete classes in the segregating populations of the crosses may result in low heritability due to segregation of several genetic factors. Furthermore, Bjarko and Line (1988) noted that the lack of normal distribution is a result of the presence of dominance, epistasis, and probably the linkage between resistance genes. Other progenies, the χ^2 values were significantly larger than the critical χ^2 value in the Table 6, indicating the involvement of more than one recessive gene conditioning resistance to CABMV. The F_2 populations Blackcowpea \times IT82D-889, Ecirikukwai \times IT82D-889, Ebelat \times IT82D-516-2, Ebelat \times IT82D-889, Ebelat \times IT85F-2841, Blackcowpea \times IT82D-516-2 and Blackcowpea \times SECOW-2W had significant χ^2 values larger than critical value, indicating the presence of more than one gene controlling resistance in these populations. A survey of literature indicated that quantitative resistance to CABMV has not been previously reported in cowpeas. However, the involvement of single or few genes has been previously reported. Shukler et al. (1978) and Pal et al. (1991) reported that resistance to yellow mosaic virus in cowpea was conditioned by double recessive genes. In the case of Taiwo et al. (1981) reported that inheritance of resistance to CABMV was conditioned by a single recessive gene.

Conclusions

The predominance of GCA effects, mainly due to the female parents, in the F_1 generation and the continuous distribution of F_2 progenies according to disease severity, suggested the involvement of many minor genes conditioning CABMV resistance in F_1 crosses. Results also suggested that a single recessive gene conditioned CABMV resistance in seven populations in F_2 , and in four populations in the BC_1F_1 generations. The progenies of Blackcowpea \times IT82D-889,

Ebelat \times IT82D-889 and Blackcowpea \times SECOW-2W did not fit to 1R:3S expectations in F_2 generation, supporting observation of polygenic inheritance. The backcross populations Ecirikukwai \times SECOW-2W and Ecirikukwai \times IT82D-516-2 showed a good fit in segregation ratio of 1R:1S, which supported monogenic inheritance. However, progenies of backcross populations of Blackcowpea \times SECOW-2W and Blackcowpea \times IT82D-889 did not fit a 1R:1S expectations, adding credence to the observation of polygenic inheritance for CABMV resistance in these cowpea genotypes. Both additive and non-additive gene action were important in determining CABMV resistance. It can be concluded that the resistance in the cowpea crosses was controlled by a single gene in seven populations and more than one gene in eight populations in this study.

Acknowledgments The Rockefeller Foundation, New York provided the funding through the African Centre for Crop Improvement under the management of Professor Mark Laing as the director of the program in South Africa is greatly acknowledged. We are also grateful to the director of the National Semi-Arid Resources Research Institute (NaSARRI) for hosting the research study by providing facilities and ample environment to undertake the research work. Part of the germplasm was provided by IITA-Nigeria, Makerere University Department of Crop Science, Uganda and NaSARRI that enabled this research study to be carried out effectively well. Mr. Walter Anyanga and Dr. Nelson Wanyera at NaSARRI, Soroti, Uganda are also appreciated for providing the reading materials for the study.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Anilkumar TB, Chandrashekar M, Saifulla M (1994) Assessment of partial resistance in cowpea cultivars to leaf spot (*Septoria vignicola* Rao). Trop Agric (Trinidad) 71:36–40
- Arshad M, Bashir M, Sharif A, Malik BA (1998) Inheritance of resistance in cowpea (*Vigna unguiculata* (L.) Walp.) to blackeye cowpea mosaic potyvirus. Pak J Bot 30:263–270
- Atiri GI, Ekpo JA, Thottappilly G (1984) The effect of aphid resistance in cowpea on infection and development of *Aphis craccivora* Koch and the transmission of cowpea aphid-borne mosaic virus. Ann Appl Biol 104:339–346
- Bashir M, Hampton RO (1996) Sources of genetic resistance in cowpea (*Vigna unguiculata* (L.) Walp.) to cowpea aphid-borne mosaic potyvirus. Eur J Plant Pathol 102:411–419

- Bashir M, Ahamad Z, Ghafoor A (2002) Cowpea aphid-borne mosaic potyvirus: a review. *Int J Pest Manage* 48:155–168
- Behnckken GM, Maleevsky L (1977) Detection of cowpea aphid-borne mosaic virus in Queensland. *Aust J Exp Agric Anim Husb* 17:674–678
- Bjarko ME, Line RF (1988) Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. *Phytopathology* 78:457–461
- Byoung-Cheorl K, Yeom I, Jahn MM (2005) Genetics of plant virus resistance. *Annu Rev Phytopathol* 43:581–621
- Edema R, Adipala E, Florini DA (1997) Influence of season and cropping system on the occurrence of cowpea diseases in Uganda. *Plant Dis* 81:465–468
- Emechebe AM, Lagoke STO (2000) Recent advances in research on cowpea diseases. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M (eds) Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the world cowpea conference III held at the international institute of tropical agriculture, Ibadan, 4–8 Sept 2000, pp 94–123
- Fisher ML, Kyle MM (1994) Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. III. Cosegregation of phenotypically similar dominant responses to nine potyviruses. *Theor Appl Genet* 89:7–8
- Fisher ML, Kyle MM (1996) Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. IV. Inheritance, linkage and environment relation to four potyviruses. *Theor Appl Genet* 92:204–212
- Fraser RSS (1992) The genetics of plant-virus interactions: implications for plant breeding. *Euphytica* 63:175–185
- Hanson PM, Hanudin Licardo O, Wang J, Chen JT (1998) Diallel analysis of bacterial wilt resistance in tomato derived from different sources. *Plant Dis* 82:74–78
- International Institute of Tropical Agriculture (IITA) (1998) Cowpea-cereal systems: improvement in the dry savannas. Annual Report 1998. International Institute of Tropical Agriculture, Ibadan
- Jatasra DS (1980) Combining ability for grain weight in cowpea. *Indian J Genet Plant Breed* 40:330–333
- Kaiser WJ, Mossahebi GH (1975) Studies with cowpea aphid-borne mosaic virus and its effect on cowpea in Iran. Food and Agriculture Organization. *Plant Prot Bull* 27:27–30
- Mali VR, Thottappilly G (1986) Virus diseases of cowpea in the tropics. *Trop Plant Pathol* 3:361–403
- Menkir A, Ayodele M (2005) Genetic analysis of resistance to gray leaf spot of mid-altitude maize inbred lines. *Crop Sci* 45:163–170
- Pal SS, Dhaliwal HS, Bains SS (1991) Inheritance of resistance to yellow mosaic virus in some *Vigna* species. *Plant Breed* 106:168–171
- Patel PN, Mlingo JK, Leya HK, Kuwite C, Mmbaga ET (1982) Sources of resistance, inheritance and breeding of cowpea for resistance to a strain of cowpea aphid-borne mosaic virus from Tanzania. *Indian J Genet* 42:221–229
- Provvidenti R, Gonsalves D, Taiwo MA (1983) Inheritance of resistance to blackeye cowpea mosaic and cowpea aphid-borne mosaic viruses in *Phaseolus vulgaris*. *J Hered* 74:60–61
- Rybicki E, Pietersen G (1999) Plant virus disease problems in the developing world. *Adv Virus Res* 53:128–175
- Shoyinka SA, Thottappilly G, Adebayo GG, Anno-Nyako FO (1997) Survey on cowpea virus incidence and distribution in Nigeria. *Int J Pest Manage* 43:127–132
- Shukler GP, Pandey BP, Singh DP (1978) Inheritance of resistance to yellow mosaic to mungbean. *Indian J Genet* 38:358–360
- Singh RK, Chaudhary BD (1985) Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, pp 211–213
- Taiwo MA, Provvidenti R, Gonsalves D (1981) Inheritance of resistance to blackeye cowpea mosaic virus in *Vigna unguiculata* (L.) Walp. *J Hered* 72:433–434
- Umaharan P, Ariyanayagam RP, Haque SQ (1997) Identification of resistance to cowpea severe mosaic virus (Trinidad isolate) in cowpea (*Vigna unguiculata* (L.) Walp.). *Trop Agric* 74:324–328
- Van Boxtel J, Singh BB, Thottappilly G, Maule AJ (2000) Resistance of (*Vigna unguiculata* (L.) Walp.) breeding lines to blackeye cowpea mosaic and cowpea aphid-borne mosaic potyvirus isolates under experimental conditions. *J Plant Dis Prot* 107:197–204