

## SEVERITY OF INFECTION OF THREE UNRELATED VIRUSES ON COWPEA CULTIVARS

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### Abstract

A study was conducted to determine the severity of three unrelated viruses; *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea mottle virus* (CMeV), and *Bean southern mosaic virus* (SBMV) on symptoms induced in three cowpea cultivars, "OLO II", "OLOYIN", and IT86D-719. The rating done on a scale of 0-5 with respect to symptomatology indicated that the viruses have significant ( $P=0.05$ ) severe effects on the virus inoculated plants compared to the control. The age of plants at the time of inoculation also have drastic effect on the symptoms induced on plants. Inoculations of plants at early age of 10 days after planting (DAP) resulted in more severe symptoms than inoculations at later age of 30 DAP. More so as the days of observation increased from 10 days after inoculation (DAI) to 30 DAI, the severity of infection also increased. The severity of the viruses on symptom rating of cultivars indicated that CABMV induced the greatest effects in IT86D-719 at 10 DAP with ratings of 3, 4 and 5 at 10, 20 and 30 DAI respectively; CMeV had the highest severity on "OLOYIN" while SBMV caused more severe effects in "OLO II" which rated 4, 4, and 5 at 10, 20 and 30 DAI respectively for plants inoculated at 10 DAP.

Keywords: Cowpea cultivars, severity, inoculation, symptomatology, virus.

### Introduction

Cowpea (*Vigna unguiculata*) (L.) Walp) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Summerfield *et al.*, 1974). It provides an extremely significant portion of the dietary protein of Africans and plays an important nutritional role in developing countries of the tropics and subtropics especially in sub-Saharan Africa (Rachie, 1985). The chemical composition of cowpea seeds corresponds with that of most edible legumes (Coetzee, 1995). The seeds contain small amounts of  $\beta$ -carotene equivalents, thiamin, riboflavin, vitamin A, niacin, folic acid and ascorbic acid (Kay, 1979). The use of cowpea as a seed vegetable provides an inexpensive source of protein in the diet. The dried pulse may be cooked together with other vegetable to make a thick soup or ground into a meal or paste, before preparation in a variety of ways (Allen, 1983; Quass, 1995). Similarly, fresh, immature pods may be boiled as a vegetable. Fresh leaves and growing points are often picked and eaten in the same way as spinach (Quass, 1995).

There have been reports of *Cowpea aphid-borne mosaic virus* (CABMV) (Huguenot *et al.*, 1994), *Cowpea mottle virus* (CMeV) (Rossel and Thottappilly, 1985) and *Bean southern mosaic virus* (SBMV) (Shoyinka *et al.*, 1997) existing in Nigeria. CABMV belongs to the family Potyviridae and genus *Potyvirus*. Symptoms due to infection by CABMV include severe mosaic, the severity depending on host cultivar and virus strain (Thottappilly and Rossel, 1985). Diseased cowpea plants show variable amount of dark green vein-banding or interveinal chlorosis, leaf distortion, blistering and stunting (Bock and Conti, 1974).

CMeV belongs to the *Carmovirus* group (Brunt *et al.*, 1990) and infection is often manifested as bright yellowing (Shoyinka *et al.*, 1978). In tolerant varieties symptoms usually consist of mottling only. The virus also induces distortion and reduction in leaf size and witches broom syndrome in cowpea.

SBMV is the type-member of the *Sobemovirus* group (Sehgal, 1981). The systemic symptoms induced by SBMV included vein-clearing followed by a mild to severe mottling or coarse mosaic leaf pattern.

Viral diseases of cowpea impact more deleterious effects on cowpea production compared to diseases due to fungi, bacteria and nematodes. This study was carried out to investigate the severity of viruses on symptoms induced in cowpea hosts and to assess the influence of plant age and time of inoculation on expression of viral symptoms on host.

## Materials and Methods

### *Sources of Seeds and Virus Isolates*

Three cowpea cultivars were used for this study. The two local cultivars; “OLO II” and “OLOYIN” were obtained from Mushin market, Lagos State while IT86D-719 was obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The virus isolates comprising CABMV, CMeV and SBMV were also obtained from IITA. Each isolate was maintained on tissue culture of *Vigna unguiculata* in the green house. The viruses were transferred to healthy *V. unguiculata* cultivar, Ife brown (an adapted local cultivar by IITA) in order to increase the viruses and to ensure their availability.

### *Preparation of Viral Inoculum*

Inoculum was prepared by grinding each of CABMV, CMeV and SBMV-infected cowpea leaves separately in 0.05M phosphate buffer at pH 7.5, using mortars and pestles. Leaf-to-buffer ratio was 1:5 (1g of infected leaf to 5ml buffer).

Plants were mechanically inoculated at 10, 20 and 30 days after plating (DAP). Inoculation was carried out by dusting the leaf surface of plants with an abrasive; carborundum (180 grit) and then applying the inoculum to the leaf with the pestle. The control plants were inoculated with the buffer only. After inoculation, carborundum was rinsed off the leaves to improve light interception. Systemic symptoms of virus infections on plants were rated at 10, 20 and 30 days after inoculation (DAI). The rating was on a scale of 0-5 (0=no symptoms; 1=slight mosaic or slight mottle on leaves, 2=mosaic or mottle leaf patches and / or necrotic spots on leaves, 3=blisters on leaves, yellow and green vein banding; 4= defoliation, leaf reduction, leaf reduction, leaf deformation, witches broom and leaf chlorosis; 5= apical necrosis, internode shortening, stunting or planting death).

The one hundred and eight (108) pots used for the experiment were arranged in a randomized complete block design (RCBD). There were three blocks for each cowpea cultivar and each block consisted of 36 pots. Inoculations were performed at 10, 20 and 30 DAP. The treatments were CABMV, CMeV, SBMV and buffer inoculated control. There were 3 replicates for each treatment.

### *Statistical Analysis*

Data obtained were analysed using statistical package for social scientists (SPSS) version 16. Duncan's multiple range test was used to determine the level of significance between the virus treatments.

## Results

Effect of Age of Plant at Time of Viral Inoculation and Rating for Symptom Development in Cowpea Cultivar “OLO II”.

Symptoms of virus rating showed that as the days after inoculation increased from 10 to 30 DAI the symptoms severity also increased, with the exception of buffer inoculated control plants in which no symptom was observed at all the stages of inoculation. The age of plant at inoculation showed significant differences in viral severity especially at 10 and 30 DAP and DAI. The severity was higher at 10 DAP and reduced at 30 DAP (Table 1). Results in Table 1 further showed that, at 10 DAP; SBMV had the greatest severity on cultivar “OLO II” with ratings of 4, 4, and 5 at 10, 20 and 30 DAI respectively.

Table 1: The effect of age of plant at time of viral inoculation and rating for symptom development in cowpea cultivar "OLO II"

Inoculum	DAP <sup>x</sup>	Trifoliolate Leaves		
		10 <sup>y</sup>	20 <sup>y</sup>	30 <sup>y</sup>
CABMV	10	3 <sup>c</sup>	4 <sup>d</sup>	5 <sup>e</sup>
	20	2 <sup>b</sup>	4 <sup>d</sup>	4 <sup>d</sup>
	30	2 <sup>b</sup>	4 <sup>d</sup>	4 <sup>d</sup>
CMeV	10	2 <sup>b</sup>	3 <sup>c</sup>	4 <sup>d</sup>
	20	2 <sup>b</sup>	3 <sup>c</sup>	4 <sup>d</sup>
	30	0 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>
SBMV	10	4 <sup>d</sup>	4 <sup>d</sup>	4 <sup>d</sup>
	20	2 <sup>b</sup>	3 <sup>c</sup>	3 <sup>c</sup>
	30	2 <sup>b</sup>	2 <sup>b</sup>	2 <sup>b</sup>
BUFFER (CONTROL)	10	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
	20	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
	30	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>

DAP<sup>x</sup> -Inoculation at 10, 20 and 30 days after planting; <sup>y</sup> -Days after inoculation; CABMV - *Cowpea aphid-borne mosaic virus*; CMeV- *Cowpea mottle virus*, SBMV- *Bean southern mosaic virus*; 0=no symptoms; 1=slight mosaic or slight mottle on leaves, 2=mosaic or mottle leaf patches and / or necrotic spots on leaves, 3=blisters on leaves, yellow and green vein banding; 4= defoliation, leaf reduction, leaf reduction, leaf deformation, witches broom and leaf chlorosis; 5= apical necrosis, internode shortening, stunting or planting death).

Each value is the mean of three replicates. In each column means followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

**Effect of Age of Plant at Time of Viral Inoculation and Rating for Symptom Development in Cowpea Cultivar "OLOYIN".**

The result of viral severity on this cultivar revealed that the three viruses had significant effects on the virus inoculated plants but none on the buffer inoculation plants. Inoculation with CABMV at 10 DAP did not induce any symptoms at early age of observation of 10 and 20 DAI; however symptoms were seen on plant at 30 DAI. Symptoms observed on plants at both 30 DAP and 20 DAI were always mild with a rating of 2 whereas progressions in severity were observed at 30 DAI in plants inoculated with CMeV and CABMV at 30 DAP (Table 2). The effect of severity of viruses on "OLOYIN" cultivar showed that the highest induction of symptoms was produced by CMeV.

Table 2: The effect of age of plant at time of viral inoculation and rating for symptom development in cowpea cultivar "OLOYIN"

Inoculum	DAP <sup>x</sup>	Trifoliolate Leaves		
		10 <sup>y</sup>	20 <sup>y</sup>	30 <sup>y</sup>
CABMV	10	0 <sup>a</sup>	0 <sup>a</sup>	4 <sup>d</sup>
	20	2 <sup>b</sup>	3 <sup>c</sup>	4 <sup>d</sup>
	30	2 <sup>b</sup>	2 <sup>b</sup>	3 <sup>c</sup>
CMeV	10	2 <sup>b</sup>	4 <sup>d</sup>	5 <sup>e</sup>
	20	2 <sup>b</sup>	4 <sup>d</sup>	4 <sup>d</sup>
	30	2 <sup>b</sup>	4 <sup>d</sup>	4 <sup>d</sup>
SBMV	10	3 <sup>c</sup>	3 <sup>c</sup>	4 <sup>d</sup>
	20	3 <sup>c</sup>	3 <sup>c</sup>	2 <sup>b</sup>
	30	2 <sup>b</sup>	2 <sup>b</sup>	2 <sup>b</sup>
BUFFER (CONTROL)	10	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
	20	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
	30	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>

DAP<sup>x</sup> -Inoculation at 10, 20 and 30 days after planting; <sup>y</sup> -Days after inoculation; CABMV – *Cowpea aphid-borne mosaic virus*; CMeV- *Cowpea mottle virus*, SBMV – *Bean southern mosaic virus*; 0=no symptoms; 1=slight mosaic or slight mottle on leaves, 2=mosaic or mottle leaf patches and / or necrotic spots on leaves, 3=blisters on leaves, yellow and green vein banding; 4= defoliation, leaf reduction, leaf reduction, leaf deformation, witches broom and leaf chlorosis; 5= apical necrosis, internode shortening, stunting or planting death).

Each value is the mean of three replicates. In each column means followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Effect of Age of Plant at Time of Viral Inoculation and Rating for Symptom Development in Cowpea Cultivar IT86D-719. Table 3 showed the effect of virus severity on IITA line IT86D-719. The control plants produced no symptoms but the virus inoculated plants produced varying degree of symptom severity, the only exception was observed at 10 DAI when the plants were inoculated with CMeV at 30 DAP. CABMV induced the most severe effect on this line while the effects of the other viruses were mild.

Table 3: The effect of age of plant at time of viral inoculation and rating for symptom development in IT86D -719

Inoculum	DAP <sup>x</sup>	Trifoliolate Leaves		
		10 <sup>y</sup>	20 <sup>y</sup>	30 <sup>y</sup>
CABMV	10	3 <sup>c</sup>	4 <sup>d</sup>	5 <sup>d</sup>
	20	2 <sup>b</sup>	4 <sup>d</sup>	4 <sup>c</sup>
	30	2 <sup>b</sup>	4 <sup>b</sup>	4 <sup>c</sup>
CMeV	10	2 <sup>b</sup>	3 <sup>c</sup>	4 <sup>c</sup>
	20	2 <sup>b</sup>	3 <sup>c</sup>	4 <sup>c</sup>
	30	0 <sup>a</sup>	2 <sup>b</sup>	3 <sup>b</sup>
SBMV	10	4 <sup>d</sup>	4 <sup>d</sup>	4 <sup>c</sup>
	20	2 <sup>b</sup>	3 <sup>c</sup>	3 <sup>b</sup>
	30	2 <sup>b</sup>	2 <sup>b</sup>	3 <sup>b</sup>
BUFFER (CONTROL)	10	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
	20	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
	30	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>

DAP<sup>x</sup> -Inoculation at 10, 20 and 30 days after planting; <sup>y</sup> -Days after inoculation; CABMV – *Cowpea aphid-borne mosaic virus*; CMeV- *Cowpea mottle virus*, SBMV – *Bean southern mosaic virus*; 0=no symptoms; 1=slight mosaic

or slight mottle on leaves, 2=mosaic or mottle leaf patches and / or necrotic spots on leaves, 3=blisters on leaves, yellow and green vein banding; 4= defoliation, leaf reduction, leaf deformation, witches broom and leaf chlorosis; 5= apical necrosis, internode shortening, stunting or planting death).

Each value is the mean of three replicates. In each column means followed by the same letter are not significantly different ( $P=0.05$ ) according to Duncan's multiple range test.

### Discussion

This study has shown that the cowpea cultivars are susceptible to the three unrelated viruses; CABMV, CMeV and SBMV used in the investigation. This corroborates the work of Owolabi *et al.*, (1988) who reported the susceptibility of two other Nigerian commercial cowpea cultivars to *Cowpea mosaic virus* genus *Comovirus* and *Blackeye cowpea mosaic virus* genus *Potyvirus*. There have also been reports of the three viruses existing in Nigeria. Shoyinka *et al.* (1977), stated that in survey carried out throughout Nigeria; CABMV was found in all ecological zones is now considered to be the most widespread as well as the most economically important viral disease of cowpea in Nigeria.

The complete loss of an irrigated cowpea crop in Northern Nigeria was tentatively attributed to CABMV (Raheja and Leleji, 1974). Shoyinka *et al.* (1997) reported that CMeV was consistently moderate both in incidence and prevalence in a survey carried out in 1991 to 1993 in the Sudan and Sahel Savanna of Nigeria and yield reductions of more than 75% in cowpea production in Nigeria have been attributed to CMeV (Shoyinka *et al.*, 1978). Shoyinka *et al.* (1977) reported that SBMV had the highest incidence (27.4%) and prevalence (66.7%) in Nigeria in 1991 planting season.

Early infection (10 DAP) of cultivars by viruses resulted in more severe symptoms than infection at later stage (30DAP) of growth. This finding is in agreement with the report of Agrios *et al.* (1985) which stated that the younger the plants at the time of viral infection, the greater the severity of disease symptoms.

At 10 DAI, "OLOYIN" was the most tolerant of all the three cultivars because it had the least symptom rating and this is substantiated by the report of Taiwo *et al.* (2007) which stated that; on the basis of the cultivars' response to the viruses in single and mixed infections, "OLO II" "White" and TVU 76 appeared to be more susceptible than "Oloyin".

The severity of the viruses on symptom rating of cultivars showed that CABMV induced the highest symptom in IT86D-719, CMeV had the highest severity on "OLOYIN" while symptoms induced in "OLO II" was more pronounced with SBMV. This observation corroborates the findings of Kareem and Taiwo (2007) which reported that the response of the three cultivars to the three viruses in single and mixed infections showed CABMV having more severe effects in IT86D-719, SBMV had the greatest effect on "OLO II" while CMeV induced the greatest effect on "OLOYIN" whereas yield was greatly reduced in double infections involving CABMV in combination with either CMeV or SBMV in "OLOYIN" and "OLO II", however, there was complete loss in yield of IT86D-719.

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