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Response of Cowpea Cultivars Against Cowpea Mottle Virus in Mokwa, Nigeria

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Abstract: The present study was undertaken in 2017 cropping season at the Screen House of Niger State College of Agriculture, Mokwa to investigate the effects of inoculating five cowpea cultivars: "Langbazo", "Ezomilkigi", "Ezobokun", "Ezokapangi" and IT07K-277-2 with Cowpea mottle virus [CPMoV], genus Carmovirus on their growth performances. The trial was laid out in Complete Randomised Design [CRD] with five treatments and an uninoculated control and set up in three replicates. Results show that growth parameters of the buffer inoculated control plants were significantly higher than those of the virus inoculated. The average values of plant height and number of leaves produced by cowpea cultivars Langbazo and IT07K-277-2 were significant compared to the other treatments. The resistant traits from Langbazo and IT07K-277-2 can be incorporated with high yielding genotypes to develop CMoV resistant varieties. Further studies are needed to screen the cultivars on their defense abilities for use in hybridisation studies.

Keywords: Hybridization studies, Resistant varieties, Cowpea cultivars, Virus free seeds, Viruses

1. Introduction

Cowpea [*Vigna unguiculata* [L.] Walp] is reported to be an important legume for human consumption in sub-Saharan Africa [SSA], Central Asia, and South America [1]. It is also reported to be widely grown for its grain and livestock feed in many rural areas of Africa [2, 3]. Cowpea is reported to be rich in protein and essential amino acids that are deficient in cereals [4]. It is known to be consumed singly or as a complement to cereal food crops such as rice and maize and its haulm is extensively fed to livestock in form of fodder [5, 6]. Many workers have reported that it contributes appreciably to improve soil fertility and plant growth by fixing atmospheric nitrogen into the soil [7, 8]. In West Africa, cowpea is reported to be second in importance after groundnuts, with Nigeria accounting for over 70 % of the total world production [9].

Cowpea cultivation is also reported to be widely adopted by millions of smallholder farmers in Nigeria partly owing to its compatibility with traditional cropping systems [10] where it is intercropped with cereals such as maize, sorghum and millet. Increased interest in cowpea production is attributable to high demand from local and external markets, and the quest for foreign earnings [11-14].

White seeded cowpea varieties and black-eyed types are commonly grown for grain and table use [15]. While viny varieties that mature late are preferred for, forage and can be grown on wide range of soil types and under a diversity of climatic and cultural conditions [15]. The author reported that highest yields of forage are obtained in sandy loam soils supplemented with proper irrigation. However, for seed purposes, cowpea reasonably performs well on soils with low fertility.

Cowpeas are known to be susceptible to a wide range of pests and pathogens that attack it at all stages of growth [16]. These include insects, bacteria, fungi and viruses. Estimated losses due to virus infection have been variously put at between 10 and 100% [16], depending on the virus-host-vector relationships as well as the prevailing epiphytological factors. Among these, CPMoV is the most infective.

Many workers [17 - 20] have reported that CPMoV is a positive sense single-stranded RNA, unipartite, isometric virus, 30 nm in diameter. The reports opined that virions contain 20% and 80%

nucleic acid and protein, respectively. They also indicated that the thermal inactivation point is 60°C longevity *in vitro* 1 day and has dilution end of 10^{-6}. Cytoplasm of infected cells contains bundles of particles. The pathogen is distributed in all ecological zones of Nigeria, particularly in the riverine areas of the middle belt, which has a southern Guinea Savanna climate and where a lot of Bambara groundnut is grown [16].

Infected plants display severe mosaic, mottling or bright yellow mosaic, leaf distortion and reduction in leaf size sometimes leading to a witches' broom appearance in cowpea occurs [21]. Thus, being a common cowpea pathogen in Nigeria, and recently reported in Mokwa Southern Agro ecological zone, it is necessary to evaluate various local cowpea genotypes for their resistance to this pathogen in order to identify resistant, tolerant and susceptible cultivars. The present study, was, therefore, set up to evaluate the reaction of different cowpea cultivars inoculated with cowpea mottle virus [*CPMoV*] in Mokwa to ascertain the levels of their resistance for subsequent utilization in hybridization studies.

2. Materials and Methods

2.1 Study area

The trial was conducted in the screen house of the Niger State College of Agriculture, Mokwa. Mokwa is located on latitude 09° 18'N and longitude 05° 04'E of the equator. It is situated on the Southern Guinea Savanna agro ecological zone of Nigeria. It is characterized by unimodal rainfall distribution with annual rainfall of 1179.5 mm and an average temperature of about 33.6 °C [22].

2.2 Source of inoculum and its multiplication

The cowpea mottle virus [CPMoV] inoculum was obtained from the stock in the Department of Crop Production, Federal University of Technology, Minna, Nigeria. The isolates were maintained on silica gels in vial bottles stored at room temperature. They were multiplied by propagating in TVU 76 cowpea seedlings through sap transmission in a screen house. Extract for inoculation was prepared by grinding each leaf isolate in extraction buffer, 0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine per litre of distilled water, with pH 7.2 at the rate of 1g/mL as described by [23]. Two microlitres of β-mercapto ethanol was mixed with the extract just before use. Seedlings were inoculated at 10 days after sowing [DAS] by rubbing the virus extract on the upper surface of the leaves dusted with carborundum powder with 600-mesh.

The inoculated plants were rinsed with sterile distilled water and thereafter left in the screen house for symptom expression. Symptomatic leaves were harvested and subjected to Enzyme-Linked Immunosorbent Assay [ELISA] for virus confirmation. Virus positive leaves were preserved on silica gels in vial bottles and were used for inoculation of the seedlings at 10 days after planting [DAP].

Inoculation of the cowpea was carried out after grinding 1 g of CPMoV infected leaf in 1ml of buffer solution with pH 7.2 in 0.1M sodium phosphate dibasic, 0.1 M potassium phosphate monobasic, 0.01M ethylenediamine tetra acetic acid and 0.001 M L-cystine per litre of distilled water in a pre-cooled sterilised mortar and pestle. One ml of β-mercapto-ethanol was added to the extract just before use to help the virus to penetrate the cell wall of the plants.

The inoculated leaves were earlier dusted with carborundum powder of 600 mesh to cause abrasion on them following the method of [23], before the inoculum extract was finally rubbed on the upper surface. The inoculated leaf on each plant was thereafter rinsed with cold distilled water immediately after being inoculated to forestall burning because of excess inoculum application. Emerged leaves of the plants inoculated were monitored until the virus disease symptoms fully appeared at 2-3 weeks after inoculation when the infected leaves were ready for harvest.

2.3 Preservation of inoculum and source of seeds

The matured virus infected cowpea leaves harvested from the preliminary studies in the screen house were kept and preserved in the inoculating vials. The norm was 1 g of the leaf vial. A vial contains 5-6 g silica gel as moisture absorber. This was covered with non -absorbent cotton wool of 0.8-1 g. One gramme of the infected leaf was kept on top of the cotton wool and sealed up for future use as described by [23].

Improved cowpea seeds of cultivar IT07k — 277 -2 were obtained from the Genetic Resources Unit, International Institute for Tropical Agriculture, [IITA], Ibadan while local commercial cultivars namely, Langbazo, Ezomilkigi, Ezobokun and Ezokapangi were purchased from Mokwa Central market, Niger State.

2.4 Screening site, treatments and experimental design

The test cowpea cultivars were evaluated against CPMoV during the 2017 cropping season at the Screen house of the College of Agriculture, Mokwa, Niger State on Latitude 6.44675°E, Longitude 9.51715°N, 220 m^2 above sea level. Two independent trials were conducted simultaneously, for CPMoV virus and a control. In each trial, the five-cowpea cultivars constituted the treatments. The screen house was

cleaned and pots arranged according to the treatments and design in a Completely Randomised Design [CRD] in three replicates. The gross plot for each cultivar consisted of four pots each and measuring $3m^2$ while the net plot was made up of 2 mid-pots.

Each cultivar was evaluated on the mid-pots, the net-plot. Seeds were sowed during the cropping season in the study year and inoculated at 10 days after sowing [DAS]. Three cowpea seeds were planted after dressing with Mecalaxy + Carboxin + Furathiocarb at the rate of 3g per 10kg seeds to protect them against soil borne pathogens and were later thinned to one per stand at 2 WAP. Manual weeding by hand pulling was carried out to control weeds in the screen house and the insecticide D-D Force [Cypermethrin [50g] plus Dimethoate [250g]] was applied at flower and bud formation, and pod initiation at the rate of 1.5 kg/ha using a hand-operated sprayer to check pest population.

Data collection was taken at 3, 6 and 9 weeks after inoculation [WAI] from randomly selected and tagged plants on disease incidence, plant height using a meter rule from the base of the plant to the apical tip and average calculated per plant, leaf number was recorded using a tally counter.

2.5 Preparation of Enzyme Extracts

Peroxidase [PO] was extracted and assayed following the procedure of [24]. Samples of cowpea leaves weighing 1 g were homogenised in 3 ml of 50 mMphosphate buffer at pH 7.0 containing 0.1 N NaCl, 1% PVP [Sigma] and 1 mMascorbate [Sigma] at 4°C. After centrifugation at 15,000 × g for 15 min, the supernatant was collected and stored at -20◦C until assayed for enzyme activity. For the photometric assay, 20 μl of the enzyme extract was mixed with 300 μl H_2O_2 [30 mM],400μl guaiacol [30 mM] and 780 μl sodium phosphate buffer at 27◦C. The change in the optical density of the reaction mixture was immediately recorded at 470 nm for 5 min with 15 s intervals [Novaspec II spectrophotometer, Amersham Pharmacia Biotech, Amersham,UK]. The blank consisted of the reaction mixture without the enzyme extract. The PO activity was determined from the linear part of the reaction curve over time and expressed as the change in optical density per second and per protein content of the sample [OD470 nm·s-1·mg protein-1].

Similarly, Polyphenol oxidase [PPO] activity was determined by colour changes in intensity of pyrroloxidation products according to [24]. The reaction mixture consisted of 20 µl of the enzyme extract of each sample which was added to 1.5 ml of 0.2 M sodium acetate buffer with pH 5 at 4˚C and modified by adding 200 µl of 0.02 M pyrogallol in place of catechol and the activity expressed as changes in absorbance at 410 nm. A blank was prepared from the reaction substrate without enzymes extract.

2.6 Statistical Analysis

All data collected were subjected to analysis of variance [ANOVA] using SPSS to verify if there were significant differences among the genotypes. Significance of the difference between inoculated and un-inoculated plants of each genotype was determined at 5 % level of probability.

3. Results and Discussion

3.1 Cowpea mottle virus on cowpea cultivars

The result of the trial on cowpea cultivars as affected by CMoV is presented in Table 1. There were significant differences in the incidence of cowpea mottle virus among the cowpea cultivars. Results showed that at one week after inoculation [WAI], cultivar Ezobokun recorded the highest incidence of cowpea mottle virus of 2.5% followed by Ezokapangi with 2.4% and the lowest incidence was recorded in cultivar IT07K-277-2 with 2.1%. On the other hand, at 5WAI, highest incidence of cowpea mottle virus was again recorded in cultivar IT07K-277-2 with 4.4 followed by cultivar, Ezobokun with 4.1 and the lowest virus incidence of 3.6% was recorded in cultivar Langbazo [Table 1].

Table 1. Disease incidence of cowpea mottle virus on cowpea cultivars

Treatment	% incidence at W1	% incidence at W2	% incidence at W3	% incidence at W4	% incidence at W5
Langbazo	2.2ab	2.6a	2.9	2.7	3.6c
Ezomilkigi	2.1b	2.4d	2.5	2.9	3.9bc
Ezobokun	2.5a	2.9b	3.1	3.7	4.1ab
Ezokapangi	2.4ab	2.9b	3.0	3.5	4.0b
IT07K-277-2	2.1b	3.4a	3.1	3.6	4.4a
SE	0.29	0.50	NS	NS	0.42

3.2 Plant Vigour

The effect of CPMoV on yield components, plant height and number of leaves is presented in Table 2. Cultivars Langbazo and IT07K-277-2 had taller plants and higher leaf number throughout the period of the study, while lower plant heights and leaf number were recorded in Ezokapangi. Similarly, percentage reduction of the measured parameters as affected by CPMoV ranged from -0.7 to -10.2% for plant heights and -4.5 to -11.1% for leaf number respectively.

At 3WAI cultivar Ezokapangi recorded the lowest plant height of 23.5cm followed by cultivar Ezobokun with 23.9 cm tall plants and highest plant height was recorded by cultivar Langbazo with 28.3cm. On the other hand, at 9WAI, the tallest plants were recorded in cultivar IT07K-277-2 measuring 42.5cm followed by cultivar Ezomilkigi with 37.4.3cm and the shortest plants were recorded in cultivar Ezobokun with 33.4cm [Table 2]. The result also showed that at 9WAI maximum plant height reduction was recorded in cultivar Ezobokun with -6.4 % followed by cultivar Ezokapangi with -6.4% compared to control treatment. Thus minimum relative plant height reduction was recorded in cultivar Ezomilkigi with -5.1 % followed by IT07K-277-2 with -5.12 % compared to the control treatment.

3.3 Number of Leaves

There were also significant differences in the number of leaves among the cowpea cultivars. Results presented in Table 3 show that at 3WAI, cultivar Ezobokun produced the lowest number of leaves of 15.0 followed by Ezokapangi with 15.1 and the highest number of leaves was produced by cultivar IT07K-277-2 with 28.26. On the other hand, at 6WAI, the highest number of leaves was recorded in cultivar IT07K-277-2 with 40.8 followed by cultivar Ezomilkigi with 22.4 and the lowest number recorded was in Ezobokun with 18.6 leaves. The result also showed that at 6WAI the maximum leaf number reduction was recorded in cultivar Ezobokun with -9.7% followed by Langbazo, also with -9.7% compared to the control treatment. Minimum relative leaf number reduction was found in IT07K-277-2 with -4.7% compared to the control treatment.

Table 2. Effect of cowpea mottle virus inoculum on plant height, percentage reduction of different cowpea cultivars in Mokwa

Plant height									
Treatment	3WAI	% reduction	Control	6WAI	% reduction	Control	9WAI	% reduction	Control
Langbazo	28.3ab	-7.8	30.7	29.8b	-0.67	30.1ab	35.6ab	-5.8	37.8ab
Ezomilkigi	24.7b	-8.5	27.0	26.2bc	-8.07	28.5b	37.6ab	-5.1	39.6a
Ezobokun	23.9b	-8.8	26.2	24.8bc	-8.49	27.1b	33.4b	-6.4	35.7b
Ezokapangi	23.5b	-9.3	25.9	23.9c	-8.78	26.2c	33.5b	-6.4	35.8b
IT07K-277-2	25.6b	-10.2	28.5	32.8a	-6.55	35.1a	42.5a	-5.1	44.8a
SE 4.02	NS	7.80	6.2	10.1	6.1				

Means within rows that share the same letter are not-significant at p = 0.05 using Duncan's Multiple Range Test.

Table 3. Effect of cowpea mottle virus inoculum on number of leaves of cowpea cultivars

Treatment	3 WAI	% reduction	Control	6 WAI	% reduction	Control
Langbazo	16.0ab	-11.11	18.00b	19.1b	-9.7	21.1b
Ezomilkigi	16.9b	-10.57	18.93b	22.4b	-7.9	22.4b
Ezobokun	15.0b	-11.76	17.00b	18.6b	-9.7	20.6b
Ezokapang	14.3b	-12.6	16.32c	17.4b	-9.6	19.3b
IT07K-277-2	28.3a	-4.53	29.60a	40.8a	-4.6	42.8a
SE	3.02	3.02	4.5	5.80	5.80	3.4

Means within rows that share the same letter are non-significant at p = 0.05 Using Duncan's Multiple Range Test WPI=weeks after inoculation,

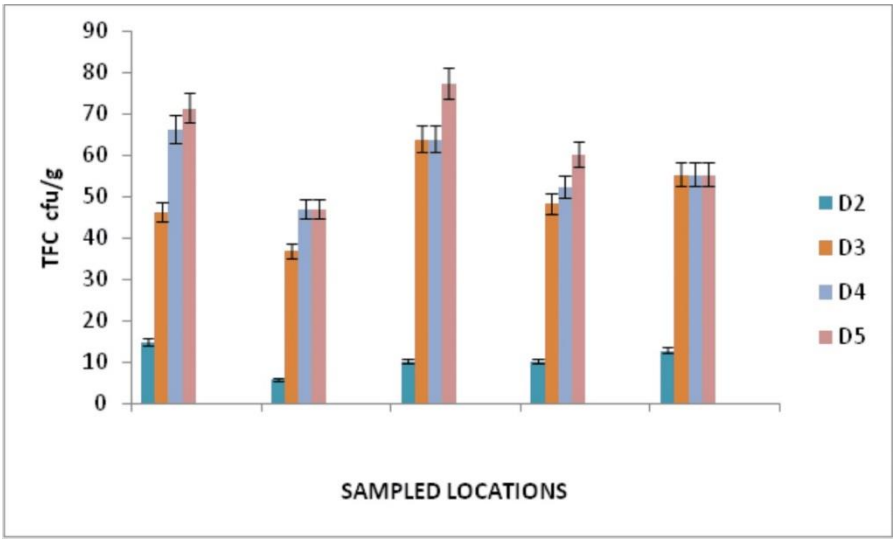

Figure 1. Peroxidase activity in leaves of cowpea cultivars infected by CPMoV

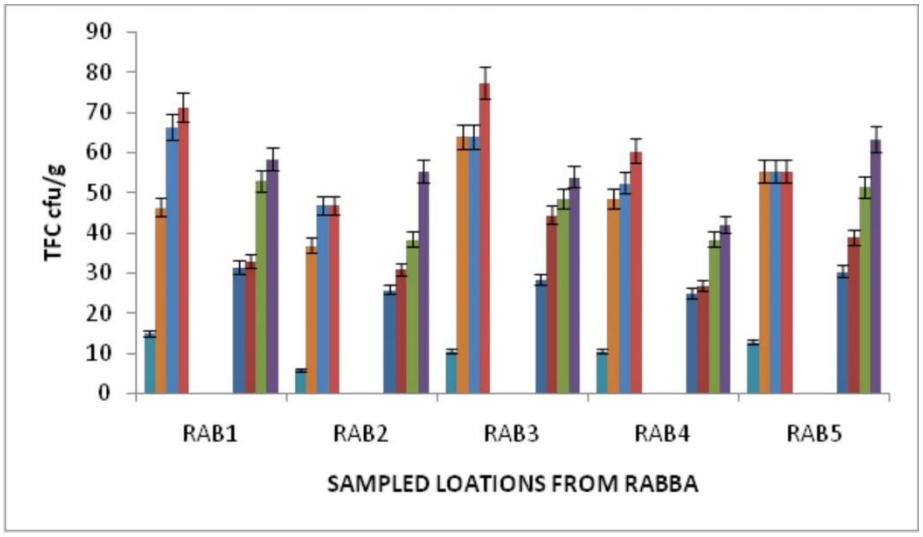

Figure 2. Polyphenol oxidase activity in leaves of cowpea cultivars infected by CPMoV from Rabba

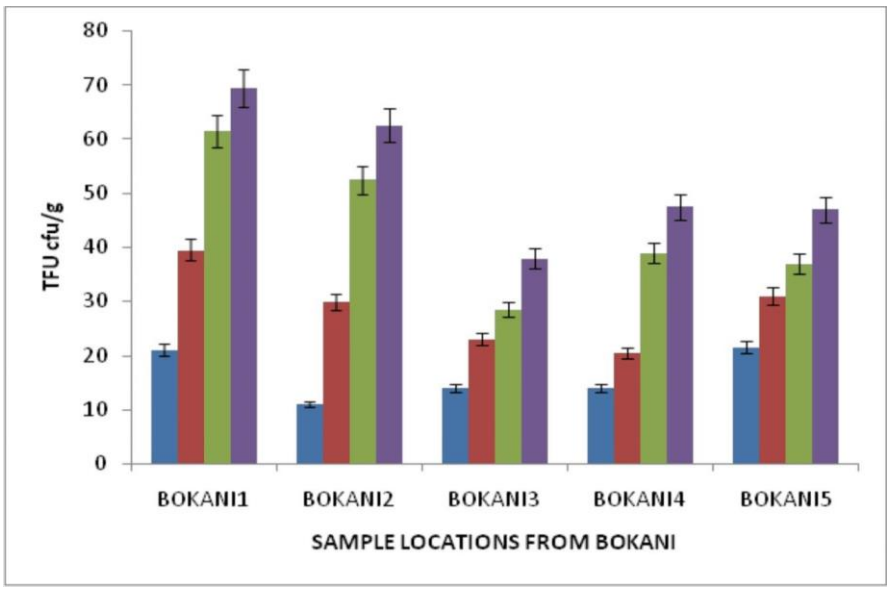

Figure 3. Peroxidase activity in leaves of cowpea cultivars infected by CPMoV sampled from Bokani

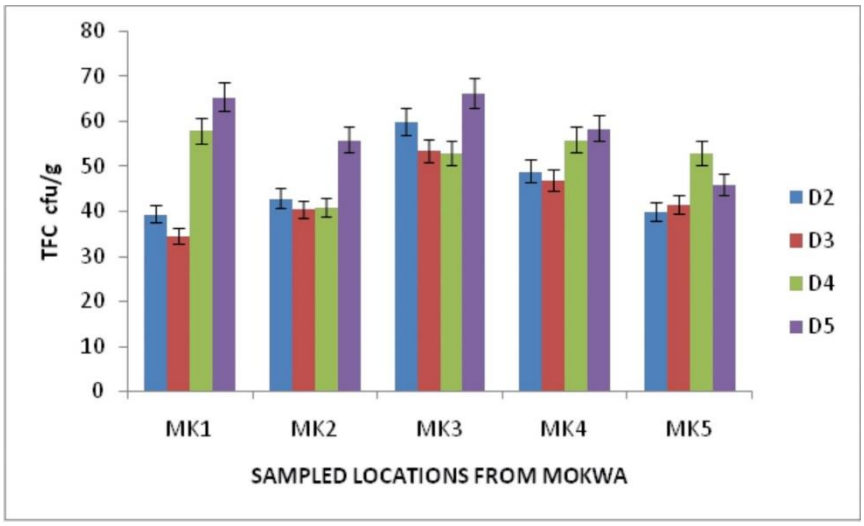

Figure 4. Peroxidase (PO) and polyphenol oxidase (PPO) activity in cowpea cultivars inoculated with CPMoV sampled from Mokwa

The activities of peroxidase and polyphenol oxidase in leaves of cowpea cultivars infected by *CPMoV* were analysed, as these enzymes are known to be involved in the defense responses of plants infection [25]. The results of the peroxidase and polyphenol oxidase activity in the cowpea cultivars infected by *CPMoV* are shown in Figs 1-4. There was a significant increase in PO and PPO activity from day 1 to 3rd day after inoculation in all the cultivars, except in Langbazo, which had low enzyme secretion.

According to reports by cowpea contains significant amounts of phenolic compounds including phenol acids, flavonoids and tannins. However, reported that the total phenolic content in cowpea varieties is dependent on the seed coat phenotype [25-28].

The resistance of cultivars Langbazo and IT07K-277-2 from the present study agrees with and contradicts earlier reports by several workers [25-27]. This is because cultivar Langbazo recorded low PO and PPO secretions, which are known enzymes that provide defense to plants against infection by pathogens and still resisted *CPMoV* infection in the present study. On the other hand, the result confirms reports by the same workers [25-27] for cultivar IT07K-277-2 which recorded high enzyme secretion [Figs. 1 & 2].

However, the low enzyme content level by cultivar Langbazo and it still being resistant to *CPMoV* in this study can be from the composition of its seed coat phenotype [28]. The workers reported that the total phenolic content in cowpea varieties is dependent on their seed coat phenotypes, which must have necessitated the differential reaction of the studied cowpea cultivars to the CPMoV pathogen in this study. Thottappilly and Rossel, [29] reported that in Nigeria, the most economical, practicable and effective method of management for legume viruses is through the use of resistant varieties. Cowpea lines with individual or combined resistance to severe cowpea viruses have been identified at IITA [30]. Sources of resistance have also been identified in soyabean [16]. Such legume lines are being tested in different localities for selection of the best locally adapted 6 varieties with multiple virus resistance [30]. However, the rate of acceptance and utilisation of such resistant varieties is rather poor, but use of resistant varieties of crops remains the best and environmentally acceptable management tool for pathogenic organisms including *CPMoV*. Thus, cultivars Langbazo and IT07K-277-2 identified to be resistant to *CPMoV* from this study adds to the IITA germplasm, which should be exploited in hybridization programmes for the development of new *CPMoV* resistant varieties for planting by cowpea farmers. The update report by [31] on cowpea viruses in Southwest Nigeria may have added to the number of further strains to be exposed by the two identified cultivars in future studies.

4. Conclusion

The identification of two resistant cowpea cultivars from the present study is a significant addition to the IITA cowpea germplasm. The Nigerian cowpea growers are to be immediate beneficiaries to the would be new varieties to be evolved using Langbazo and IT07K-277-2 cultivars as donor traits in hybridization studies.

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Conflict of interest

The authors declare that no conflict of interest exists.

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