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Germination and Longevity of Some Cowpea Cultivars Affected by Single and Mixed Virus Infections in Niger State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MTS managed the analyses of the study. Authors ACW and HI managed the literature searches and improved on several aspects of the work. All authors read and approved the final manuscript.

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ABSTRACT

Cowpea being a dependable source of protein for human growth and development is widely cultivated in sub-Saharan Africa including Nigeria. In spite of its numerous uses, infection by viruses constitutes serious problems to its productivity and once plants are infected, there is no remedy as is with other pathogens such as bacteria, fungi and nematodes. A field trial was conducted to investigate the reactions of twenty five cowpea cultivars to single and mixed infections with two unrelated viruses: *Blackeye cowpea mosaic virus* (BlCMV) and *Cowpea mottle virus* (CPMoV) on seed germination and longevity. The trial was conducted at the Teaching and Research Farm of the Ahmadu Bello University (ABU), Zaria, Mokwa Station and set up in a randomized complete block design (RCBD) with three replicates. For the single virus infection, seedlings of the twenty five cultivars were inoculated at 10 days after sowing (DAS), while for the mixed virus infections, seedlings were inoculated at 10 and 17 DAS. Results showed that all the cultivars were susceptible to single and mixed infections but to seemingly different extents.

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Germination of seeds was generally high before storage but was short lived indicating that conservation of infected seeds of the cultivars was impaired. Seeds of cultivars IT04K-267-8 and IT07K-222-2 recorded germination percentages of 54.6 and 53.7% respectively, while cultivars IT96D-610 and IT04K-291-2 had germination values of 52% which did not differ from each other. Viability of seeds amongst the 25 test cowpea cultivars from single infection with CPMoV alone and BICMV alone did not differ in some instances. Percent germination in cultivars IT98K-205-M8, IT90K-277-2 and IT07K-222-2 inoculated with BICMV + CPMoV were not much affected. Test of accelerated ageing germination (AAG) percentage for four weeks showed that seed vigour was greatly impaired in cultivars IT07K-292-2-10, IT06K-124 and IT90K-277-2 infected with BICMV + CPMoV compared to the lowest AAG percentage of 31.6 recorded in seeds of cultivar IT99K-377-1. Constant monitoring of legume fields through regular field sanitation and disease surveys to identify new and emerging viruses as facts obtained from this study are good starting point for legume virus diseases diagnosis.

Keywords: Cowpea seeds; germination; infections; viability; viruses.

1. INTRODUCTION

Cowpea (Vigna unguiculata L. Walp) is a major leguminous crop grown in Africa [1]. It is a drought tolerant and warm-weather crop. The largest cowpea production is in the moist and dry savannas of sub-Saharan Africa, where it is intensively grown as an intercrop with other cereal crops like millet, sorghum and maize due to the advantages of residual nitrogen emanating from the decay of roots and root nodules [2,3]. Cowpea has the capability to improve nitrogen content of its immediate environment for growth and development [4]. Cowpea seed contains 24 % crude protein, 53% carbohydrate and 2% fat [5]. The seeds are a major source of plant protein and vitamins for man, feed for animals, and also a source of cash income. It has been estimated that the annual world cowpea crop is grown on 12.5 million hectares, and the total grain production is 3.9 million tonnes5. More than 8 million hectares of cowpea are grown in West and Central Africa. Also, it is known that Nigeria is the largest producer with 4 million hectares [6]. Nigeria, being the largest producer accounts for 45% of the total on 1.15 million hectares annually. The major cowpea producing areas in Nigeria include Niger, Kwara, Kaduna, Borno, Taraba and Yobe States in the northern part while Oyo, Ogun and Ondo also produce appreciable quantities in the southern part of the country [7].

Virus diseases are considered to be a major limiting factor for the production and productivity of legumes in the tropical and sub-tropical countries [8]. Out of more than 20 viruses reported on legume from different parts of the world, nine are known to infect cowpea naturally in Nigeria. Blackeye cowpea mosaic virus (BICMV) genus Potyvirus, family potyviridae was first reported on cowpea in the U.S. in 1959. It is distributed in all ecological zones and cowpea-growing areas of Nigeria. Local symptoms appear as large reddish lesions that spread along the veins, while systemic symptoms appear as severe mottle, mosaic, vein-banding, veinal chlorosis, distortion and stunting of the plant. Disease symptoms vary with virus strain and host cultivar. Incidence varies from 1-40% on farmers' fields. Yield losses due to the virus vary from 10-85% on individually infected plants and vary with the time of sowing. Cowpea mottle virus (CPMoV) genus Gammacarmovirus, family Tombusviridae is a positive sense single-stranded RNA, unipartite, isometric virus, 30 nm in diameter [9]. 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 [9]. Infected plants display severe mosaic, mottling or bright yellow mosaic, leaf distortion and reduction in leaf size.

Seed-borne viruses can aggravate other transmission methods and cause disease to spread rapidly. Seed-borne and seed transmitted viruses are also damaging to cowpea productivity owing to inherent primary inoculum and potential for their widespread damage to the crop [10]. Information on germination of infected seeds and survival of resulting plants, virus disease progress during the growing season, magnitude of yield loss and amount of infection in harvested seeds in replicated field experiments is required to establish acceptable threshold levels of seed-borne infections. The study is essential to develop preventive and management measures for cowpea virus diseases in Niger State.

Therefore, this research work is aimed at examining germination and longevity of some cowpea cultivars affected by single and mixed virus infections to provide information for plant breeders for the development of cowpea resistant varieties to these viruses.

2. MATERIALS AND METHODS

2.1 Field Trial

Field trial was conducted during the 2017 wet session at the Teaching and Research farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Mokwa Station (09°211' N and 5°135' E, 201 m above sea level) in the Southern Guinea Savanna agro - ecological zone of Nigeria. The site used was under continuous mixed cropping with maize, guinea-corn and soyabean between 2012 till the commencement of the experiment.

2.2 Treatments and Experimental Design

The treatments consisted of 25 cowpea cultivars which were photosensitive and high yielding under virus free conditions. Four independent trials were conducted simultaneously, for single (BICMV and CPMoV) and mixed infections (BICMV + CPMoV and CPMoV + BICMV) respectively. The cowpea cultivars namely Ife Brown, IT90K – 277 – 2, IT96D – 610, IT97K – 499 – 35, IT97K – 568 – 18,IT97K – 573 – 2 –1, IT98K – 205 –M8, IT98KD – 288, IT99K – 316 – 2, IT99K – 377 – 1, IT00K – 901 – 5, IT03K – 337 – 6, IT04K – 267 – 8, IT04K – 291 – 2, IT04K – 321 – 2, IT04K – 332 – 1, IT06K – 124, IT06K – 137 – 1, IT07K – 211 – 1 – 8, IT07K – 222 – 2, IT07K – 243 – 1 – 10, IT07K – 251 – 3 – 3, IT07K – 292 – 1 – 10, IT07K – 299 – 6, IT07K – 318 – 33). The trial was arranged as randomized complete block design (RCBD) and replicated three times.

2.3 Source of Inoculum and Multiplication

The BICMV and CPMoV isolates previously identified by DAS-ELISA were obtained from the Department of Crop Production, Federal University of Technology, Minna Niger State. The virus isolates were extracted by grinding 1g/1 ml of each isolate in extraction buffer containing 0.1 M sodium phosphate dibasic, 0.1 M potassium phosphate monobasic, 0.0lM ethylene diamine tetra acetic acid and 0.00lM-cystine per litre of distilled water using a pre-cooled sterilized mortar and pestle as described by Kumar [11]. Two microlitres of β- mercapto-ethanol was added to the extract just before used. Thereafter, cowpea seedlings were infected with BICMV and CPMoV inoculum at 10 days after sowing (DAS) by rubbing the virus extracts on the upper surface of the leaves that was dusted with carborundum powder of 600- mesh. The leaves of inoculated plant were rinsed with sterile distilled water. Symptomatic cowpea leaves were collected from the infected plants at 3 weeks after inoculation (WAI) and used for inoculation during the main experiment. The leaves were preserved at room temperature in airtight via bottle on silica gels covered with a thin layer of non-absorbent cotton wool.

2.4 Land Preparation, Sowing, Inoculation and Disease Severity Score

The field was manually cleared of the previous plant remains, ploughed, harrowed and ridged with tractor at 0.75 m apart then marked out into plots and replications in the second week of August, 2017. Each cultivar was evaluated in 0.375 m ridge wide, 3 m long and 0.75 m apart giving a total plot size of 18.75 m with a total land area of 900 m^2. Cowpea seeds were sown one week after the land preparation. Three cowpea seeds of each cultivar were sown after dressing with Apron – star (methylthiuram + metalaxyl + carboxin) at the of rate 3.0 kg seed per 10 g sachet of the chemical to protect seed against soil borne pathogens. The sowing was carried out at an intra and inter–row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS). The BICMV and CPMoV infected cowpea leaves previously preserved on silica gels were used for inoculation. For the single virus infection, seedlings of the twenty five cultivars were mechanically inoculated singly with BICMV or CPMoV at 10 days after sowing while for the mixed virus infections, seedlings were inoculated at 10 and 17 DAS. Weeds were manually controlled through hand weeding at 4 and 6 weeks after sowing. Insect pests were controlled by spraying D-D force (Cypermethrin plus Dimethoate) and pods were harvested at physiological maturity. Disease severity scores on the infected cowpea cultivars were assessed at 2 and 5 weeks after inoculation (WAI).

Disease severity based on a visual scale of 1 – 5 was used as described by[12] Where 1 = no symptoms, 2 = slight mosaic; 3 = moderate mosaic, 4 = severe mosaic, leaf distortion and stunting, 5 = severe mosaic, stunting and plant

death, as follows Disease severity (Table 1) was based on a visual scale (1 – 5) as described by Nsa and Kareem [12] Where, 1 = no symptoms, 2 = slight mosaic; 3 = moderate mosaic, 4 = severe mosaic, leaf distortion and stunting, 5 = severe mosaic, stunting and plant death. The pods were processed and packaged for seed quality assessment in the laboratory.

2.5 Assessment of Virus Infection on Seed Quality

Seed lots from the various virus treatments were subjected to viability and longevity test and determined by germination test after harvest and at four weeks of storage at the Crop Production Laboratory, Department of Crop Production, Federal University of Technology, Minna. Twenty-five cowpea seeds of the different cultivars were placed in distilled-water moistened filter paper lined in petri-dish in three replicates. The filter paper in the petri-dishes was kept moist as found necessary. The petri-dishes were arranged inside the seed germination chamber. Germination counts were taken at 1, 2, 3, 4 and 5 days after sowing. Seeds were considered germinated when the tip of the radicle had grown free from the seed coat [13]. Germination percentage (GPCT) was calculated as follows:

GPCT= (Total number of seedlings that emerged on the final day /Total number of seeds planted) × 100

Cowpea seeds were also subjected to accelerated ageing (AA) tests at two and four weeks as described by Balla et al. [13] for vigour determination. The seeds of all the treatments were stored in open plastic plates and arranged inside an incubator at 35^{o}C and 86% relative humidity. This was aimed at acce0lerating the ageing of the seeds so that the relative longevity of the seed samples could be determined. Twenty five seeds from each treatment that were artificially aged in three replications were counted and placed on layer of distilled water moistened-filter paper placed in petri-dishes over a wire mesh screen inside a growth chamber at 30^{o}C. Germination count was taken as described above.

2.6 Data Analysis

Disease severity (Table 1) was based on a visual scale of 1 – 5 described by Nsa and Kareem [12]. On the scale, 1 = no symptoms, 2 = slight mosaic; 3 = moderate mosaic, 4 = severe mosaic, leaf distortion and stunting, 5 = severe mosaic, stunting and plant death, while data gathered from germination test were subjected to analysis of variance (ANOVA) using Statistical Analysis System [14] to verify if there were significant differences among the cultivars. Significance was determined at 5% level of probability. Where the F-test ratio was significant, means were separated using Student-Newman-Keuls (SNK) test.

3. RESULTS

3.1 Symptom Severity Induced by Single and Mixed Virus Infections on Cowpea Plants

Disease severity differed significantly ($p \leq 0.05$) amongst the 25 cowpea cultivars investigated irrespective of the four virus treatments. The progress of infection in the cowpea plants inoculated with each virus and the virus combinations is shown in Table 1. Disease severity increased progressively after inoculation, at 2 WPI, the symptoms observed on plants inoculated with BICMV + CPMoV were not much different from those of BICMV alone, and the symptoms observed on CPMoV + BICMV were also like those of CPMoV. Disease severity values remained constant between 3 and 4 WPI irrespective of the virus treatment, but not the same in all the cowpea cultivars.

At 2 WAI, disease severity was significantly ($p<0.05$) highest in Ife brown with 3.6 score,IT97K-568-18, IT06K-124 and IT07K-292-1-10 had a symptom score of 2 in BICMV infected cowpea plants, IT03K-337-6 exhibited a mean severity score of 1.0 and moderate level of severity symptom score of 3.0 was observed in the other cultivars. In CPMoV infected cowpea plants, disease severity ranged between 1 and 3.6. The lowest symptom score of 1was observed in IT90K-277-2, IT96D-610, IT04K-332-1 and IT07K-243-1-10. Disease severity was mild in IT07K-222-2 and IT07K-292-1-10 with a score of 2 while IT03K-337-6, IT04K-291-2, IT04K-321-2, IT06K-124, IT06K-137-1, IT07K-211-1-8, IT07K-251-3-3 and IT07K-299-6 recorded a mean severity score of 3.The other cultivars: Ife brown, IT97K-573-2-1 and IT07K-318-33 had a mean symptom score of 3.6. Generally, disease severity in the mixture of BICMV + CPMoV infected cowpea cultivars did not differ significantly ($p>0.05$) from those of BICMV infected cowpea cultivars alone (Table 1).

Table 1. Severity of single and mixed infections of Blackeye cowpea Mosaic Virus (BICMV) and Cowpea Mottle Virus (CPMoV) on cowpea plants at Mokwa, southern guinea savannah, Nigeria

Cultivar	2 week post infection				5 weeks post infection			
	BICMV	CPMoV	BI + CP	CP + BI	BICMV	CPMoV	BI + CP	CP + BI
Ife brown	3.6^{a}	3.6^{a}	3.6^{a}	3.6^{a}	4.3^{a}	4.3^{a}	4.3^{a}	4.3^{a}
IT90K-277-2	2.6^{abc}	1.0^{d}	3.6^{a}	3.6^{a}	4.0^{a}	1.6^{c}	4.3^{a}	4.3^{a}
IT96D-610	3.0^{ab}	1.0^{d}	1.0^{b}	3.6^{a}	4.3^{a}	1.6^{c}	1.6^{b}	4.3^{a}
IT97K-499-35	3.0^{ab}	3.0^{ab}	1.0^{b}	3.0^{ab}	4.3^{a}	4.3^{a}	1.6^{b}	4.3^{a}
IT97K-568-18	1.6^{cd}	3.0^{ab}	3.0a	2.0^{b}	1.6^{b}	4.3^{a}	4.3^{a}	2.6^{abc}
IT97K-573-2-1	2.6^{abc}	3.6^{a}	1.0^{b}	3.0^{ab}	4.3^{a}	4.3^{a}	1.6^{b}	4.3^{a}
IT98K-205-M8	3.0^{ab}	2.6^{abc}	3.0^{a}	3.0^{ab}	4.3^{a}	4.3^{a}	4.0^{a}	4.3^{a}
IT98KD-288	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}	3.6^{a}	3.6^{ab}	4.3^{a}	3.6b
IT99K-316-2	2.6^{abc}	1.0^{d}	2.6^{a}	2.6^{ab}	4.3^{a}	1.6^{c}	4.0^{a}	4.3^{a}
IT99K-377-1	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}	4.3^{a}	4.3^{a}	4.0^{a}	4.3^{a}
IT00K-901-5	2.6^{abc}	3.0^{ab}	2.6^{a}	2.6^{ab}	4.0a	4.3^{a}	4.3^{a}	4.3^{a}
IT03K-337-6	1.0^{d}	2.6^{abc}	2.6^{a}	2.6^{ab}	1.6^{b}	4.3^{a}	4.3^{a}	4.3^{a}
IT04K-267-8	3.0^{ab}	1.0^{d}	2.6^{a}	2.6^{ab}	4.3^{a}	1.6^{c}	4.0^{a}	4.3^{a}
IT04K-291-2	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}	3.6^{a}	3.6^{ab}	4.0^{a}	3.6^{ab}
IT04K-321-2	2.6^{abc}	2.6^{abc}	2.6^{a}	2.6^{ab}	4.3^{a}	4.3^{a}	4.3^{a}	4.3^{a}
IT04K-332-1	3.0^{ab}	1.3^{cd}	1.0^{b}	2.0^{b}	4.3^{a}	1.6^{c}	1.6^{b}	2.6^{abc}
IT06K-124	2.0^{bcd}	3.3b	3.0^{a}	3.0^{ab}	2.3^{b}	3.6^{ab}	4.3^{a}	4.3^{a}
IT06K-137-1	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}	4.3^{a}	4.0^{a}	4.0^{a}	4.3^{a}
IT07K-211-1-8	2.6^{abc}	3.0^{ab}	2.6^{a}	2.6^{ab}	4.3^{a}	4.3^{a}	4.3^{a}	4.3^{a}
IT07K-222-2	3.0^{ab}	2.0^{bc}	3.0^{a}	2.0^{b}	4.3^{a}	2.3^{bc}	4.2^{a}	2.3^{bc}
IT07K-243-1-10	2.6^{abc}	1.0^{d}	2.6^{a}	2.6^{ab}	4.0^{a}	1.6^{c}	4.3^{a}	4.3^{a}
IT07K-251-3-3	2.6^{abc}	2.6^{abc}	2.6^{a}	2.6^{ab}	4.3^{a}	4.3^{a}	4.0^{a}	4.3^{a}
IT07K-292-1-10	2.3^{bc}	2.0^{bc}	1.0^{b}	1.0c	2.6^{b}	2.6^{abc}	2.0^{b}	1.6^{c}
IT07K-299-6	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}	4.0^{a}	4.3^{a}	4.3^{a}	4.0^{a}
IT07K-318-33	2.6^{abc}	2.6^{abc}	2.6^{a}	2.6^{ab}	4.3^{a}	4.0^{a}	4.3^{a}	4.3^{a}
+ SEM	1.9	0.2	0.21	0.2	0.30	0.37	0.34	0.32

Means with the letter (s) within the same column are not significantly ($p \leq 0.05$) different by Student-Newman-Keuls (SNK) test; +SE: Standard error

3.2 Effects of Single and Mixed Virus Infections on Seed Quality

Variations in seed viability of cowpea cultivars with respect to virus infections are presented in Table 2. This showed that there were significant impairments in germination before and after four weeks of storage of the 25 cultivars of cowpea both in single and mixed infections of the viruses used. The difference between the lowest and highest mean value for seed viability was wide and significant ($p<0.05$) on seed germination test (SGT) prior to seeds storage, Percent seed germination varied from 77.4 to 98.7% for CPMoV infected cultivars, 77.4 to 99.7% for the BICMV infected cultivars, 74.8 to 98.5% for BICMV + CPMoV infected cultivars and 78.6 to 98.5% for CPMoV + BICMV inoculated cultivars (Table 1).Seeds obtained from IT97K-568-18, IT04K-332-1 and IT07K-292-1-10 cultivars of cowpea infected with BICMV resulted to significantly ($p<0.05$) higher percent germination of 99.7 which was statistically similar to 97.6 and 97.3% germination obtained from seeds of cowpea cultivars IT07K-243-1-10 and IT03K-337-6 respectively. Cowpea seeds from cultivars IT90K-277-2, IT07K-211-1-8 and IT06K-124 had germination values of 94.7, 94.3 and 93.7% respectively which were not significantly different among each other. Seeds of cultivars IT07K-251-3-3 and IT07K-222-2 recorded germination values of 92.3 and 92.5 % respectively which were statistically similar, while seeds from the remaining cowpea cultivars recorded germination percentages ranging between 77.4 and 91.3.

Seed germinability of 98.7% was highest in cultivar IT90K-277-2 with CPMoV infected cowpea seeds which was not significantly ($p > 0.05$) different from seeds obtained from cultivars IT04K-332-1 with 98.5%, IT07K-243-1-10 with 98.4%, IT04K-267-8 with 98.2% and IT96D-610 with 97.7%, while significantly the lowest seed germination percentage of 77.4 was recorded in seeds of cowpea cultivar IT07K-292-1-10 (Table 2).

Table 2. Cowpea seed quality as affected by single and mixed infections of Blackeye Cowpea Mosaic Virus (BICMV) and Cowpea Mottle Virus (CPMoV) at Mokwa in 2017

Cultivar	Viability test (%)				Accelerated ageing germination (%) 4 weeks of storage			
	BICMV	CPMoV	BI + CP	CP + BI	BICMV	CPMoV	BI + CP	CP + BI
Ife Brown	93.5^{bcd}	90.5^{c-f}	86.7^{f}	86.5^{gh}	77.9^{a}	69.0^{b}	56.6^{b}	46.2^{l}
IT90K – 277 – 2	94.7^{bc}	98.7^{a}	78.5^{c}	96.0^{bc}	76.6^{a}	68.0^{bc}	58.4^{a}	51.6^{e}
IT96D – 610	87.3^{g}	97.7^{a}	97.6^{b}	96.0^{bc}	70.3^{b}	66.5^{d}	52.0^{e}	52.0^{de}
IT97K – 499 – 35	88.0^{fg}	86.9^{i}	97.6^{b}	92.0^{e}	61.5^{de}	60.0^{g}	55.0^{c}	56.0^{ab}
IT97K – 568 – 18	99.7^{a}	91.2^{c}	81.3^{j}	98.5^{a}	69.5^{b}	57.2^{h}	48.0^{h}	41.2^{l}
IT97K – 573 – 2 – 1	87.8^{g}	93.4^{b}	94.8^{d}	94.5^{d}	50.6^{l}	57.1^{hi}	45.5^{i}	35.6^{m}
IT98K – 205 –M8	87.6^{g}	89.2^{efg}	77.5^{m}	96.0^{bc}	57.5^{gh}	70.6^{a}	56.3^{b}	41.5^{l}
IT98KD – 288	91.3^{c-g}	90.7^{cde}	82.6^{i}	96.0^{bc}	48.0^{m}	62.7^{f}	48.3^{gh}	51.3^{ef}
IT99K – 316 – 2	92.1^{c-f}	93.4^{b}	85.0^{gh}	97.3^{ab}	53.3^{jk}	64.0e^{f}	57.3^{ab}	46.0^{j}
IT99K – 377 – 1	88.9^{efg}	90.8^{cd}	85.4^{gh}	92.0^{e}	60.0ef	60.0^{g}	50.6^{f}	31.6^{n}
IT00K – 901 – 5	88.8^{efg}	86.1^{i}	81.3^{j}	89.3^{f}	70.6^{b}	65.0^{e}	52.0^{e}	47.0^{ij}
IT03K – 337 – 6	97.3^{ab}	89.4^{d-g}	84.6^{h}	89.3^{f}	50.5^{l}	66.8^{cd}	46.4^{i}	41.4^{l}
IT04K – 267 – 8	92.2^{c-f}	98.2^{a}	81.3^{j}	86.5^{gh}	56.0^{hi}	62.6^{f}	49.5^{fg}	54.6^{bc}
IT04K – 291 – 2	87.8^{g}	86.9^{i}	89.3^{e}	87.7^{g}	54.6^{ij}	58.7^{g}	57.4^{ab}	52.0^{de}
IT04K – 321 – 2	90.5^{c-g}	93.8^{b}	85.3^{gh}	78.6^{k}	58.6^{fg}	56.3^{hi}	48.0^{h}	50.6^{efg}
IT04K – 332 – 1	99.7^{a}	98.5^{a}	98.5^{a}	96.0^{bc}	60.0^{ef}	53.4^{k}	49.3^{fgh}	48.1^{hi}
IT06K – 124	93.7^{bc}	90.1^{c-g}	80.0^{k}	81.2^{j}	46.6^{m}	56.8^{hi}	58.6^{a}	49.6^{fgh}
IT06K – 137 – 1	77.4^{h}	87.2^{hi}	78.8^{l}	80.0^{jk}	52.0^{kl}	56.0^{hij}	54.5^{cde}	44.0^{k}
IT07K – 211 – 1 – 8	94.5^{bc}	88.5^{gh}	89.3^{e}	78.6^{k}	53.3^{jk}	56.0^{hij}	53.4^{d}	49.3^{gh}
IT07K – 222 – 2	92.5^{cde}	93.0^{b}	74.8^{n}	96.0^{bc}	54.2^{j}	56.4^{jk}	45.3^{ij}	53.7^{bc}
IT07K – 243 – 1 – 10	97.6^{ab}	98.4^{a}	89.3^{e}	94.8^{cd}	57.5^{gh}	54.7^{jk}	50.5^{f}	50.8^{efg}
IT07K – 251 – 3 – 3	92.3^{cde}	88.5^{gh}	82.8^{i}	85.3^{h}	57.3^{gh}	56.6^{ij}	44.0^{k}	47.0^{ij}
IT07K – 292 – 1 – 10	99.7^{a}	77.4^{j}	96.0^{c}	81.3^{j}	62.1^{d}	57.3^{h}	58.3^{a}	57.3^{a}
IT07K – 299 – 6	80.3^{h}	89.0^{gh}	86.8^{f}	82.6^{i}	64.4^{c}	64.0^{ef}	49.7^{f}	44.0^{k}
IT07K – 318 – 33	89.3^{d-g}	77.6^{j}	85.6^{g}	80.0^{jk}	59.8^{f}	58.7^{g}	44.9^{jk}	46.5^{ij}
+SE	1.27	0.5	0.26	0.43	0.54	0.46	0.42	0.61

Means with the letter (s) within the same column are not significantly ($p \leq 0.05$) different by Student-Newman-Keuls (SNK) test; ±SE: Standard error

On the other hand, co-infections of cowpea seeds significantly ($p<0.05$) affected seed germinability across the cowpea cultivars investigated. BICMV + CPMoV infected IT04K-332-1 exhibited the highest germination percentage of 98.5% than all other cultivars, whereas IT96D-610 and IT97K-499-35 gave 97.6% each. Seeds of cultivars IT07K-292-1-10 and IT97K-573-2-1 had 96.0 and 94.8% germination respectively, while seeds of cultivar IT07K-222-2 recorded the lowest germination percentage of 74.8. Seeds obtained from cultivar IT97K-568-18 infected with CPMoV + BICMV recorded the highest germination percentage of 98.5 before storage which was not significantly ($p > 0.05$) different from 97.3% obtained from seeds of cultivar IT99K-316-2.

Next to these with high germination percentage of 96 were seeds obtained from cultivars IT90K-277-2, IT96D-610, IT98K-205-M8, IT98KD-288, IT04K-332-1 and IT07K-222-2 whereas the significantly lowest germination percentage of 78.6 was recorded in seeds of cowpea cultivars IT04K-321-2 and IT07K-211-1-8 (Table 2).

Similarly, the difference between the lowest and highest percentage mean values for seed vigour traits was also wide and significant ($p<0.05$). When seeds were stored for four weeks for accelerated ageing, significantly highest germination percentage of 77.9 was recorded in seeds of BICMV infected Ife Brown followed by those of cultivars IT90K-277-2, IT00K-901-5 and IT96D-610 with 76.6, 70.6 and 70.3 germination percentage respectively. Seeds of cultivars IT97K-568-18, IT07K-292-1-10 and IT07K-299-6 recorded germination values of 69.5, 64.4 and 62.1%, respectively whereas the least germination value of 46.6% was obtained from seeds of IT06K-124. Mean value for accelerated ageing germination (AAG) on CPMoV infected cowpea cultivars showed that seeds of cultivar IT98K-205-M8 had 70.6% germination. This was closely followed by seeds of Ife Brown with 69 % while 68, 66.8 and 66.5% germination were obtained from cultivars IT90K-277-2, IT03K-337-6 and IT96D-610, respectively. The germination capacity of 64% was recorded from seeds of cultivar IT99K-316-2 while cultivar IT07K-299-6 and the remaining cultivars had AAG percentages ranging from 53.4 to 62.7% (Table 2).

In the mixed infection treatments, germination value of 58.6% was obtained from cultivars IT90K-277-2, IT06k-124 and IT07K-292-1-10 in BICMV + CPMoV infections. The 58.6% germination was significantly ($p<0.05$) higher than the values obtained from seeds of other cultivars. Seeds from cultivars IT98K-205-M8, IT97K-499-35, IT06K-137-1 and IT07K-211-1-8 recorded germination values of 56.5, 55, 54.5 and 53.4% respectively. Seeds of cultivars IT96D-610 and IT00K-901-5 recorded similar germination percentage of 52 while the remaining cowpea cultivars had germination percentages of between 44.0 and 50.6. Also, seed germinability of 57.3% was highest in IT07K-292-1-10 in CPMoV + BICMV infections which was statistically ($p>0.05$) similar to the performance of seeds of cultivar IT97K-499-35 with 56%. Seeds of cultivars IT04K-267-8 and IT07K-222-2 recorded 54.6 and 53.7% germination respectively, while cultivars IT96D-610 and IT04K-291-2 had germination values of 52% which did not differ from one another. The lowest AAG percentage of 31.6 was recorded in seeds of cowpea cultivar IT99K-377-1 (Table 2).

Seed germinability of 98.7% was highest in cultivar IT90K-277-2 with CPMoV infected cowpea seeds which was not significantly ($p > 0.05$) different from seeds obtained from cultivars IT04K-332-1 with 98.5%, IT07K-243-1-10 with 98.4%, IT04K-267-8 with 98.2% and IT96D-610 with 97.7%, while significantly the lowest seed germination percentage of 77.4 was recorded in seeds of cowpea cultivar IT07K-292-1-10 (Table 2).

4. DISCUSSION

The imperative of understanding the impact of virus management strategies and management for quality seed production stems from the paucity of information on the agronomy of seed production [15], more so that seed production efforts are judged on the basis of quality of the produce rather than quantity [16]. Blackeye cowpea mosaic virus and Cowpea mottle virus diseases cause significant yield losses in cowpea and several crops of economic importance [17]. In the present study, disease severity varied among the cowpea cultivars owing to the differences in their genotypes and genetic architecture [18]. This corroborates the findings of [19], who recorded substantial negative consequences in their CPMoV infected Arabidopsis thaliana. The quality of seed is influenced by the environment where it is produced, while seed viability and vigour determine the performance capability of seed lot. Nematodes, fungi, bacteria, viruses among other

pathogens are integral components of the environment of any seed crop and failure to effectively manage their competition can result in poor yield [20] as found in this study.

The result of the present study shows a clear negative influence of virus infection on cowpea seed quality. Also the differential ranking of the virus infection treatments in both germination and longevity tests is an indication of the effect of the competing virus infection and the developing seeds on the mother plant situations. Differences in time of flower initiation, pod setting, seed formation and maturity due to virus infections are critical factors in tropical farming [21]. Results obtained from this study show that there was a variation in germination percentage before and after four weeks of storage which is a measure of seed quality. When seed that has this trait is sown on the field for production, it exhibits a wide variation in performance after sowing due to the differences in quality [22].

It is known that cowpea seedlings are susceptible to virus infection at different stages of development [23]. This is agrees with the differential responses of cowpea seeds harvested from the different virus treatment seed lots in the present study. The initial general high germination percentage recorded in seeds of all treatment combinations in this study is an indication that the seeds did not exhibit dormancy contrary to what is known with most vegetable seeds when freshly harvested. The rapid germination recorded by the cultivars after harvest also shows that the activities of the viruses whether single or mixed on the seeds were not severe enough to impair germination [24]. According to Mandhare and Kand [25] and Pazarlar et al. [26], mosaic infected soyabean seeds at harvest recorded high seed germination but a significant sharp decline in germination percentage of the seeds was recorded following four weeks of storage at 32^{o}C and 50% relative humidity.

In the present study also after storage of the cowpea seeds for four weeks, a sharp decline in their germination capability from all the treatment combinations was recorded. However, this sharp decline in the quality of seeds is abnormal according to the normal and natural seed ageing process observed by Hamim et al. [27]. Thus, the reason may be that the pathogen activities must have been activated in the seeds which resulted in the sudden and heavy decline in the germination percentages which agrees with the findings of [28]. In addition, the variation in germination percentages amongst the cultivars and treatments as shown in this study suggests genetic superiority [23] and tolerant levels of the cultivars over each another. Thus, cultivars IT04K-332-1, IT07K-243-1-10, IT04K-267-8 and IT96D-610 can be exploited by plant breeders to develop resistant cowpea varieties against Blackeye cowpea mosaic virus and Cowpea mottle virus in the study area and other locations.

5. CONCLUSION AND RECOMMENDATIONS

As plant growth parameters were impaired by the viruses, so also was the seed quality and the magnitude of the effects varied with the cultivars. These differences which became evident from the first day after sowing were sustained for the rest of the study period. The study showed significant impairments in germination before and after four weeks of storage of the 25 cowpea cultivars in both single and mixed infections of the two viruses. Viability of seeds from infected plants was generally high before storage; the high initial germination percentage was however short lived indicating that conservation of infected seeds of all the cultivars was impaired. Percent germination in cultivars IT98K-205-M8, IT90K-277-2 and IT07K-222-2 with BICMV + CPMoV infections were not much affected. After storing the seeds for four weeks, a sharp decline in their germination capability from all the treatment combinations was recorded, seed vigour was seriously impaired in cultivars IT07K-292-2-10, IT06K-124 and IT90K-277-2 infected with BICMV + CPMoV as compared to the other three virus treatments. Therefore, constant monitoring and management of the studied viruses reported here are necessary for sustainable cowpea production. Constant monitoring of legume fields through regular field sanitation, disease surveys to identify new and emerging viruses will also provide valuable data for legume virus diseases diagnosis in the study area. Ensuring availability of acceptable and desirable cowpea cultivars with high level of resistance to the cowpea viruses will assist Nigeria and other cowpea producing countries to sustain high level cowpea productivity.

DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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