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OCCURRENCE OF CUCUMBER MOSAIC VIRUS WITHIN TOMATO SEED LOTS

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is an important crop whose fruit is widely consumed globally. However, its yield is affected by cucumber mosaic virus (CMV) and can cause total crop failure. The virus is mainly transmitted by aphid vectors, but data on its spread via seeds are limited. Thus, the occurrence of CMV within tomato seed lots obtained from different sources was investigated. Seven tomato accessions and varieties were collected from five sources and evaluated for seed transmission of CMV. One hundred seeds each were sown in a plastic tray, and germination rates were recorded. The incidence and severity of virus symptoms were observed at 3, 6, and 9 weeks after sowing (WAS), while leaves were tested for CMV using antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA). The germination percentage ranged from 60% in accession 'V4' to 92% in 'UC-82B'. Infection rates were 100%, 71.74%, 70.31%, 45.90%, and 8.33% in 'Roma-VF', 'UC-82B', 'NG/AA/SQ/09/053', 'V2', and 'V4', respectively. 'Kerewa' and 'Alausa-Long' exhibited zero infection rates and tested negative for CMV using ACP-ELISA. Eighty percent of test plants became symptomatic at 6 and 9 WAS, although accession 'NG/AA/SQ/09/053' tested positive for CMV despite showing no symptoms. Tomato seeds from commercial stores, research institutes, and farmers' fields tested positive for CMV, while seeds from the market were negative at 9 WAS. The results from this study confirm the transmissibility of CMV through seeds in tomatoes, although the rate of seed transmission is cultivar dependent.

Keywords: *Solanum lycopersicum* L., Seed Sources, Seed Transmission, ACP-ELISA.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and consumed vegetables worldwide (Arah, 2015). It is an annual herbaceous fruiting plant widely cultivated in Nigeria by subsistence farmers (Olanrewaju, 2017). It is considered an important cash and industrial crop in many parts of the world (Ajagbe *et al.*, 2014). The crop is believed to have its origin in the South American Andes (Naika *et al.*, 2005) in present-day Peru, where it grows in the wild at the foot of hills. In 2020, over 186 million tonnes of tomatoes were harvested worldwide from plantings on over 5 million hectares, with Africa producing more than 20 million tonnes (FAOSTAT, 2021). According to Ebimieowei and Ebideseghabofa (2013), Nigeria is the second largest producer of tomatoes in Africa after Egypt and 13th in the world, with an annual production of 6 million tonnes before 1990. Despite the numerous importance of the crop, some viruses, such as tomato spotted wilt virus, tomato yellow leaf curl virus, and cucumber mosaic virus (CMV), cause severe damage to the crop, leading to a drastic reductions in yield (Hanssen *et al.*, 2010).

Cucumber mosaic virus is widespread and causes immense yield losses in many important crops (Jacquemond, 2012), including tomatoes. The symptoms that occur in diseased plants range from mosaics and chlorosis in leaves to necrosis, shoe-strings, and general stunting. Symptom intensity is known to be host- and strain-dependent (Jacquemond, 2012), with viral strains characterized into subgroups I and II (Roossinck *et al.,* 1999), while putative new categories are now being postulated (Tepfer *et al.,* 2016; Adediji, 2019, Apalowo *et al.,* 2022). The virus can be spread across and within fields by up to 80 different aphid species (Palukaitis and García-Arenal, 2003). It is also known to be mechanically and seed transmissible in some hosts (Palukaitis *et al.*, 1992; Arogundade *et al.,* 2018).

The occurrence of CMV is well documented. However, its epidemiology is yet to be fully understood. Although CMV is known to be seed

transmissible (Jalender *et al.*, 2018), its transmissibility within tomato seeds is yet to be properly studied. Hence, this study sought to evaluate the possibility of CMV presence in tomatoes from various sources and to evaluate seed transmission rates of CMV within different tomato cultivars.

MATERIALS AND METHODS

Seed Sources and Sample Collection

Tomato seeds were sourced from Ibadan, Nigeria: two research institutes (National Institute for Horticultural Research, NIHORT, and National Centre for Genetic Resources and Biotechnology, NACGRAB), a commercial seed store, a market, and two farmers' fields (Table 1). One hundred seeds from each source were planted in nursery trays (42 cm x 51 cm x 6 cm) filled with sterilized topsoil, and the germination percentage was observed for each seed lot. Each tomato plant per lot was transplanted to 5 L pots of sterilized topsoil in the screenhouse three weeks after sowing (WAS), and CMV-induced symptoms were monitored on the plants. Leaf samples were

Table 1: Sources of tomato seeds used for the determination of seed transmission of cucumber mosaic virus.

Varieties/Accession	Sources	Botanical properties
Roma VF	NIHORT[1], Ibadan, Nigeria	Oblong fruit and determinate growth
UC-82B	Agrotropical Ltd., Ibadan, Nigeria	Round fruit and determinate growth
Kerewa	Bodija Market, Ibadan, Nigeria	Round fruit and determinate growth
Alausa Long	Bodija Market, Ibadan, Nigeria	Oblong fruit and determinate growth
V2	Farmer field, Ido, Ibadan, Nigeria	Oblong fruit and determinate growth
V4	Farmer field, Ido, Ibadan, Nigeria	Round fruit and determinate growth
NG/AA/SQ/09/053	NACGRAB[2], Ibadan, Nigeria	Round fruit and determinate growth

[1]NIHORT = National Horticultural Research Institute.

Determination of Disease Incidence and Severity

The disease incidence was evaluated by obtaining the ratio of symptomatic plants in each seed lot to the total number of plants and expressed as a percentage, (Vetten and Allen, 1983) i.e.

$$\frac{\text{Number of symptomatic plants}}{\text{Total number of plants sampled}} \times 100$$

The disease severity rating scale described by Kone *et al.* (2017) was used to evaluate the plants. Briefly, 0 represented no observable disease symptoms; 1, very mild yellowing on 10-25% of the leaf surface and some leaf distortions; 2, mild chlorosis on 50% onset of mosaic and leaf curling; 3, mottling, interveinal shoe strings and the onset of cupping or leaf rolling; 4, intense mottling and shoe strings with stunting; and 5, very intense chlorosis, abnormal growth, stunting and plant death. From the scale, the average disease severity score per variety or accession was calculated as the sum of disease severity scores divided by the number of plants showing

$$\text{Average disease severity} = \frac{\text{sum of disease severity scores}}{\text{number of plants with symptoms}}$$

Both disease incidence and severity on each variety and accession were evaluated at 8, 12, 16, 20, and 24 days after sowing (DAS).

Detection of CMV Using Enzyme-Linked Immunosorbent Assay

At 6 and 9 WAS, fresh leaf samples were obtained from the test plants, and a CMV polyclonal antibody was used in an antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) as described by Kumar (2009). Briefly, approximately 0.1 g of leaf samples were ground in 1 mL of coating buffer (0.015 M $NaCO_3$, 0.0349 M $NaHCO_3$, pH 9.6) and dispensed into each well within the polystyrene ELISA plate. After incubation at 37 °C for 1 h, the plate was washed three times with 1X phosphate buffered saline Tween (PBST: 137.00 mM NaCl, 1.46 mM KH_2PO_4, 7.75 mM Na_2HPO_4, 2.68 mM KCl, pH 7.4, 0.05% v/v Tween-20) for 3 min between each wash. Cross adsorption of virus antiserum was

made by grinding 1 g of healthy plant samples in 20 mL of conjugate buffer (0.05 M PBS, 0.05% v/v Tween-20, 0.2% w/v egg albumin, 2% w/v polyvinylpyrrolidone, pH 7.4). The universal cucumovirus antiserum (Agdia, Inc., Indiana, USA) was diluted at 1:3,000 in the adsorption solution, and 100 µL of the antiserum polyclonal antisera was added to wells of the ELISA plates and again incubated at 37 °C for 1 h. The removed plates were washed three times with 1X PBST, while 100 µL of protein-A alkaline phosphatase conjugate, diluted at a ratio of 1:15,000 in conjugate buffer, was added per well, and plates were incubated at 37 °C for 1 h. The plates were washed three times with PBST, and 100 µL of p-nitrophenyl phosphate substrate (0.001 g/mL) in substrate buffer (10% diethyl ethanolamine, 0.2 g $NaNO_3$, pH 9.8) was added per well and incubated again at 37 °C for 1 h. During the incubation period, plates were protected with ELISA cover plates to avoid edge effects and to maintain a uniform temperature. The healthy and diseased plant samples were used as controls. Absorbance was measured at 405 nm using an ELISA plate reader (BioTek Inc., Vermont, USA) after 1 h incubation. The samples were considered positive when the average optical density value obtained was more than twice the average readings of the corresponding healthy controls.

RESULTS

Germination rates and virus symptoms

The germination rates among tomato seed lots from various sources revealed moderate to high levels. The highest rate (92%) was observed with 'UC-82B', followed by 'Kerewa' (89%) and 'Alausa-Long' (88%) (Table 2). Moreover, the lowest germination rates (60% and 61%) were noted for variety 'V4' and accession 'NG/AA/SQ/09/053', respectively. However, the infection rate was highest in 'Roma-VF' at 100%, while 'UC-82B' and 'V2' had 71.4% and 70.3% infection rates, respectively. Varieties 'Kerewa' and 'Alausa-Long', both obtained from farmers' fields, exhibited zero infection rates (Table 2). Virus symptoms were observed in all tomato seed lots from the five locations (Figure 1), except on 'NG/AA/SQ/09/053'. At 9 WAS, symptoms of leaf chlorosis, stunting, and shoe-stringing were observed on 'Roma-VF', while 'UC-82B' produced leaf chlorosis, leaf curl, and stunting. 'Kerewa', 'Alausa-Long' and accessions 'V2', 'V4', and 'NG/AA/SQ/09/053' were mainly symptomless (Figure 1).

Table 2: Germination and infection rates of cucumber mosaic virus within tomato seeds.

Variety or Accession	Number of seeds planted	Number of seeds germinated	Number of infected plants	Infection rates (%)
Roma VF	100	87	87	100.0
UC-82B	100	92	66	71.4
Kerewa	100	89	0	0.0
Alausa-Long	100	88	0	0.0
V2	100	64	45	70.3
V4	100	60	5	8.3
NG/AA/SQ/09/053	100	61	29	45.9

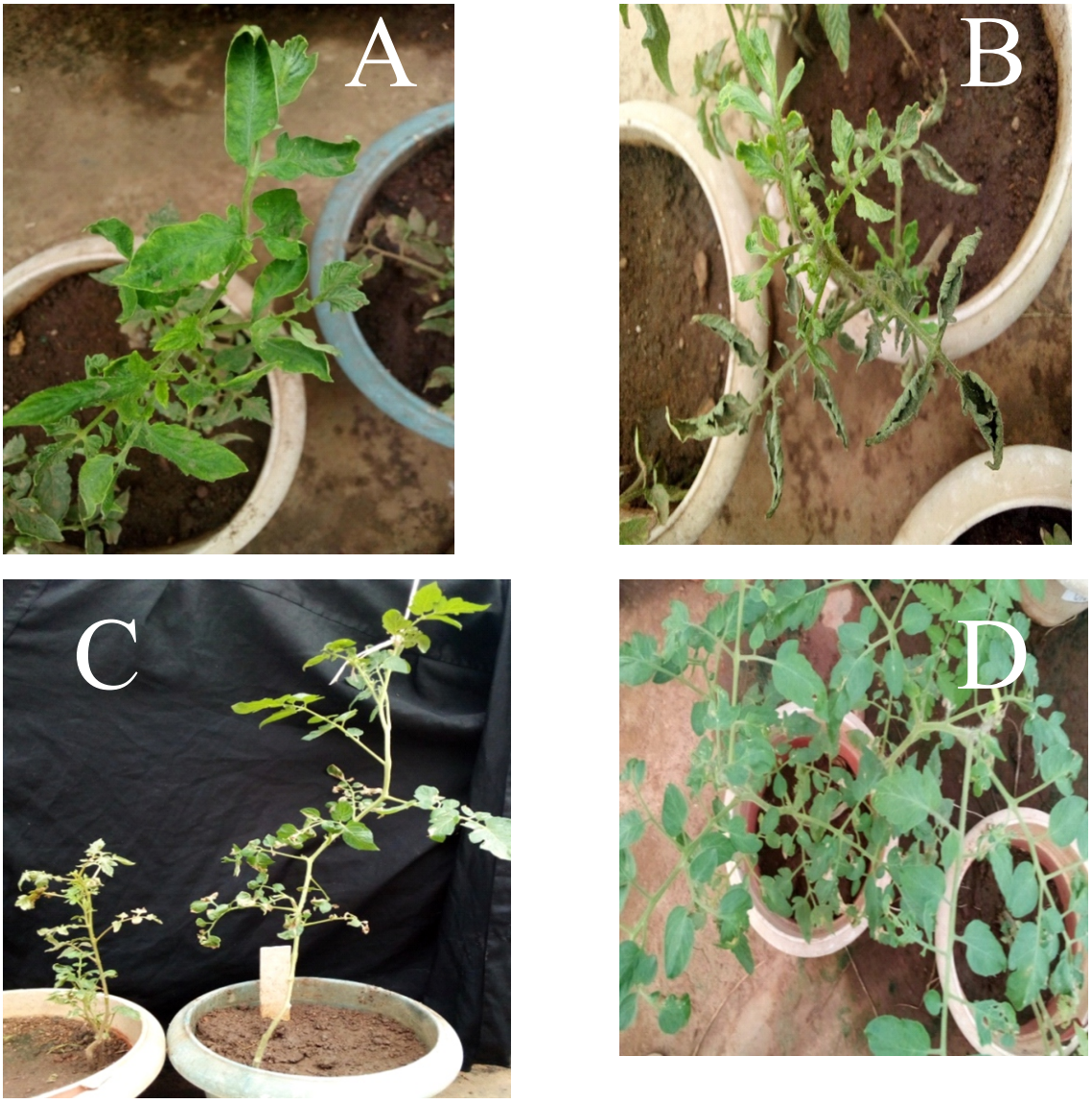

Figure 1: Symptoms of cucumber mosaic virus infection on tomato varieties. A: Leaf curling on 'UC-82B'; B: Leaf curling on 'Roma-VF'; C: Stunted and healthy 'UC-82B' plants; D: Healthy, uninfected 'Kerewa'.

Disease incidence and severity of CMV symptoms within tomato seedlings

At 16 DAS, 'Roma-VF' had the highest disease incidence (27%), while 'UC-82B' recorded a disease incidence of 10% (Figure 2). Varieties 'Kerewa', 'Alausa-Long' and accessions 'V2', 'V4', and 'NG/AA/SQ/09/053' had no disease incidence recorded at 16 DAS. Additionally, at 20 DAS, the disease incidence for 'Roma-VF' and 'UC-82B' increased to 60% and 25%, respectively, while accession 'V2' had a 4% disease incidence. No disease incidence was recorded for 'Kerewa', 'Alausa-Long', 'V4', and 'NG/AA/SQ/09/053' at 20 DAS (Figure 2). At 24 DAS, 'Roma-VF' had an 80% incidence, 54% for 'UC-82B' recorded 54%, 17% for 'V2', and 3% for 'V4' had 3% (Figure 2). The accession 'NG/AA/SQ/09/053' had no symptoms.

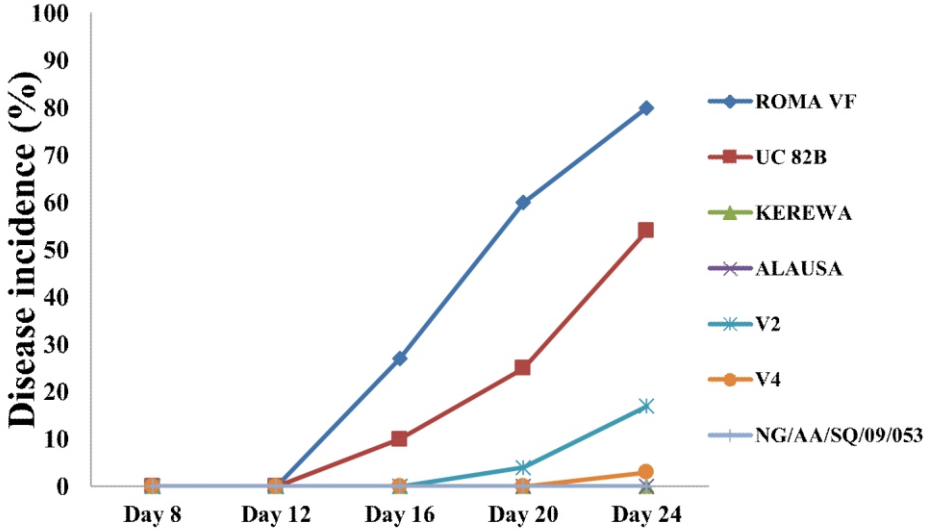

Figure 2: Incidence of cucumber mosaic virus infection within tomato seeds.

There were no symptoms at 8 and 12 DAS, but at 16 DAS, 'Roma-VF' and 'UC-82B' had average disease severities of 2.00 and 1.40, respectively (Figure 3). There were no symptoms observed on other varieties and accessions. Furthermore, at 20 DAS, the average disease severity of 'Roma-VF' and 'UC-82B' increased to 2.98 and 2.00, respectively, while 'V2' recorded 1.00. No symptoms were observed on 'Kerewa', 'Alausa-Long', and accessions 'V4' and 'NG/AA/SQ/09 /053' at 20 DAS. At 24 DAS, the average disease severity of 'Roma-VF', 'UC-82B' and 'V2' rose to 3.59, 2.33, and 1.82, respectively, while V4 had 1.67. Accession 'NG/AA/SQ/09/053' did not record any disease severity.

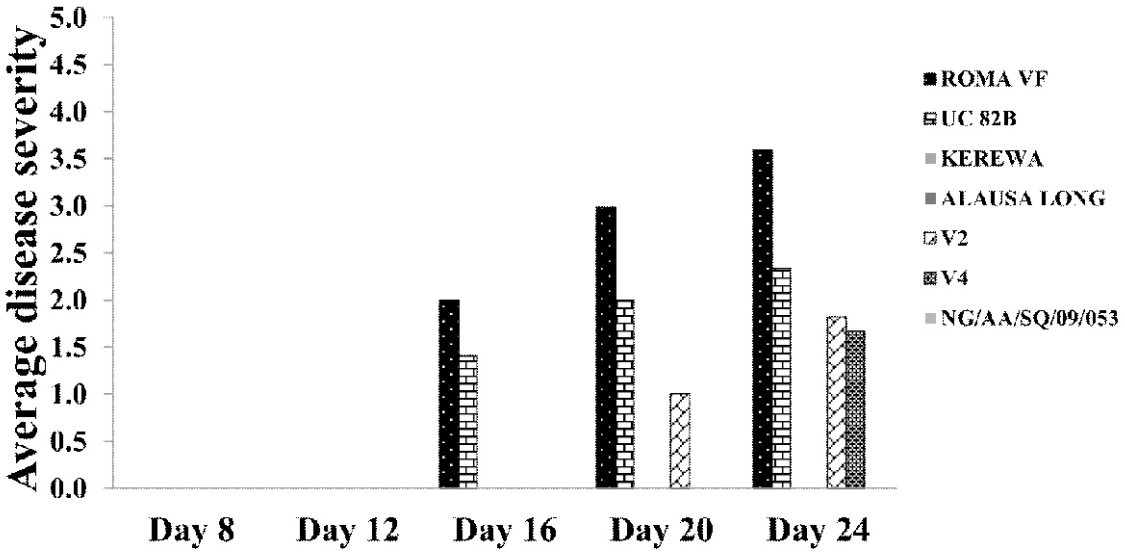

Figure 3: Average disease severity of cucumber mosaic virus infection in tomato plants.

Evaluation of CMV in tomato seed lots using an enzyme-linked immunosorbent assay

The mean absorbance values from the ELISA plates at 405 nm wavelength for leaf samples obtained at 6 WAS showed that 'UC-82B' had the highest CMV titre (1.692), followed by 'Roma-VF' (0.794), 'NG/AA/SQ/09/053' (0.505) and 'V2' (0.495). The leaf samples from these plants were confirmed to be positive, as the mean absorbance values were more than twice the values for the healthy controls (0.244). 'Kerewa', 'Alausa-Long', and 'V4' tested negative at 6 WAS with absorbance values of 0.247, 0.214, and 0.428, respectively. Similar trends were observed for ELISA values recorded at 9 WAS: 'UC-82B' recorded 3.65, while 'Roma-VF', 'NG/AA/SQ/09/053' and 'V2' recorded 0.979, 1.199, and 1.229, respectively. An exception was accession 'V4', which tested positive with 1.193 at 9 WAS. The varieties 'Kerewa' and 'Alausa-Long' tested negative at 9 WAS with absorbance values of 0.381 and 0.360, respectively.

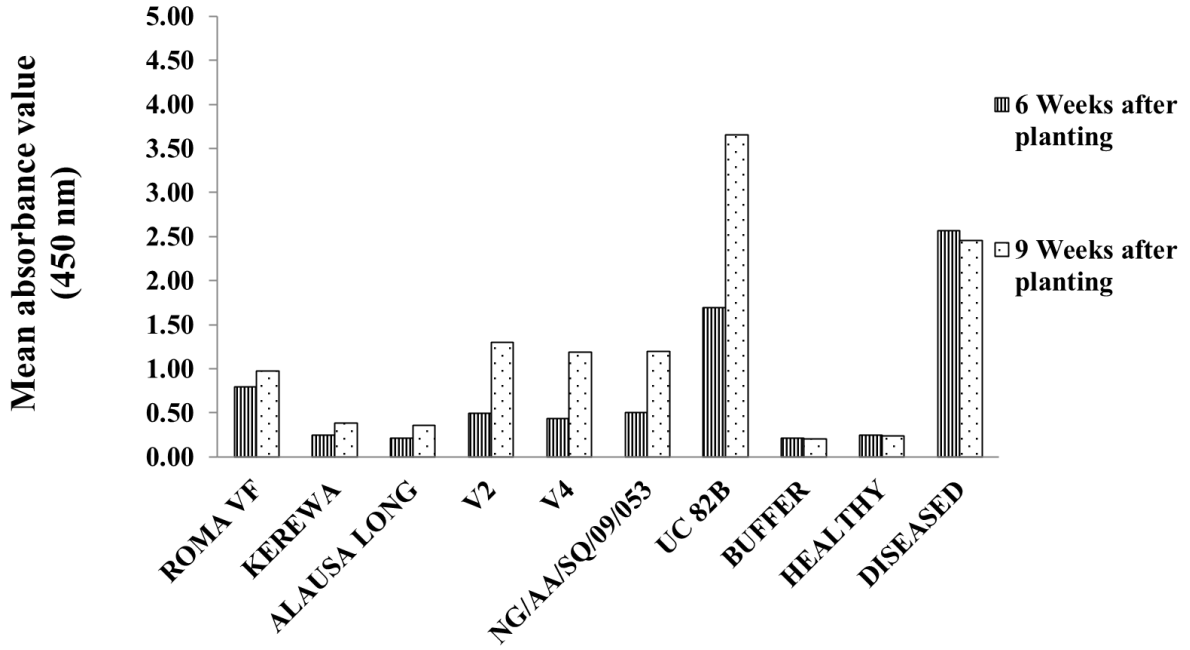

Figure 4: Mean absorbance values (450 nm) of tomato varieties and accessions infected with cucumber mosaic virus at six and nine weeks after sowing.

DISCUSSION

Plant virus transmission through seeds has epidemiological implications worldwide, and this study aimed to reveal the occurrence of CMV in tomato seeds. Seed transmission plays a pivotal role in virus survival (Johansen *et al.,* 1994), and even at a very low rate, it can be important for virus perpetuation in the environment and from season to season, as well as for long-range dissemination. It provides an initial source of inoculum for vector transmission of the virus that may have a considerable impact on overall crop yield. The positive evidence of seed transmission on CMV in this study agrees with the findings of Soler *et al.* (2010), who identified seed transmission with tomatoes in Spain. In the USA, Ali and Kobayashi (2009) detected CMV in seed coats and embryos of peppers, another important solanaceous plant, while Arogundade *et al.* (2018) also reported CMV transmission within pepper in Nigeria. Conversely, previous findings of zero seed transmission of CMV in some solanaceous plants, including tomatoes, have been reported (Dafalla, 2000; Jalender *et al.,* 2018).

In this study, typical CMV symptoms such as chlorosis, stunted growth, shoe string, and leaf curl (Zitter and Murphy, 2009) were observed on infected tomato plants. Additionally, germination rates varied across the varieties and accessions evaluated. Low germination rates were observed in tomato seeds obtained from farmers' fields.

Similar findings were reported by Bortey *et al.* (2011), who noted low germination rates from tomato seeds sourced among Ghanaian farmers. Accession 'NG/AA/SQ/09/053' was obtained from NACGRAB, and its low germination may be due to prolonged seed storage, which is known to negatively affect tomato germination (Kong and Zhang, 1998). Thus, this study provides evidence that seed sources influence CMV occurrence in tomato seeds.

The disease incidence and severity were more prevalent in 'Roma-VF' and 'UC-82B' till maturity. Park and Cha (2002) reported 'Roma-VF' and 'UC-82B' to be highly susceptible to virus infections, and our results agreed with Saluadeen *et al.* (2018), who reported 'UC-82B' as a tomato variety with high CMV incidence and susceptibility. Surprisingly, zero infection rates were observed on 'Kerewa' and 'Alausa-Long' from market sources. Additionally, these two varieties did not show symptoms or disease incidence and tested negative for CMV using ELISA. This could have epidemiological implications, as seeds from local markets could be recommended to farmers for planting instead of recycling seeds from their fields. Thus, seed sources also influence infection rates, as high CMV occurrence was found in seeds from research institutes and farmers' fields, while low virus occurrence was found in seeds sourced from the market.

It was observed that although accessions 'V2' and 'NG/AA/SQ/09/053' were asymptomatic within the screenhouse, the accession tested positive for CMV using ELISA at 6 WAS and 9 WAS. This could be attributed to host factors that mask the expression of symptoms in these accessions. The lack of symptoms in some CMV-infected seedlings derived from seeds is not unusual. Many viruses, including CMV, have been reported to produce asymptomatic seedlings that are infected through seed transmission (Gallitelli, 2000). Hampton (1983) reported that more than 300 plant viruses are seed-borne in one or more host species, and the number is expected to rise with the evolution of viruses (Apalowo *et al.*, 2022) and reports of new viruses emerging or discovered.

CONCLUSION

The results from this study detected CMV in tomato seeds and can serve as a source of virus inoculum in cultivated tomato farms. The virus can be seed-borne and transmitted from one generation to another. Tomato seeds may serve as a potential inoculum source for the long-distance movement and local spread of CMV in the field and could affect tomato production. Therefore, farmers should adopt some cultural practices, such as host plant resistance, use of cover cropping, mulching, eradication of alternate weed hosts, and use of resistant cultivars, for adequate disease management. Additionally, rigorous seed testing regimes should be implemented in commercial seed stores and research stations to reduce the high prevalence of seed-borne viruses in seed lots.

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