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ORIGINAL RESEARCH ARTICLE

Screening of sweet potato feathery mottle virus resistant sweet potato (*Ipomoea batatas* L., Lam.) cultivars in Kebbi State, Nigeria

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ABSTRACT

Sweet potato is a food security crop because of its ability to withstand adverse climatic conditions. This security, however, is being threaten by viral diseases and use of resistant cultivars remain the best management. This research was conducted to screen cultivars of sweet potato against sweet potato feathery mottle virus (SPFMV) infection. The treatments consisted of five cultivars coded as CV1, CV2, CV3, CV4 and CV5, respectively. The asymptomatic experimental plants were established, maintained under screen house conditions and graft-inoculated using infected vines which were tested SPFMV positive using both Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) and Polymerase Chain Reaction (PCR). The experiment was laid out in Completely Randomized Design (CRD) and replicated three times. Results obtained from disease incidence and symptom severity indicated that there was significant difference (P <0.05) among cultivars in their reaction to SPFMV infection. CV2 had the highest mean disease incidence (60.67%) while, CV3 had the lowest mean disease incidence (36.67%). CV1, CV3, and CV5 have the same lowest mean symptom severity score of 2.00 while, CV2 had the highest mean severity score of 4.00. Based on the reaction of the cultivars after inoculation, it could be concluded that, all the cultivars screened were susceptible to SPFMV but CV1, CV3 and CV4 cultivars have some degree of resistance to SPFMV infection and were therefore recommended for use by the farmers in the study area. This is the first research that screened sweet potato cultivars for resistance to SPFMV in Kebbi State, Nigeria.

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INTRODUCTION

Sweet potato (*Ipomoea batatas* L., Lam.) is a dicotyledonous plant belonging to morning glory family *Convolvulaceae* (Nandhini *et al.*, 2021; Oke and Workneh, 2013). Based on its life cycle, sweet potato is classified as perennial plant but cultivated as an annual crop throughout the tropics and warm temperate regions of the world for its starchy storage roots, which can provide nutrition, besides energy (Ngailo *et al.*, 2013; Padmaja *et al.*, 2012). In Africa, it is considered to be a major food crop and consumed in major households of many African countries. Its edible tuberous root is either long and tapered, ovoid or round with skin color ranging from white, brown, purple to red and the flesh color ranging from white, pale cream, orange to purple (Padmaja *et al.*, 2012). The plant is also valued for its green tops, which are considered as leafy vegetable and consumed by many people in Africa (Nguyen *et al.*, 2021). It is a source of many essential vitamins and minerals for both human and livestock (Nguyen *et al.*, 2021). Despite the health and nutritional benefit of the crop, sweet potato production is

constrained by several biotic factors such as viral diseases, insect pests, weeds, and abiotic factors (Abrham *et al.*, 2021; Clark *et al.*, 2012; Ngailo *et al.*, 2013). Diseases and insects of paramount importance are sweet potato virus diseases and sweet potato weevil respectively (Ngailo *et al.*, 2013). Sweet potato virus disease (SPVD) caused by the dual infection and synergistic interaction of Sweet potato chlorotic stunt virus (SPCSV) and Sweet potato feathery mottle virus (SPFMV) is distributed worldwide (Mbewe *et al.*, 2021; Mukasa *et al.*, 2006). Sweet potato virus disease is the most devastating disease of the crop, causing reduction in crop growth and storage root yields (Jones, 2021; Ngailo *et al.*, 2013). Furthermore, it limits the length of time the roots can be kept in the ground and shortens the storage duration of the harvested crop (Jones, 2021). The damage caused by SPVD can be up to 98% (Jones, 2021; Ngailo *et al.*, 2013). It has been reported that, the best management of plant virus diseases is the use of resistant varieties/cultivars, and some varieties/cultivars are naturally resistant to some viral diseases. Whether sweet potato cultivars cultivated in Kebbi State are resistant to SPFMV infection are not known because, no scientific research reported any of the sweet potato cultivars as resistant to SPFMV infection in the state. Screening sweet potato cultivars for resistant to SPFMV infection in Kebbi State will be of paramount important to farmers, plant breeders, virologists, extension agents, and other researchers. Use of resistant cultivars as a cultural method of plant virus disease management have been reported to be less expensive and environment friendly; therefore, the outcome of this research will assist farmers in minimizing the cost of management by using resistant cultivar(s). The main aim of the research was to screen sweet potato cultivars for resistance to SPFMV infection in the study area. Although several virus diseases have been reported to infect sweet potato worldwide (Adikini *et al.*, 2015; Clark *et al.*, 2012; Wasswa, 2012), but, resistance of sweet potato cultivars to these virus diseases in different agro-ecological zones have not been fully researched.

MATERIALS AND METHODS

Source of planting material

A total of five asymptomatic sweet potato cultivars were sourced from Aliero and Jega Local Government Areas (LGAs) (Figure 1) of Kebbi State from farmers' fields. Leaf samples were collected from each of the cultivar and tested negative of SPFMV using DAS-ELISA and PCR before their vines were planted in planting plots and managed under screen house condition at Kebbi State University of Science and Technology, Aliero (KSUSTA). The cultivars and their designation were 'Danmadakala' (V1), 'Dangote' (V2), 'Dangwaronyo' (V3), 'Danayi' (V4), and 'Danolonyo' (V5).

Virus isolates and source

Vines of diseased sweet potato plant showing typical symptoms of SPFMV which include vein clearing, mottle on leave, and leaf chlorosis were sourced from farmers' fields in Aliero and Jega LGAs of Kebbi State (Figure 1). Leaf samples from the infected vines were tested SPFMV positive using DAS-ELISA and PCR.

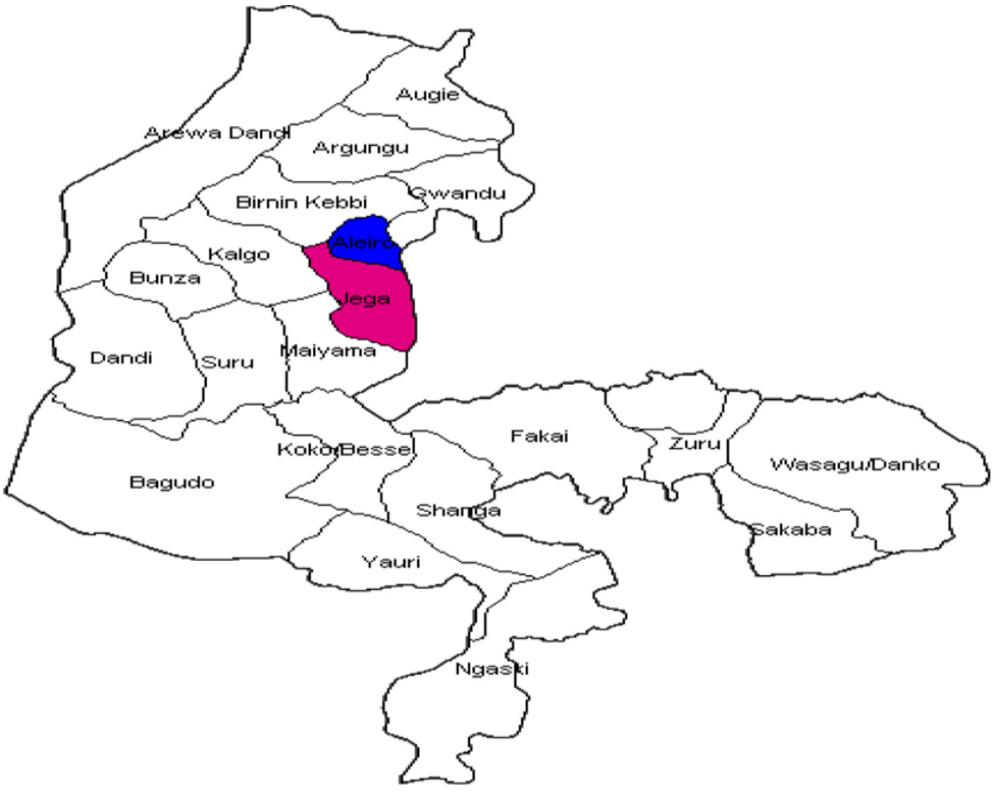

Figure 1. *Map of Kebbi State showing the two LGAs where planting materials and SPFMV isolate were sourced.*

Virus transmission

Virus transmission was achieved by graft-inoculation method of Mohammed *et al.* (2012). All the five cultivars were graft-inoculated with vines of SPFMV-diseased sweet potato. Vines of about 10 cm length were used for virus transmission. Scions were cut, and all the leaves were removed except for the first unopened and second opened leaves while the buds were left intact. Scion was immediately inserted in freshly cut rootstocks plant. The scion and rootstock plants were secured by wrapping gently but tightly with long strips of plastic grafting tape. On each scion, 1 – 2 young leaves were retained to encourage the exchange of water and nutrients, thus virus movement within the rootstock was guaranteed.

Disease incidence and severity

Disease incidence (DI) of each cultivar was calculated using the formula of Mohammed *et al.* (2017) and Sseruwagi *et al.* (2004):

$$DI\ (\%) = \frac{number\ of\ diseased\ plants}{total\ number\ of\ plants\ assesed/sampled} \times 100$$

Disease severity of each cultivar was scored by the arbitrary score: 1-5 (Mohammed *et al.*, 2017; Sseruwagi *et al.*, 2004) indicating the degree of symptom development of each sampled plant. Where: 1 = Symptomless (no symptom development), 2 = Mild (symptoms but no pronounced development), 3 = Moderate (pronounced symptom on about one thirds of the leaves), 4 = Severe (symptoms on about two thirds of the leaves) and 5 = Very severe (symptoms on almost all the leaves).

RNA extraction

Extraction of nucleic acid from the asymptomatic and symptomatic leaves of the five cultivars was carried out at the Molecular Biology Laboratory of KSUSTA using a CTAB method (Cetyl Trimethyl Ammonium Bromide) as originally described by Lodhi *et al.* (1994) and later modified by Abarshi *et al.* (2012). Firstly, the CTAB extraction buffer made up of [2% (w/v) CTAB, 1.4M NaCl, 0.2% (v/v) 2- mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0] was preheated to 60°C for 10 minutes. Mercaptoethanol was added freshly to the buffer. About 0.1 g of diseased plant leaf was weighed and placed into a polythene bag. The leaf was then ground using a roller and about 1 ml of CTAB extraction buffer was added. About 750 µl of the sample was transferred into a 1.5 ml eppendorf tube which was then incubated at 60°C for 30 minutes using water bath. Approximately 750 µl of Phenol: chloroform: isoamyl alcohol (25:24:1) was then added, vortexed briefly and centrifuged at 13000 rpm for 10 minutes. About 500 µl of the aqueous solution was transferred into a new 1.5 ml eppendorf tube. The nucleic acid was precipitated by adding 300 µl of cold isopropanol and incubated at -20°C for one hour. The samples were centrifuged at 13000 rpm at 4°C for 10 minutes and the supernatant was discarded. The pellet was washed in 0.5 ml 70% ethanol by vortexing and then centrifuged for 5 minutes at 13000 rpm. The ethanol was removed, and pellet was vacuum dried for 5 minutes. The dried pellet was suspended in 100 µl 1X TE buffer and stored at -20°C. Extractions were diluted 1:100 fold in sterile distilled water (SDW) before being used in PCR amplification.

RT– PCR and primers

SPFMV is an RNA virus and therefore reverse transcriptase polymerase chain reaction (RT - PCR) was conducted as described by Opiyo *et al.* (2010). Initial denaturation was carried out at 94°C for 5 minutes followed by 35 thermal cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 1 minute. Final extension was done at 72°C for 5 minutes. The PCR was done in 14.2 µl reaction mixture of 5 µl of PCR-buffer, 2 µl of dNTP solution mix, 0.2 µl of Taq polymerase, 4 µl of $MgCl_2$, 2 µl of cDNA templates, 0.5 µl of forward and reverse primers (SPFMV-F GGACGAGACAC-TAGCAA and SPFMV-R TTCTTCTTGCGT GGAGACGT) (Opiyo *et al.*, 2010). PCR products were processed by agarose gel electrophoresis using the protocol of (Abarshi *et al.*, 2012). Pictures were then taken using a gel documentation system.

Data analysis

Data obtained was analyzed using SAS software and means were separated using Least Significant difference (LSD), thereafter, the results were presented in charts using Excel 2016.

RESULTS AND DISCUSSION

Establishment count (%) and percentage disease incidence (%)

The experiment was established and maintained under screen house conditions. In order to determine the cultivar that can survive under screen house conditions, establishment count was recorded in percentage. The results showed that CV1 had the highest establishment count two weeks after transplanted in screen house while, CV5 had the lowest establishment count (Figure 2). The highest establishment count recorded by CV1 might be due to varietal differences that enable the cultivar to fully be established under screen house conditions. Fabunmi (2013), reported a successful sweet potato vines production under screen house conditions at University of Agriculture Abeokuta, Ogun State, Nigeria. All the five cultivars screened were infected when graft inoculated with SPFMV isolate albeit at varying rate of infection. Sweet potato feathery mottle disease (SPFMD) incidence significantly ($P<0.05$) varied among the cultivars screened. CV2 recorded the highest mean disease incidence of 60.67%, while CV5 recorded average mean disease incidence of 40.67% and CV3 recorded the lowest mean disease incidence was 36.67% (Figure 2). This result is in line with the work of Adikini *et al.* (2016) who previously reported SPFMD incidence among the varieties of sweet potato screened. Mbewe *et al.* (2021), also reported higher SPFMD incidence in Malawi.

Time taken for symptoms appearance weeks after inoculation (WAI), types of SPFMV symptoms observed, and symptom severity

A successful virus transmission using graft-inoculation method was achieved because SPFMV symptoms appeared on CV1 and

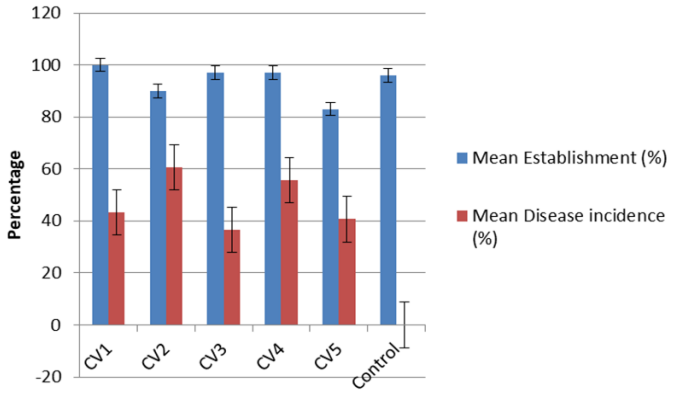

Figure 2. *Means establishment count and disease incidence of the five cultivars weeks after inoculation. Bars indicate standard error of means at 5 % probability level.*

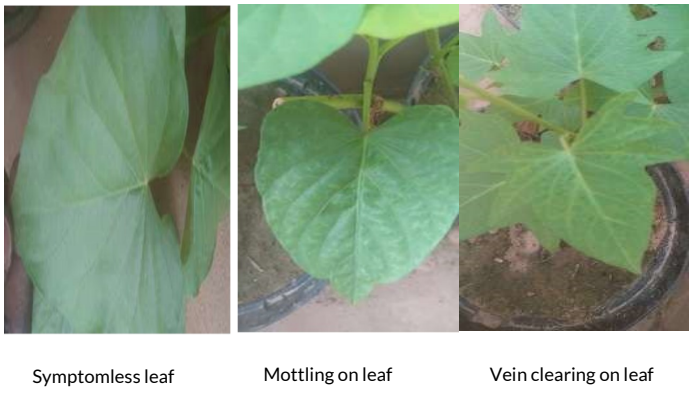

Figure 3. *Symptoms of SPFMV as observed on the experimental plants.*

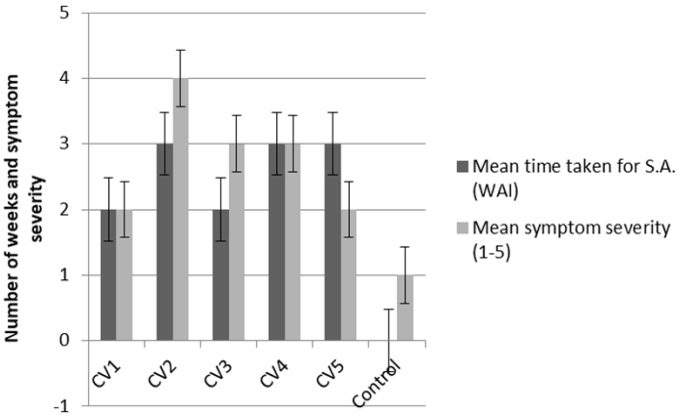

Figure 4. *Mean of time taken for symptoms appearance weeks after inoculation and SPFMV symptom severity. Bars indicate standard error of means at 5 % probability level.*

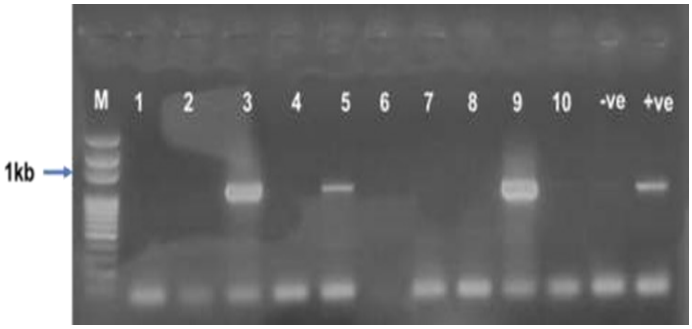

Figure 5. *Samples 1 and 2 (CV3), 3 and 4 (CV4), 5 and 6 (CV4), 7 and 8 (CV5) and 9 and 10 (CV2), M = 1 kb marker, -ve = known negative control and +ve (known positive control).*

CV3 cultivars two weeks after inoculation while; it took CV2, CV4, and CV5 three weeks after inoculation for SPFMV symptoms to appear. Bednarek *et al.* (2021), reported a successful SPFMV transmission on Ipomoea setosa using graft-inoculation method. Mohammed *et al.* (2016), also reported a successful graft-inoculation of *Cassava brown streak virus* (CBSV) in cassava. Mottling, chlorotic, and vein clearing were the major symptoms induced by SPFMV that were observed on the sweet potato cultivars screened when they were graft-inoculated with SPFMV inoculum. Similar symptoms of SPFMV were reported by other researchers in Nigeria and in other African countries (Musa *et al.*, 2021; Rodamilans *et al.*, 2021; Wasswa, 2012). In this present study, mottling was the most common symptom observed on all the sweet potato cultivars screened while, Bednarek *et al.* (2021), reported vein clearing as the most common symptom in their study. SPFMV symptom severity on infected sweet potato cultivars screened varied within the cultivars. Amongst the five cultivars screened, the highest mean symptom severity score of 4 was recorded on CV2 while the average symptom severity score of 3 was recorded on CV4, and the least mean symptom severity score of 2 was recorded on CV1, CV3 and CV5 respectively (Figure 4). The findings of this study are similar with the findings of Cuellar *et al.* (2015) and Adikini *et al.* (2016). Mbewe *et al.* (2021), also reported similar results.

Based on symptoms observed which were earlier reported to be induced by SPFMV (Mbewe *et al.*, 2021), SPFMD incidence, and symptom severity recorded, and successful detection of SPFMV from leaf samples of all the cultivars screened, this revealed that all the cultivars screened were susceptible to SPFMV infection but with some degree of resistance. This is in line with the work of Ngailo *et al.* (2013). The molecular and serological detection of SPFMV before and after the inoculation indicated that, SPFMV is common in the study area. Musa *et al.* (2021) and Wasswa (2012) have earlier reported that SPFMV is common anywhere sweet potato is cultivated. SPFMV was detected by RT-PCR using novel primers SPFMV-F and SPFMV-R, which specifically amplified SPFMV in CV1, CV4, and CV (Figure 5). No amplifications were obtained from RNA extracted from virus-free plants (-ve), CV3 and CV5. Although, mild symptoms of SPFMV were observed on CV3 and CV5 but no amplifications were obtained from RNA from symptomatic leaf samples of these cultivars. The non-amplification from these cultivars might be as a result of low virus concentration in these cultivars. Similar results have been reported by different researchers in the world (Bednarek *et al.*, 2021; Mbewe *et al.*, 2021).

Conclusion

From the findings of this study particularly on resistance to SPFMV infection by the sweet potato cultivars screened, it could be concluded that all the cultivars screened in this study were susceptible to SPFMV infection because SPFMV symp-

toms were observed on all the cultivars weeks after inoculation. CV1, CV3 and CV5 have some degree of resistance to SPFMV as they recorded the same mean symptom severity score of 2 indicating mild symptom of SPFMV. It can also be concluded that SPFMV occurred in the study area as it was detected from leaf samples sourced from the farmers' fields which was then used for virus transmission on the research plant. Based on this finding, CV1, CV3 and CV5 were recommended for use by farmers in the study area. Considering the limitation of this study as it only screened five local cultivars of sweet potato in the study area where sweet potato is predominantly cultivated, there is need for a research that will screen more sweet potato cultivars in the state that were not screen in this research. A research survey is also recommended to assess the incidence and distribution of sweet potato virus diseases in the state to develop management strategies for the disease. State extension agents are encouraged to disseminate this finding to sweet potato farmers in the state.

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Conflict of interests

The authors have not declared any conflict of interests.

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