Growth and yield assessment of *Sphenostylis stenocarpa* affected by virus infection

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Abstract

African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms. is an underexploited legume with the potential of becoming an alternative food staple to alleviate the problem of food security. An assessment was carried out on the growth and yield of AYB affected by Telfairia mosaic virus (TeMV). Telfairia mosaic virus caused severe mosaic, leaf distortion, and malformation on AYB. Seeds of AYB were cultivated and on germination, inoculated with TeMV and the virus effect at different stages of growth and yield was measured. Results analyses revealed that TeMV caused significant (P=0.05) reduction in growth parameters of AYB including leaf area, shoot height, number of primary shoot branches, number of leaves produced, leaf fresh weight (FW) and dry weight (DW), relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR). Results depicted differences between healthy and infected plants with percentage reduction in leaf area, shoot height, and leaf fresh and dry weight at 120 DAI of 41.5%, 48.4%, 29.6%, and 25.5% respectively. Relative growth rate, NAR had reductions of 27.2%, 39.1% 30 days after inoculation (DAI) and 27.0% for LAR at 120 DAI. Infection also led to significant (P=0.05) yield loses: reduction in pod length, pod width, pod weight, number of pods per plant, number of seeds per pod, number of seeds per plant, 100 seed weight. The virus caused insignificant reduction in seed length and breadth, with significant reduction in pod length of 24.7%, pod weight (40.8%), number of pods per plant (22.1%), number of seeds per pod (25.6%), and number of seeds per plant (40.5%). Telfairia mosaic virus caused reduction in growth and yield which may limit the potential of AYB in becoming an alternative food source in Nigeria.

Keywords: African yam bean; food security; grain yield; performance; Telfairia mosaic virus

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Introduction

African yam bean (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich) Harms is a lesser known and underexploited legume that is attracting serious attention due to its rich source of nutrients. It is an indigenous African crop cultivated in different parts of the continent. African yam bean originated from Africa and can be found only in West tropical Africa (Nigeria, Ghana, Cote d'Ivoire, Togo, Guinea, Mali, and Niger), West-central tropical Africa (Burundi,

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Central African Republic, and Democratic Republic of Congo), South tropical Africa (Angola, Malawi, Zambia, and Zimbabwe), as well as East and Northeast tropical Africa (Kenya, Ethiopia, Tanzania, and Uganda) (Genetic Resources Information Network, 2009). In Nigeria, AYB is cultivated by rural subsistence farmers mostly in southern parts of the country. The cultivation of AYB in different parts of Africa has been reported (Porter, 1992, Amoatey et al., 2000, Klu et al., 2001).

Globally, food security is a key factor to the survival of any nation. Food security has become a threat to life causing the increasing dependence on a few major popular staple crops. To expand the food base, AYB, a high yielding grain legume with higher proteins than the conventional tubers and leguminous seeds (Nwokolo, 1996, Baudoin and Mergeai, 2001), higher amino acid contents than those of pigeon pea, cowpea, and bambara groundnut (Uguru and Madukaife, 2001), same tuberous proteins as yam, sweet potatoes, and other root crops (Norman and Cunningham, 2006), seeds high in vitamin B_6, potassium, and manganese, but low in saturated fat and sodium (Oagile et al., 2012), seeds rich in protein, fiber, amino acids, vitamins (A, C, B_1, B_2, B_3, and biotin), and mineral nutrients but low in fat (Mofunanya, 2016) could be added to increase the food base of consumable staple in Nigeria. The utilization of an underexploited legume species like AYB could be of assistance in alleviating the food insecurity problem and preventing food crisis in the country. The popularity of AYB has the potential for developing into a major cash crop like other legumes thereby contributing to the food security need of the country. The performance of this legume in relation to yield and other biotic stresses will aid in repositioning the status of this crop.

A few viruses and their effects have been reported on AYB, namely cucumber mosaic virus (Azizi and Shams-bakhsh, 2014) and Telfairia mosaic virus which cause an increase in alkaloids, saponins, tannins, flavonoids, reducing compounds, and phenols (Mofunanya and Nta, 2011). In spite of these studies, there is dearth of information on the growth and yield of *S. stenocarpa* as affected by TeMV infection hence, the need to assess the influence of TeMV on *S. stenocarpa* in order to ascertain the fate of the plant during infection.

Materials and Methods

Seed collection and planting

The dry seeds of *S. stenocarpa* were obtained from Akparabong, Ikom Local Government Area of Cross River State, Nigeria. The plants from which pods of AYB were harvested were monitored during growth for symptom expression. Seeds of uniform size and weight were sorted and planted in 16 cm diameter polyethylene bags filled with steam-sterilized garden soil. The seeds germinated 9 days after planting and the seedlings were staked to enhance measurement and leaf counting.

Virus source and inoculums preparation

The isolate of TeMV used in this study was supplied by Dr. H. J. Vetten of the Federal Biological Research Centre for Agriculture and Forestry Braunschweig, Germany. The TeMV infected leaf stored under liquid nitrogen was reactivated by homogenizing the leaf tissue in a sterile mortar and pestle in cold disodium phosphate buffer 0.03 M, pH 8.0. When the primary leaves of *S. stenocarpa* were fully expanded, the inoculum was applied on carborundum (800 mesh) dusted leaves, rinsed and allowed to stay for symptom development. Subsequent inoculations were conducted using TeMV infected *Telfairia occidentalis* maintained in the screen-house at 23 ± 3 ^{o}C.

Experimental design

The seedlings were arranged in two groups (infected and healthy), each group containing 15 seedlings. Prior to inoculation, the study groups were arranged in a randomized block design (RBD) in a greenhouse. All the seedlings in the first group inoculated with the virus were designated infected and those in the second group inoculated with the buffer only were designated as healthy or control.

Investigations were carried out on healthy and TeMV-infected *S. stenocarpa* plants at various periods of growth. Leaf area was determined at

30, 60, 90, and 120 days after inoculation (DAI) using leaves of the same age and position on infected and healthy plants. The flat leaf area was measured by Stickler's equation (Stickler and Pauli, 1961) as given below:

Leaf area (cm^2) = L X B X 0.747

Shoot height and number of leaves produced by infected and healthy plants of *S. stenocarpa* were obtained by measuring shoot length in centimeters from the base to the tip of the plant and leaf numbers were counted for healthy and infected plants at various stages of growth.

Fresh and dry weight of leaves were estimated by harvested leaves from 5 infected and healthy plants at an interval of 30 DAI and dried. Leaf fresh weight was taken before the samples were dried in a Hot Box Oven Gallenkamp, CHF097 XX2.5, England) to constant weight at a temperature of 60 ℃.

Fresh and dry weight of shoot of *S. stenocarpa* were recorded after a period of 135 DAI. Five plants were harvested, their leaves and shoots were removed. The shoots of both infected and healthy plants were cut off from the roots with the aid of a scissor and their fresh weights were recorded separately before drying in the Hot Box Oven (Gallenkamp, CHF097 XX2.5, England) to constant weight at 74 °C for 24 hr.

Relative growth rate measures the changes in weight of infected and healthy samples. The change in weight was taken at various stages of growth (30, 60, 90, and 120 DAI). The differences between the samples were calculated (Atwell et al., 1999).

Net assimilation rate is a function of the photosynthetic efficiency of leaves of a plant and its leafiness. The difference in the natural logarithm of leaf area over leaf area and leaf dry weight over difference in time was used to show the photosynthetic efficiency between leaves of healthy and infected samples.

The leaf area ratio for healthy and TeMV-infected *S. stenocarpa* represents the ratio of total leaf area to whole leaf dry weight over a period of time (30-day interval).

Number of pods per plant, number of seeds per pod, and number of seeds per plant were obtained by counting pods. Also, 100 seed weight, and pod length, pod width, seed length, and seed breadth, were measured.

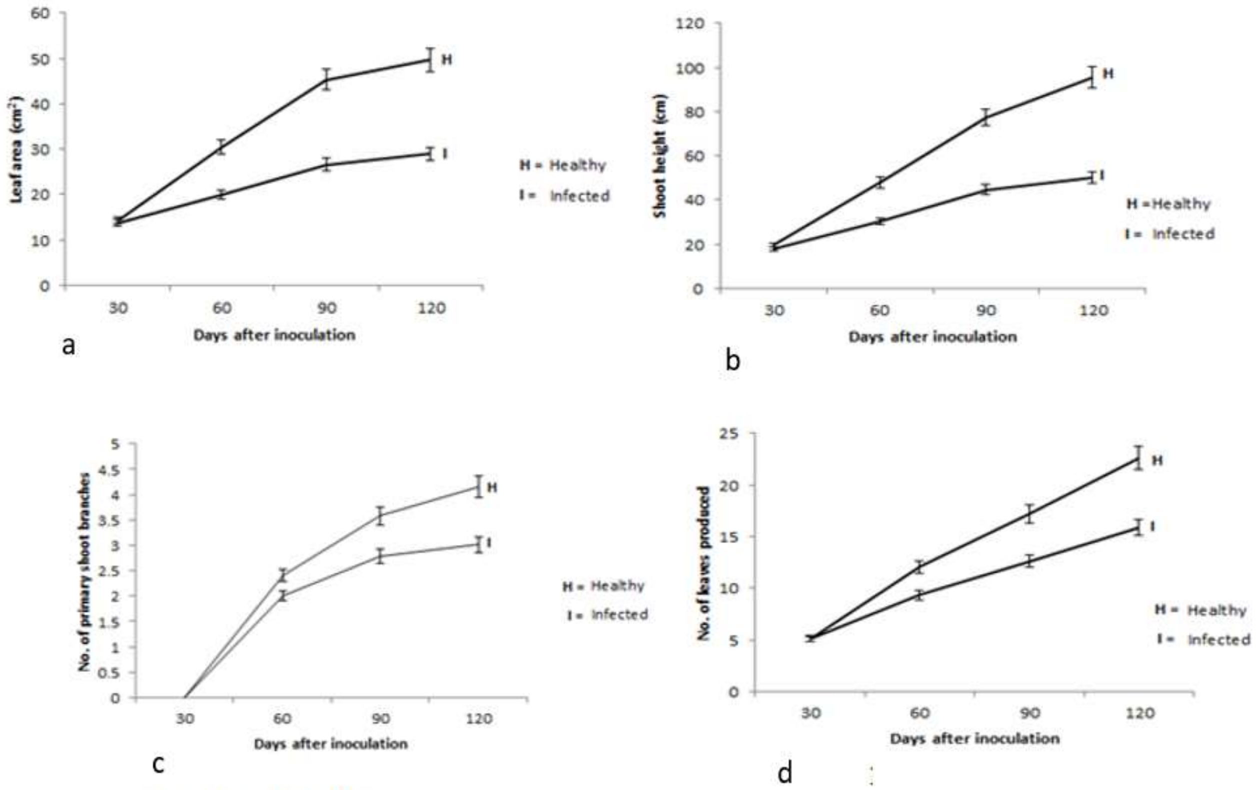
Data Analyses

Data from this study was analyzed using the t-Test statistics (Excel Statistical Package, 2010). Results were also expressed as percentage difference in growth and yield parameters and differences between mean values for infected and healthy plants were determined at 5% probability.

Results

Assessment of the effects of TeMV infection on growth parameters of *S. stenocarpa* revealed significant differences between healthy and infected plants. Analyses of results showed insignificant differences in all growth parameters at 30 DAI with the least significant differences at 60 DAI and the highest at 120 DAI. Telfairia mosaic virus infection caused significant (P=0.05) reduction in leaf area with percentage reduction values of 34.4%, 41.3%, and 41.5% at 60, 90, and 120 DAI, respectively (Fig. I. a). Shoot heights of infected plants were significantly (P=0.05) shorter than the healthy ones. At 120 DAI, mean shoot height value for healthy plants was 95.46 ± 0.088 cm as against value of 50.28 ± 0.058 cm for infected plants (Fig. I. b). There was no production of primary shoot branches 30 DAI in both healthy and infected plants. The number of primary shoot branches of TeMV infected AYB was significantly reduced with a range in mean values of healthy plants from 2.40 ± 0.002 to 4.16 ± 0.003 (60 DAI) compared to values of 2.00 ± 0.002 to 3.01 ± 0.002 (120 DAI) for infected plants (Fig. I. c). The virus engendered significant (P=0.05) reduction in number of leaves produced with a range in mean values (from 30-120 DAI) for healthy plants of 5.11 ± 0.033 to 22.65 ± 0.115 compared to infected plants values of [illegible]10 ± 0.033 to 15.90 ± 0.058 (Fig. I. d).

Stress induced by TeMV resulted in leaf fresh and dry weight decrease. The differences in leaf fresh and dry weight were significant (P=0.05) from 60-120 DAI but insignificant at 30 DAI in both healthy and TeMV infected plants. Percentage decrease in leaf FW and DW for healthy and

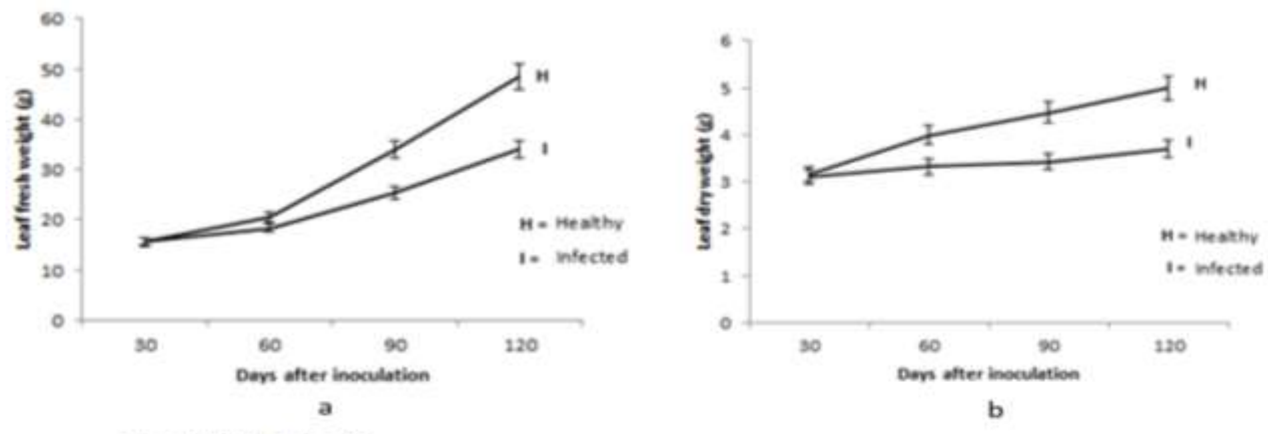


Mean ± SE, N= 10, P=0.05

Fig. I. Assessment of TeMV infection on flag leaf area (a), shoot height (b), number of primary shoot branches (c), and number of leaves produced (IV) by *S. stenocarpa*

infected plants at 60, 90, and 120 DAI were 10.0%, 25.2%, 29.6% and 16.8%, 23.5%, 25.9%, respectively (Fig. II. A and II. b).

Relative growth rate was negatively affected by TeMV infection with significant (P=0.05) reductions at all stages of growth. Results analyses revealed a progressive decrease with prolonged period of infection. The highest and lowest percentages obtained for RGR were 27.2% at 30 DAI and 12.4% at 120 DAI, respectively (Fig. III. a). The negative effect of TeMV infection on NAR followed a similar trend of decrease with increasing periods of development in healthy and infected samples. The highest mean value was obtained at 30 DAI ($9.94 \times 10^{-4} \pm 4.1 \times 10^{-7}$ g cm^{-2} day^{-1}) and the lowest value of ($2.96 \times 10^{-4} \pm 3.6$ x



Mean ± SE, N= 10, P=0.05

Fig. II. Assessment of TeMV infection on leaf FW (a) and DW (b) of *S. stenocarpa*

10^{-7} g cm^{-2} day^{-1}) at 120 DAI for healthy plant samples. Corresponding values for infected samples were 6.05 x 10^{-4} ± 5.3 x 10^{-7} and 2.30 x 10^{-4} ± 6.2 x 10^{-7} g cm^{-2} day^{-1} at the same periods of growth (Fig. III. b). Leaf area ratio showed a general trend of increase with progressive periods of growth. Percentage reduction values for LAR ranged from insignificant reduction of 3.35 at 30 DAI to significant value of 27.0% at 120 DAI (Fig. III. c).

At 140 DAI, 10 dry mature pods of *S. stenocarpa* were harvested and yield measurements were taken. Yield parameters of *S. stenocarpa* were significantly affected by TeMV infection. Pod length and pod width of the infected plants were significantly (P=0.05) reduced with mean values of 14.49 ± 0.34 and 0.93 ± 0.01 compared to 19.25 ± 0.44 and 1.10 ± 0.006. Pod weight of infected plants were severely impacted by the virus with mean value of healthy plants of 13.70 ± 0.13 compared to infected plants' value of 8.11 ± 0.02 depicting (40.8% pod weight) reduction. Infection caused significant (P=0.05) loses in the number of pods per plant, number of seeds per pod, and of seeds per plant. Telfairia mosaic virus engendered reduction in the number of pods produced per plant with mean value for infected plants of 14.76 ± 0.64 as against healthy plant value of 18.95 ± 0.021. The virus caused significant (P=0.05) loses in the number of seeds per pod with mean value for infected plant of 8.88 ± 0.34 in comparison to healthy plants' value of 11.94 ± 0.32. Number of seeds per plant was drastically reduced by TeMV infection to 134.62 ± 0.01 for infected and 226.26 ± 0.006 for healthy plants. However, reduction in seed length and seed breadth of healthy and infected plants did not differ statistically (Table 1).

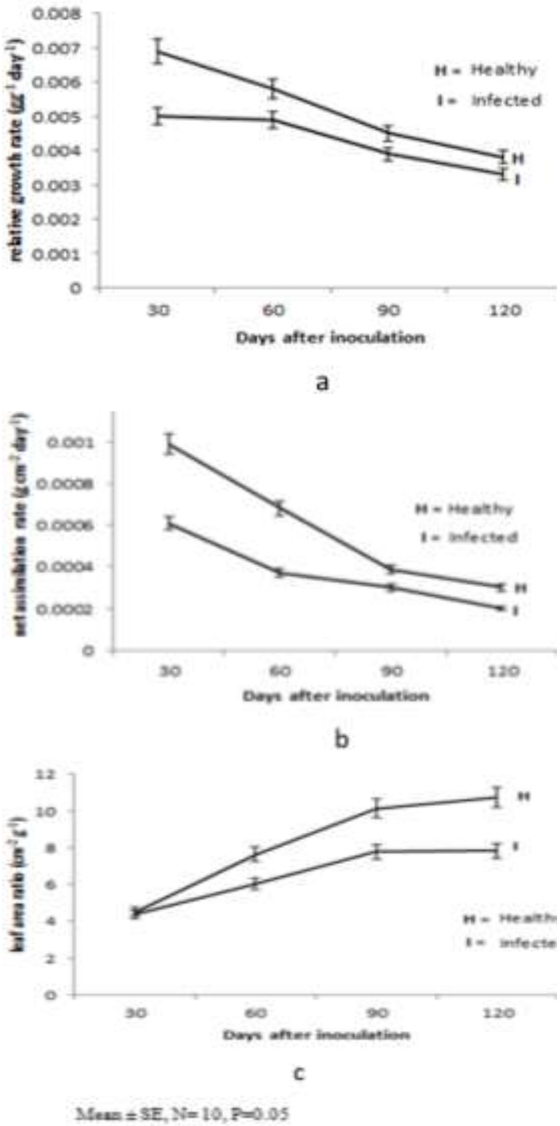


Fig. III. Assessment of TeMV infection on RGR (a), NAR (b) and LAR (c) of *S. stenocarpa*

Discussion

Growth and yield assessment of *S. stenocarpa* affected by TeMV infection was studied. Virus infected plants showed reduction in growth and yield components in response to infection. Leaf area reduction in this study was disturbing because symptoms of viral infection developed in the leaves and they were important for plant-virus interaction studies. Reduction in leaf area is also disturbing because of the significance of leaf area or size in plant physiology. The smaller the leaf is, the smaller the leaf surface area for harvesting light energy with a resultant decrease in photosynthesis. The plant leaf is a key component in maintaining life on the planet earth through the food and oxygen production process of photosynthesis. Reduction in leaf area has a direct effect on leaf weight or mass and yield. Leaves are the principal photosynthetic organs of plants (Wright et al. 2004), therefore, the size of leaves very importantly affects a variety of biological processes, such as plant growth, survival, reproduction, and ecosystem function (Koch et al., 2004; Tozer et al., 2015). Koester et al. (2014) reported that leaf area growth determines

Table 1
Assessment of the effects of TeMV infection on yield parameters of *S. stenocarpa*

Plant parameters	Healthy	Infected	% difference
Pod length (cm)	19.25 ± 0.44	14.49±0.34*	24.7
Pod width (mm)	1.10 ± 0.006	0.93 ± 0.01	15.5
Pod weight (g)	13.70± 0.13	8.11 ± 0.02	40.8
Number of pods per plant	18.95±0.021	14.76± 0.64*	22.1
Number of seeds per pod	11.94 ± 0.32	8.88 ± 0.34*	25.6
Number of seeds per plant	226.26± 0.006	134.62±0.01*	40.5
Weight of 100 seeds (g)	9.86 ± 0.12	8.04 ±0.01*	18.5
Eeeffects Length of seed (mm)	5.30 ± 0.44	4.91 ± 0.32 ^{NS}	7.4
Breadth of seed (mm)	6.09 ± 0.44	5.80 ± 0.12^{NS}	4.8

Mean ± SE, N = 10, P=*0.05*, NS= Not significant, *Significant; percentage difference was obtained by expressing the difference between the value for healthy and infected as a percentage of the healthy

the light interception and is an important parameter in determining plant productivity. Plant leaf is a critical component in the plant water transport system accounting for organic food and nutrient transport in the plant system. Leaf area can regulate leaf temperature (Niinemets et al., 2006). Shoot height reduction orchestrated by TeMV infection resulted in stunting which is the most common symptom observed in virus infected plants with remarkable effects on yield. Reduction in growth parameters due to virus infection have been documented by other researchers; Pazarlar et al. (2013) found significant decrease in growth and physiological parameters in some pepper varieties infected with TMV. Infection of *Cucurbita moschata* by a Nigerian strain of MWMV caused reduction in plant growth (Mofunanya and Edu, 2015). Effect of tomato mosaic virus caused significant reduction in plant height, fresh shoot weight, dry shoot weight, fresh root weight, and dry root weight of tomato genotypes (Ullah et al., 2017). Reduction in the number of primary shoots which is an important indicator of grain yield directly affected productivity of AYB.

The values for RGR, NAR and LAR for healthy plants of *S. stenocarpa* were higher than TeMV-infected plants. Mofunanya et al. (2015) reported a reduction in NAR, RGR and LAR in ecotypes of *T. occidentalis* infected with TeMV. Net assimilation rate is a primary physiological parameter that has a strong and positive association with area-based photosynthetic rate and leaf nitrogen content which are good predicators of plant growth. The NAR, which is the rate of dry mass gain per unit leaf area, is largely the net result of the rate of carbon gain in photosynthesis per unit leaf area and that of carbon use in respiration of leaves, stems, and roots. Decrease in NAR found in this study is of great concern because some physiological processes are linked to it. Reduction in NAR caused by TeMV infection led to reduction or stunted growth of *S. stenocarpa* due to low carbon production. The net assimilation rate provides carbon for growth (Lambers et al., 2008). The relationship between leaf area growth and growth in terms of mass will depend on how carbon is partitioned among new leaf area, leaf mass, root mass, reproduction, and respiration (Weraduwage et al., 2015). Healthy plants of *S. stenocarpa* studied had inherently higher relative growth rates than TeMV-infected plants. Relative growth rate is used physiologically to quantify the speed of plant growth. It is measured as the mass increase per aboveground biomass per day. Growth in leaf mass can result from an increase in area or thickness; total leaf mass is the sum of mass increase for leaf area growth and leaf thickening. High RGR could be associated with a high NAR. When plants grow in favorable environments they often have inherently higher maximum relative growth rates than do species from less favorable environments like the virus infected species. Plant viruses are responsible for losses in subsistence and commercial food crops production worldwide. Telfairia mosaic virus infection significantly and negatively impacted the yield components of *S. stenocarpa*. Growth and yield losses in this study are attributed to reduction in leaf area, number of leaves produced, RGR, NAR, and LAR which affected photosynthetic activities and interfered with energy production resulting in stunted growth and poor yield depicting the direct link between growth and yield. Yield component analyses of TeMV infected *S. stenocarpa* revealed that variation in pod yield can be explained by the number of pods per plant, pod length, and number of seeds per plant. The reduction in pod number which is the main yield component negatively impacted the overall number of seeds per plant. Yield loses caused by TeMV in the present study could also be due to reduction in the number of primary shoot branches which is an index of yield. The higher the number of primary shoot branches, the greater the yield. Severe mosaic symptoms of the virus also accounted for yield loses of AYB. Ameh and Okezie (2005) reported that grain yield of AYB was affected by pests and diseases. Seed number reduction orchestrated by TeMV is worrisome because the demand for food is rising and AYB is cultivated mainly for its seed and tuber but our study focused on seed production required to meet the food need of the people. The intention of broadening the food base of legumes and creating room for alternative food source will be truncated if efforts are not geared towards controlling this virus on AYB. Considering the reduction in growth and yield loses of this underutilized legume by TeMV, the development of resistant varieties of *S. stenocarpa* and controlling TeMV on the crop will inform further research. This is the first report on the effect of TeMV on growth and yield of *S. stenocarpa*.

Conclusion

Growth and yield assessment of AYB affected by virus infection was investigated. Telfairia mosaic virus severely affected the growth at different periods with significant yield loses. The effect of the virus was not significant at 30 DAI but significant from 60-120 DAI. The virus engendered significant reduction in leaf area, shoot height, number of primary shoot branches, number of leaves produced, leaf fresh and dry weight, RGR, NAR, and LAR. There was a progressive decrease in growth with prolonged periods of infection. Virus infection also led to significant yield loses, reduction in pod length, pod width, pod weight, number of pods per plant, number of seeds per pod, number of seed per plant, and 100 seed weight hence, the need for further research.

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