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Limitation on Growth and Yield of *Phaseolus vulgaris* L. Due to Telfairia Mosaic Virus

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAJM designed the study, wrote the protocol and the manuscript. Author EAE handled the data. Author BAN performed the statistical analysis. Authors FAA and AOD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess limitation on growth and yield of *P. vulgaris* due to Telfairia mosaic virus (TeMV).
Study Design: The study was conducted in a randomized block design.
Place and Duration: Department of Botany, University of Calabar, Calabar, Nigeria between April and August, 2016.
Methodology: Seeds of *P. vulgaris* were obtained, sorted, planted in polyethylene bags, and on germination, inoculated with TeMV, growth and yield limitation assessed at 2, 4, 6, 8 and 12 weeks after inoculation.
Results: Results showed that the virus decreased growth of *P. vulgaris* resulting in yield loses. Growth parameters were severely limited by TeMV with percentage reduction in Leaf area, shoot height, number of primary shoot, petiole length and number of leaves produced of 44.5%, 52.8%, 44.0%, 44.9% and 51.45 respectively at 10 weeks after inoculation (WAI). Leaf fresh weight (38.4%) and dry weight (32.3%) were significantly (*P=0.05*) reduced. Relative growth rate, net assimilation rate and leaf area ratio were negatively impacted by the virus with reductions of 26.5%, 36.7% at 4 WAI and 33.5% at 12 WAI respectively. Limitation on yield due to TeMV was

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significant (*P=0.05*) with reduction in pod length of 36.9%, number of pods per plant of 36.5%, number of seeds per pod of 26.5%, number of seeds per plant of 53.3% and weight of 100 seeds of 18.5%. Limitation induced on fresh and dry weight of shoot and root, pod width, seed length and seed width was not significant.

Conclusion: The study confirmed the threat of TeMV necessitating control of the virus on this important legume.

Keywords: Bean; constraint; performance; Telfairia mosaic virus; yield loses.

1. INTRODUCTION

Phaseolus vulgaris L. (Fabaceae) is grown and consumed widely in Nigeria, other African countries and the world at large. The high consumption of *P. vulgaris* in Nigeria is attributed to the fact that it is relatively cheap, affordable and readily available. *Phaseolus vulgaris* is one of the staple crops in many parts of Africa, Asia and Latin America and serves as a source of primary nutrition to millions of people because of its high protein content [1]. Health organizations now promote regular consumption of common bean and other pulses because it reduces the risk of diseases such as cancer, diabetes or coronary heart diseases [2].

The production of common bean is limited by virus diseases. Virus diseases pose serious challenges to crop growth, productivity, product quality, its marketability and in some cases elimination of infected plants. Bean is infected by many viruses of which some cause diseases of economic importance. Over 200 different viruses can infect *P. vulgaris*. The most commonly occurring viruses on *P. vulgaris* in the sub-Saharan Africa are Bean common mosaic virus (BCMV) genus Potyvirus, Bean leaf roll virus (BLRV) genus genus Luteovirus, Bean southern mosaic virus (SBMV) genus Sobemovirus, Bean yellow mosaic virus (BYMV) genus Potyvirus, Cucumber mosaic virus (CMV) genus Cucumovirus, Jatropha mosaic virus (JMV) genus Begomovirus, Peanut mottle virus (PeMoV) genus Potyvirus, and Tomato spotted wilt virus (TSWV) genus Tospovirus. Other naturally occurring virus diseases affecting *P. vulgaris* in sub-Saharan Africa are Alfalfa mosaic virus (AMV) genus Alfamovirus, Tobacco necrosis virus (TNV) genus Necrovirus, Tobacco streak virus (TSV) genus Itarvirus, Tobacco black ring virus (TBRV) genus Nepovirus [3].

In Nigeria, *P. vulgaris* was reported to be a susceptible host of Telfairia mosaic virus (TeMV) [4]. In an earlier study, [5] reported a reduction in saponins (89.29%), alkaloids (54.42%), phenols (37.87%), tannins (25.86%) and reducing compounds (18.35%) occasioned by TeMV infection on *P. vulgaris*. In another study, [6] documented significant reductions in fat (28.6%), fiber (20.8%), carbohydrate (14.2%), nicotinamide (32.7%), riboflavin (47.3%), sodium (95.3%), magnesium (40.6%), potassium (32.2%) and other nutrients of *P. vulgaris* due to Telfairia mosaic virus (TeMV) infection. The effects of other viruses on *P. vulgaris* are found but literature is however, lacking on growth and yield limitation of *P. vulgaris*. This study was undertaken to ascertain the level of damage on growth and yield loses caused by TeMV on *P. vulgaris* in order to advance control measures for the virus in subsequent investigation.

2. MATERIALS AND METHODS

2.1 Planting Materials

Dry pods of *P. vulgaris* from which seeds were obtained were bought from local farmers at the University of Calabar Staff Quarters whose plants were monitored on the field for symptoms expression. Two seeds each were planted in steam-sterilized soil in 16 cm diameter polyethylene bags. The work was carried out in the Botanical Garden greenhouse of Department of Botany, University of Calabar, Nigeria. The seeds germinated four days after planting (DAP) and the seedlings staked to enhance measurement of shoot height and leaf number count.

2.2 Inoculum Preparation and Plant Inoculation

Young leaves of Telfairia mosaic virus infected-*Telfairia occidentalis* were collected and ground in a sterilized mortar and pestle in disodium phosphate buffer 0.03 M, pH 8.0. The inoculum obtained was applied on *P. vulgaris* seedlings pre-dusted with carborundum (800-mesh) at two-leaf stage mechanically by rubbing the sap on the leaf. The inoculated leaves were rinsed with water and observed for symptom development under screen-house condition of 25±3^{o}C. Before *P. vulgaris* plants were inoculated, those to be inoculated and those of the control were

arranged in a randomized block design (RBD) containing a total of 40 plants. Twenty plants were inoculated with the virus and the other twenty plants were inoculated with the buffer only to serve as control. Plants inoculated with TeMV were left for symptom development such as mosaic, severe leaf malformation and distortion characteristics of TeMV infection.

2.3 Plant Growth Parameters Determination

Observations were carried out on TeMV-inoculated and control plants of *P. vulgaris* at various stages of growth. Limitation on leaf area was determined using leaves of the same age and position on inoculated and control plants at 2, 4, 6, 8, 10 and 12 weeks after inoculated (WAI). The leaf length was measured with a meter rule in centimeters along the midrib, from the base to apex excluding the petiole and leaf width in centimeters was measured perpendicular to the midrib from one end of the leaf to the other. The readings obtained were then used to estimate the leaf area by applying [7] equation.

$$A\mathit{f}LW = -27.7418 + (3.9812\ LW / \ln LW)$$

Where, L is the leaf length and W is the width.

Shoot height, number of primary shoots, petiole length and number of leaves produced on *P. vulgaris* due to TeMV were obtained by measuring shoot length and petiole length in centimeters while number of primary shoots and number of leaves produced leaves number were counted for healthy and infected plants at various stages of growth.

For leaf fresh and dry weight measurements, the leaves were harvested from inoculated and control plants and dried. Leaf fresh weight was taken before the samples were dried to constant weight at a temperature of 60^{o}C. Limitation on fresh and dry weight of shoot and root of *P. vulgaris* was carried after a period of 14 WAI. Ten plants were uprooted and placed in a bucket of water and the soil carefully washed off to prevent root damage. The shoots of both infected and healthy plants were cut off from the roots with the aid of a scissor and their fresh weights taken before drying separately in Hot Box Oven (Gallenkamp, CHF097 XX2.5, England) to constant weight at 74^{o}C for 24 h.

Limitation on relative growth rate (RGR) measured the changes in weight of control and inoculated samples. The change in weight was taken at various stages of growth. The differences between the samples were calculated [8]. Net assimilation rate (NAR) 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 the control and inoculated. The leaf area ratio (LAR) for TeMV-inoculated and control *P. vulgaris* represents the ratio of total leaf area to whole leaf dry weight over a period of time (2 weeks interval).

2.4 Yield Parameters Determined Included

Dry pods of *P. vulgaris* were harvested from control and inoculated plants at maturity indicated by pod yellowing with appearance of marked indication on the pods. The number of pods per plant was counted, the pods were opened and the number of seeds per pod and number of seeds per plant counted. The pod length, pod weight, seed length and breadth were measured with the aid of a meter rule, the weight of 100 seeds was obtained using analytical weighing balance.

2.5 Data Analysis

Data from this study was analyzed using the independent t-Test. Results were also expressed as percentage difference and differences between mean values for infected and healthy were determined at 5% probability.

3. RESULTS

3.1 Limitation on Growth Parameters per Plant of *Phaseolus vulgaris* by Telfairia Mosaic Virus

Telfairia mosaic virus significantly (*P=0.05*) limited the growth parameters of *P. vulgaris*. Results revealed that TeMV caused a progressive decrease in growth with prolonged period of infection with no significant limitation at 2 WAI for all growth parameters studied. Leaf area of *P. vulgaris* was severely reduced by the virus with mean reduction for inoculated plant at 10 WAI of 37.03 ± 0.03 cm^2 compared to control plant of 66.71 ± 0.01 cm^2. The virus caused significant (*P=0.05*) reduction in shoot height of 40.23 ± 0.02 cm compared to control plant height of 85.19 ± 0.03 cm. Mean reduction in number of primary shoot branches of *P. vulgaris* due to TeMV infection at 10 WAI was 4.03 ± 0.13 for

inoculated plant and 7.20 ± 0.10 for control plant. Limitation on petiole length and number of leaves produced of 5.26 ± 1.04 cm and 14.60± 0.02 compared to values of 9.55 ± 0.03 cm and 28.79± 0.02 for the control plants (Table 1).

3.2 Leaf Fresh and Dry Weight Per Plant of *Phaseolus vulgaris* Due to Telfairia Mosaic Virus

Leaf fresh and dry weights were severely affected by TeMV with significant (*P=0.05*) reductions. There was no change found in LFW and LDW at 2 WAI due to virus infection, LFW and LDW of 5.34±0.00, 1.03±0.01 g for control plant and 5.34±0.01, 1.03±0.01 g for inoculated plant. Mean reduction in leaf fresh weight of 17.23 ± 0.067 g and 27.95 ± 0.058 g for inoculated and control plant. Corresponding reduction for leaf dry weight was 8.88 ± 0.033 g for inoculated plant and 13.11 ± 0.033 g for control value of (Table 2).

3.3 Limitation on Relative Growth Rate, Net Assimilation Rate and Leaf Area Ratio per Plant of *Phaseolus vulgaris* by Telfairia Mosaic Virus

Telfairia mosaic virus caused a negative impact on RGR, NAR and LAR per plant of *P. vulgaris* (Table 3). The virus significantly (*P=0.05*) limited the RGR with highest and lowest reduction for inoculated plant of 0.0061 ± 1E-04 and 0.0027 ± 0.0001 (g cm^{-2} day^{-1}) as against control plant of 0.0083 ± 1E-04 and 0.0032 ± 0.0001 (gg^{-1} day^{-1}). Limitation on NAR by TeMV had decrease of 0.0007 ± 0.00001 for inoculated plant at 4 WAI compared to the control plant of 0.00011 ± 0.00001 (g cm^{-2} day^{-1}). The virus also caused reduction in LAR at 12 WAI of 8.97 ± 0.01 compared to control of 13.49 ± 0.01 (cm^{-2} g^{-1}). A trend of decrease in RGR and NAR with prolonged period of growth was observed, while LAR had a trend of increase with progressive period of growth for both inoculated and control plants.

3.4 Limitation on Fresh and Dry Weight of Shoot and Root in (g) of *Phaseolus vulgaris* by Telfairia Mosaic Virus

Limitation on fresh and dry weight of shoot and root by TeMV at the end of the study was not statistically significant. Percentage reduction values for fresh and dry weight of shoot and root were 9.2%, 6.5% and 6.0%, 3.7% respectively (Table 4).

3.5 Yield Limitation of *Phaseolus vulgaris* by Telfairia Mosaic Virus

Telfairia mosaic virus adversely limited the yield of *P. vulgaris* (Table 5). The virus caused reduction in pod length, number of pods per plant, number of seeds per pod, number of seeds per *P. vulgaris* plant with mean reduction values of 6.32 ± 0.02, 17.02 ± 0.64, 4.78 ± 0.34, 81.36 ± 0.01 compared to control plants of 10.01 ± 0.01, 26.81 ± 0.02, 6.50 ± 0.12 and 174.27± 0.06 respectively (Table 4). Pod width, length of seed and breadth of seed did not differ statistically.

4. DISCUSSION

Growth and yield limitation of *P. vulgaris* by TeMV was investigated. Telfairia mosaic virus infection significantly inhibited growth and development of *P. vulgaris*. The virus caused significant reduction in leaf area, leaf number, shoot height, number of primary shoot branches, leaf fresh and dry weight and fresh and dry weight of shoot and root of *P. vulgaris*. Telfairia mosaic virus caused similar reduction in growth and yield of *Sphenostylis stenocarpa* [9]. The reduction in growth parameters by TeMV are of great concern. Reduction in leaf area is crucial because of the significance of leaf 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 mass and yield. [10] reported that leaf area growth determines the light interception and is an important parameter in determining plant productivity. Plant leaf is a critical component in water transport system accounting for organic food and nutrient transport in the plant system. Leaf area can regulate leaf temperature. Shoot height reduction orchestrated by TeMV infection results in stunting and dwarfing which are the most common symptoms observed in virus infected plants with remarkable effect on yield. Reduction in growth parameters due to virus infection have been documented by other researchers; [11] reported reduction in plant height, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight of tomato genotypes due to Tomato mosaic virus, [12] recorded maximum reduction in leaf area index, leaf area duration and pod yield in cowpea infected by cowpea severe mosaic virus.

Table 1. Limitation on growth parameters per plant of *Phaseolus vulgaris* by Telfairia mosaic virus

WAI	Leaf area (cm^2)		Shoot height (cm)		No. of primary shoot		Petiole length		No. of leaves produced	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
2	19.49±0.01	19.48±0.01	14.53±0.03	14.52±0.01	0.00±0.00	0.00±0.00	3.10±0.02	3.09±0.01	2.10±0.00	2.10±0.01
4	38.6±0.02	28.12±0.04*	35.17±0.06	23.1±0.01*	3.16±0.01	3.10±0.00*	5.23±0.01	3.47±0.03*	4.23±0.01	4.23±0.03*
6	49.72±0.03	31.31±0.02*	56.61±0.01	32.22±0.04*	5.33±0.01	3.27±0.01*	7.48±0.02	4.06±0.03*	9.54±0.01	7.11±0.03*
8	63.57±0.01	33.2±0.04*	78.34±0.04	37.45±0.05*	6.25±0.02	3.64±0.01*	8.09±0.01	5.2±0.03*	17.65±0.01	9.33±0.01*
10	66.71±0.01	34.03±0.02*	85.19±0.01	40.23±0.01*	7.20±0.02	4.01±0.01*	9.55±0.01	5.26±0.01*	28.79±0.01	14.6±0.01*
12	66.71±0.02	35.04±0.04*	90.16±0.01	41.2±0.01*	7.24±0.02	4.03±0.01*	10.95±0.02	5.48±0.02*	30.11±0.01	15.19±0.01*

*Values are mean ± SD, n=5, P=0.05 * = statistically significant*

Table 2. Limitation on leaf fresh and dry weight per plant of *Phaseolus vulgaris* by Telfairia mosaic virus

WAI	Leaf FW (g)		Leaf DW (g)	
	Control	Inoculated	Control	Inoculated
2	5.34±0.00	5.34±0.01	1.03±0.01	1.03±0.01
4	10.71±0.01	8.84±0.01*	1.51±0.01	1.25±0.02*
6	16.08±0.01	12.23±0.02*	5.02±0.01	4.01±0.01*
8	20.53±0.01	14.14±0.01*	10.2±0.01	7.24±0.01*
10	27.92±0.01	17.22±0.02*	13.10±0.01	8.88±0.02*
12	27.95±0.01	17.22±0.00*	13.11±0.01	8.88±0.01*

Values are Mean ± SD, n=5, P=0.05 = statistically significant*

Table 3. Limitation on relative growth rate, net assimilation rate and leaf area ratio per plant of *Phaseolus vulgaris* by Telfairia mosaic virus

WAI	RGR ($gg^{-1}day^{-1}$)		NAR ($g\ cm^{-2}\ day^{-1}$)		LAR ($cm^{-2}g^{-1}$)	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
4	0.0083±1E-04	0.0061±1E-04*	0.000111±0.001	0.00070±0.00001*	5.1±0.02	4.68±0.03*
8	0.0059±0.0001	0.0047±1E-04*	0.00085±1E-05	0.00060±0.0000*	9.75±0.01	7.23±0.03*
12	0.0032±0.0001	0.0027±0.0001*	0.00052±1E-05	0.00040±0.00001*	13.49±0.01	8.97±0.01*

*Values are Mean ± SD, n=5, P=0.05, * = statistically significant, E=exponential*

Table 4. Limitation on fresh and dry weight of shoot and root in (g) of *Phaseolus vulgaris* by Telfairia mosaic virus

14 WAI	Shoot FW		Shoot DW		Root FW		Root DW	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
	52.01±0.01	45.23±0.01*	29.1±0.01	26.22±0.01	39.41±0.01	37.06±0.01	19.37±0.01	18.65±0.01

*Values are mean± SD, n=5, P=0.05, * = statistically significant, FW = fresh weight, DW = dry weight*

Table 5. Yield limitation of *Phaseolus vulgaris* by Telfairia mosaic virus

Plant parameters	Control	Inoculated
Pod length (cm)	10.01 ± 0.01	6.32 ± 0.02*
Pod width (mm)	0.90 ± 0.003	0.86 ± 0.01^{NS}
Pod weight (g)	8.64 ± 0.002	7.21 ± 0.02*
Number of pods per plant	26.81 ± 0.02	17.02 ± 0.64*
Number of seeds per pod	6.50 ± 0.12	4.78 ± 0.34*
Number of seeds per plant	174.27± 0.06	81.36 ± 0.01*
Weight of 100 seeds (g)	9.86 ± 0.12	8.04 ±0.01*
Length of seed (mm)	1.01 ± 0.44	0.95 ± 0.02^{NS}
Breath of seed (mm)	0.80 ± 0.03	0.75 ± 0.01^{NS}

*Mean ± SD, n = 5, P=0.05, NS = not significant, *= statistically significant*

Results revealed that RGR, NAR and LAR of *P. vulgaris* inoculated with TeMV were severely limited when compared to control plants. In an earlier study, [13] reported a reduction in NAR, RGR and LAR in two ecotypes of *T. occidentalis* inoculated with TeMV. Virus-induced limitation on these important indices of growth led to poor plant performance. Net assimilation rate is a primary physiological parameter that has a positive association with area-based photosynthetic rate and leaf nitrogen content which are good predicators of plant growth. The NAR is 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. Reduction in NAR caused by TeMV infection led to decreased growth due to low carbon production. Net assimilation rate provides carbon for growth [14]. Control plants of *P. vulgaris* studied had inherently higher relative growth rates than TeMV-inoculated plants. Relative growth rate is a physiological parameter used to quantify the speed of plant growth. Growth in leaf mass can result from an increase in area or thickness as found in results of leaf area increase with progressive periods of development for both control and inoculated plants; total leaf mass is the sum of mass increase for leaf area growth and leaf thickening. Control plants of *P. vulgaris* not inoculated with TeMV had inherently higher maximum relative growth rates than inoculated plants grown in the same environment. Relative growth rate of a plant is the product of LAR which is leaf area per unit total plant biomass and NAR. Leaf area ratio is the product of specific leaf area, the ratio of leaf area and leaf weight, and the leaf weight ratio, which are indicators of the fraction of total plant weight allocated to the leaves.

Viruses cause significant yield loses in crop production worldwide. Telfairia mosaic virus negatively impacted the yield components of *P. vulgaris* with reduction in pod length, pod width, pod weight, number of pod per plant, number of seed per pod, number of seed per plant and 100 g seed weight. Yield losses in this study may be ascribed to reduction in vegetative growth (leaf area, number of leaves produced, RGR, NAR and LAR) which affected photosynthetic activities and altered energy production resulting in stunted growth and poor yield exhibiting the direct link between growth and yield. From the results, variation in pod can be explained by number of pod per plant, pod weight, 100 seed weight and number of seed per plant. The reduction in pod number which is the principal yield component severely impacted the overall number of seed per plant. Reduction in vegetative growth explains reduction in yield. Yield loses could also be attributed to severe mosaic symptoms of TeMV. Pod and seed number reduction occasioned by TeMV is a potential threat to the production of *P. vulgaris* where this virus is endemic. The demand for food is rising and *P. vulgaris* is cultivated mainly for its seeds and fresh pods (used as vegetable) which are on high demand to feed millions of people worldwide. Low seed yield implies low income earning for both subsistence and commercial farmers whose income is tied to its production. Yield loses on common bean by other viruses have been reported; In the USA, crop and yield losses of 68% and 50% of infected bean by BCMV were reported [15]. Bean common mosaic virus caused pod yields and seed yields reductions of 50%, 64% and 53%, 68% respectively [16]. Tobacco mosaic virus and other viruses were reported in the US to cause an estimated $60 billion loss in common bean yields worldwide each year [17]. This is the first report on growth and yield limitation of *P. vulgaris* by TeMV. Considering the damage caused by this virus, subsequent investigation will focus on the control of TeMV on this crop of high economic value.

5. CONCLUSION

Limitation on growth and yield components of *P. vulgaris* studied were severely affected by TeMV infection. Effect of TeMV at initial growth period (two weeks after inoculation) on all the growth parameters was insignificant. The virus exhibited reduction in leaf area, shoot height, number of primary shoot branches, number of leaves produced, leaf fresh and dry weight, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR). Effect of the virus on shoot fresh and dry weight, root fresh and dry weight was not significant. There was a progressive decrease in growth parameters with prolonged periods of infection. Infection led to significant ($P=0.05$) yield loses; reduction in pod length, pod width, pod weight, number of pod per plant , number of seed per pod, number of seed per plant, 100 seed weight. These yield loses are of great concern, hence the need to control the virus on *P. vulgaris*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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