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Sensitivity of *Amaranthus hybridus* L. Physiological Parameters to Telfairia mosaic virus Infection

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ABSTRACT

The sensitivity of Amaranthus hybridus physiological parameters to Telfairia mosaic virus infection was investigated. To achieve this, seeds of A. hybridus were obtained from plants monitored on the field for virus symptoms expression. The seeds were broadcasted on nursery beds, on germination seedlings were transplanted and inoculated with Telfairia mosaic virus. Sensitivity of physiological parameters was assessed by measuring effect of the virus on growth, yield, leaf nitrogen, relative water content and photosynthetic pigments at different stages of growth for 12 weeks after inoculation (WAI). Results revealed high sensitivity of growth and yield parameters of A. hybridus to TeMV infection with significant (P=0.05) reductions. Severe leaf size reduction of (58.8%), stem height (53.7%), number of primary shoot (50.8%) and number of leaves produced (69.9%), leaf fresh weight (56.9%), leaf dry weight (58.0%) at 12 WAI. Shoot fresh weight and root dry weight had reductions of 20.3% and 20.1% at 13 WAI. Shoot DW and root FW revealed less sensitivity to TeMV infection. The virus caused reduction in relative growth rate of (42.0%), net assimilation rate (45.5%) at 4 WAI and leaf area ratio (33.1%) at 12 WAI. Leaf nitrogen content decreased with plant age in both healthy and infected plants with highest decrease of 56.1% at 2 WAI and lowest decrease of 37.1% at 12 WAI. The virus caused reduction in relative water content of 40.0% at 12 WAI. Photosynthetic pigments of A. hybridus were highly sensitive to TeMV infection with reduction in chlorophyll a of (50.8%), chlorophyll b (22.3%), chlorophyll a + b (40.6%) ratio of chlorophyll a/b (36.5%) at 12 WAI and carotenoids (55.1%) at 12 WAI. Chlorophyll a was more sensitive to TeMV than chlorophyll b. The sensitivity of A. hybridus to TeMV disrupted physiological processes which affected growth and yield with resultant effect on low income earnings of growers.

1. Introduction

Amaranthus hybridus L. (family: Amaranthaceae) is an herbaceous crop cultivated on a commercial scale in southern Nigeria. It is a crop of economic importance as it generates income to the growers both in the wet and especially in the dry season. Nutritionally, it possesses high amount of protein, fat, fibre, ash, mineral elements, vitamins and amino acids (Mofunanya *et al*., 2015) required for the maintenance of the body, growth, reproduction and health. In recent times, vegetables have been recognized as indispensable constituent in the diet of the people. The diet of the poor people of Nigeria and other developing countries of the world are dominated by highly starchy foods, to compensate nutrients deficit of starchy foods, demand for the cultivation of *A. hybridus* commonly called green vegetable has increased and production done on a sustainable basis to meet the dietary needs of the people.

However, the cultivation of this highly nutritious vegetable is limited by viral diseases. Amaranthus mosaic virus was reported in Lagos, Nigeria to be highly prevalent in commercially cultivated *A. hybridus* causing great economic losses (Taiwo and Owolabi, 2004b). Telfairia mosaic virus is another virus reported to cause reduction in the nutrients of *A. hybridus* (Mofunanya et al., 2015). Plants are highly sensitive to virus infection. Infected plants are sensitive to the multiplication of virus particles which alter physiological processes with effect on crop growth, yield, leaf pigments etc. Disturbances in growth often result in

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morphological abnormalities, manifested in symptoms such as mosaic, chlorotic, leaf distortion etc. Considering the nutritional potential of *A. hybridus* in improving the food needs of the people which are mainly starchy in nature and the economic value, there is need to study TeMV which is one of the viruses that limits its production. No information exists on the sensitivity of TeMV on growth, yield and other physiological parameters. The present study was conducted to elucidate the responses of *A. hybridus* to TeMV infection in order to ascertain its severity on some basic physiological processes with the virus control in view.

2. Materials and methods

2.1 Seed collection and planting

Seeds of *A. hybridus* used in this study were obtained from plants monitored on the farm for symptoms expression in the University Staff Quarters. The seeds were grown in the Department of Botany screenhouse located in the Botanical Garden of the University of Calabar, Nigeria (latitude 4.952'N and longitude 8.341'E) at 25±3^{0}C. Seeds were broadcasted on the nursery beds, on germination the young seedlings were transferred one seedling per polyethylene bag.

2.2 Inoculum preparation and plant inoculation

Telfairia mosaic virus isolate used was obtained from young symptomatic leaves of *Telfairia occidentalis* where the virus was maintained. The leaves were macerated in a sterilized mortar and pestle in disodium phosphate buffer 0.03 M, pH 8.0. The sap was then applied mechanically by conventional leaf-rub on *A. hybridus* dusted with carborundum (800-mesh). Before inoculation, plants to be inoculated and the healthy ones were arranged in a randomized block design (RBD) containing a total of 60 plants. Thirty plants were inoculated with the virus and the other thirty inoculated only with the buffer to serve as healthy. Plant virus inoculation was done at two leaf stage and the leaves rinsed with water and allowed to stay for symptom expression of mosaic, severe leaf malformation and distortion characteristics of TeMV infection.

2.3 Growth and yield parameters

Growth and yield parameters used in studying the sensitivity of *A. hybridus* to TeMV infection were; leaf area, shoot height, number of primary shoot, number of leaves produced, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), fresh and dry weight of leaves, shoots and roots. Initial measurement was taken 2 weeks after inoculation, and subsequently at four weeks interval for all parameters except for RGR, NAR and LAR. Leaf area measurement was taken by measuring the leaf length along the leaf midrib, from the base to apex excluding the petiole; and leaf width measuring perpendicularly to the midrib from one end of the leaf to the other. The readings obtained were then used to determine the leaf area (Erlacher *et al*. 2016). Shoot height was measured in (cm) from the soil level to the terminal bud. Leaves were counted to ascertain the number of leaves produced by *A. hybridus* inoculated with TeMV compared to control plants. At the end of experiment, the whole plant was uprooted, shoots and roots separated and weighed before drying to constant weight. Relative growth rate (RGR) was determined by measuring the changes in weight of inoculated and control samples. The change in weight was taken at various stages of growth. The differences between the samples were calculated (Atwell *et al*., 1999). Net assimilation rate (NAR): 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 inoculated and control. Leaf area ratio (LAR): The ratio of total leaf area to whole leaf dry weight over a period of time (4 weeks interval). Leaf fresh and dry weight: Leaves were harvested at 2, 4, 8 and 12 WAI, fresh weight measurements taken before drying to constant weight in a Hot Box Oven (Gallenkamp, CHF097 XX2.5, England).

Shoot and root fresh and dry weight: At the end of experiment (13 WAI), the shoots and roots of inoculated and control plants were harvested, weighed and dried in order to investigate their sensitivity to TeMV infection.

2.4 Relative water content determination

Leaves of the same physiological age and position on the plants were used for relative water content estimation. Control and inoculated leaf samples of *A. hybridus* were collected from experimental plots. Ten leaf pieces measuring 2 cm in diameter were cut and weighed immediately to determine the fresh weight (FW). Turgid weight (TW) was determined by weighing the leaf segments after which were immense in distilled water for 24 h in a sealed flask at room temperature. The dry weight (DW) was determined by weighing the leaves after drying in a Hot Box Oven (Gallenkamp, CHF097 XX2.5, England) at 70^{o}C for 48 hours (Efeoglu *et al*., 2009). The RWC (%) of each harvested leaf was calculated with the following formula:

$$RWC\ (\%) = FW\text{-}DW/TW\text{-}DW$$

Where, FW, DW and TW are the fresh, dry and turgid weights of tissue respectively

2.5 Chlorophyll and carotenoids estimation

To determine the sensitivity of leaf chlorophyll to TeMV infection, chlorophyll estimation was conducted using 0.2 g leaf samples obtained from healthy and infected plants of *A. hybridus* at different stages of growth. The leaves were placed in a mortar and macerated with the aid of a pestle. From the macerate, 4 ml of 80% acetone was mixed thoroughly with 2 ml ethanol (2:1 v/v) to ensure complete contact of plant material. The mixture was allowed to stand for 30 minutes in the freezer in the dark, centrifuged for 10 minutes at 2000 rpm. The test tubes were covered with aluminum foil and 5 ml of acetone/ethanol (2:1 v/v) was added and stirred for 1 minute. The blank was set with acetone/ethanol (2:1 v/v), absorbance readings taken with a spectrophotometer (Model 722S, England) at 663 nm and 645 nm for chlorophyll *a* and chlorophyll *b* in mg per g tissue. Readings obtained were substituted in the formulas described by Gu et al. (2016).

$$\text{Chlorophyll } a\ (mg\ g^{-1}) = (12.7 \times A663) - (2.59 \times A645)$$

$$\text{Chlorophyll } b\ (mg\ g^{-1}) = (22.9 \times A645) - (4.7 \times A663)$$

Where A663 and A645 are the absorbance measured at 663 and 645.

The fresh leaf extract prepared for chlorophyll estimation was used for carotenoids determination and absorbance measured at 450 nm, setting up equations using specific absorption coefficients for carotenoids. The amount of chlorophylls and carotenoids present in the extracts were calculated and expressed in mg per g tissue using the following equations:

mg carotenoids per g tissue = (4.07 x 450 nm) x (0.0435 x chl *a*) + (0.367 x chl *b*) x V x W

Where;

A = absorbance at specific wavelength

V = final volume of pigments extract in 80% acetone/ethanol

W = fresh weight of tissue

3. Results

3.1 Sensitivity of growth parameters per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

Growth parameters of *A. hybridus* were highly sensitive to TeMV infection as expressed by significant (*P=0.05*) reductions. The virus did not caused meaningful effect at initial period of 2 WAI effect however, became significant with prolonged infection. Results revealed that infected plants had lower reductions at different periods of growth than healthy plants. At 12 WAI, infected plants exhibited decrease in leaf area, shoot height, number of primary shoot and number of leaves produced with values of 32.78±0.03 cm^2, 28.48±0.01 cm, 3.11±0.01 and 19.19±0.01 respectively. Corresponding values for healthy plant were 79.55±0.01 cm^2, 61.50±0.01 cm, 6.32±0.01 and 63.8±0.01 (Table 1).

3.2 Sensitivity of leaf fresh and dry weight per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

Results depicted drastic reduction in yield orchestrated by the sensitivity of *A. hybridus* to TeMV at the different periods of growth. Table 2 presented results of leaf biomass sensitivity to Telfairia mosaic virus infection. There was a significant (*P=0.05*) gradual reduction in leaf biomass of *A. hybridus* at different stages of virus infection. The LFW and LDW of virus infected plant at 8 WAI were 23.05±0.0 g and 5.65±0.01 g compared to control values of 59.86±0.012 g and 10.19±0.01 g.

3.3 Sensitivity of shoot and root per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

At the end of the study, the shoots and roots were harvested, weighed and analyzed. The virus caused significant (*P=0.05*) decrease in shoot FW per plant of 29.65±0.01 g as against healthy plant of 37.21±0.01 g. Infected root DW had value of 1.67±0.01 g compared to healthy plant value of 2.09±0.01 g. However, decrease in shoot DW and root FW was not significant (Table 3).

3.4 Sensitivity of relative growth rate, net assimilation rate and leaf area ratio per plant *of Amaranthus hybridus* to Telfairia mosaic virus infection

Telfairia mosaic virus affected the growth and development of *A. hybridus* with significant (*P=0.05*) reduction in components. Sensitivity of the vegetable to TeMV led to decrease in RGR, NAR and LAR per plant. Highest sensitivity to TeMV was accompanied by highest reduction in RGR and NAR which occurred at 4 WAI and at 12 WAI for LAR. Highest mean reduction value for RGR for infected plant was 0.0069 ± 5.8E-07 in comparison to the healthy value of 0.0119 ± 1.53E-06 gg^{-1} day^{-1}. Corresponding reduction in NAR for infected plant was 0.00054 ± 5.77E-07 as against healthy plant of 0.00099 ± 5.53E-07 g cm^{-2} day^{-1}. Infected plant had mean reduction for LAR of 17.56 ± 0.06 when compared to the healthy value of 26.24 ± 0.00 cm^{-2} g^{-1} (Table 4).

3.5 Sensitivity of relative water content and leaf nitrogen content per plant of of *Amaranthus hybridus* to Telfairia mosaic virus infection

Leaf nitrogen and RWC were severely decreased *A. hybridus* due to sensitivity to TeMV infection. Decrease in leaf nitrogen was highest at initial stage of growth (2 WAI) and lowest at the later stage (12 WAI). Relative water content content showed a gradual decrease with increasing period of growth. Mean reduction in leaf nitrogen per plant at 2 WAI and 12 WAI for infected plants were 30.23 ± 0.01 and 19.24 ± 0.01 as against healthy plants values of 68.79 ± 0.01 and 30.58 ± 0.01. At 12 WAI, RWC depicted decrease in infected plants of 50.74 ± 0.01 in comparison to 84.61 ± 0.01 for healthy plants (Table 5).

3.6 Sensitivity of photosynthetic pigments per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

Photosynthetic pigments of *A. hybridus* showed high sensitivity to TeMV infection. Chlorophyll *a, b*, and carotenoids decreased in content with prolonged period of infection. In both healthy and infected plants, pigments content increased with progressive periods of development but were considerably lower in infected *A. hybridus* compared to healthy plant. Mean reduction in chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, ratio of chlorophyll *a*/*b* per plant at 12 WAI for infected plants were 2.69 ±0.01, 2.39±0.01, 5.08 ±0.01 and 5.08 ±0.01 mg g^{-1} FW respectively. Corresponding mean reduction for healthy plants were 5.47 ± 0.02, 3.08 ±0.01, 8.55 ± 0.02 and 8.55 ±0.02 mg g^{-1} FW. Carotenoids were significantly (*P=0.05*) decreased due to its sensitivity to TeMV infection with mean value for infected plants of 0.035 ± 0.01 mg g^{-1} FW when compared to healthy value of 0.078 ±0.01 mg g^{-1} FW at 12 WAI (Table 6).

4. Discussion

Growth parameters of *A. hybridus* showed high sensitivity to TeMV infection. The vegetable suffered reduction in leaf area, shoot height, number of primary shoot branches, number of leaves produced, leaf fresh and dry weight and fresh and dry weight of shoot and root due to its sensitivity to the virus. Growth parameters reductions by TeMV influenced performance of major plant organs (leaf, stem and root). Decrease in leaf area affected leaf size by

Table 1: Sensitivity of growth parameters per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

WAI	Leaf area (cm^2)		Shoot height (cm)		Number of primary shoot		Number of leaves produced	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
2	4.10±0.02	4.07±0.01	5.80±0.01	5.69±0.00*	0.00±0.00	0.00±0.00	2.12±0.01	2.12±0.01
4	14.29±0.01	10.31±0.01*	13.23±0.01	9.56±0.02*	2.65±0.01	2.1±0.01*	7.64±0.01	5.41±0.01*
8	50.73±0.01	23.85±0.01*	49.69±0.01	19.27±0.01*	4.58±0.00	2.21±0.01*	36.91±0.01	12.48±0.01*
12	79.55±0.01	32.78±0.01*	61.50±0.01	25.48±0.01*	6.32±0.01	3.11±0.01*	63.8±0.01	25.19±0.01*

Values are Mean ± SD, * = Significant, n=3, *P=0.05*

Table 2: Sensitivity of leaf fresh and dry weight per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

WAI	Leaf fresh weight (LFW) (g)		Leaf dry weight (LDW) (g)	
	Healthy	Infected	Healthy	Infected
2	2.08±0.02	2.07±0.01	0.05±8.5E-18	0.05±8.5E-18
4	8.47±0.01	7.13±0.01*	1.08±0.01	1.05±0.02
8	59.86±0.01	23.05±0.00*	10.19±0.01	5.65±0.01*
12	69.29±0.01	29.84±0.01*	14.55±0.01	6.1±0.01*

Values are Mean±SD, * = Significant, n=3, *P=0.05*

Table 3: Sensitivity of shoot and root per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

WAI	Plant	Shoot (g)		Root (g)	
		Fresh weight	Dry weight	Fresh weight	Dry weight
13	Healthy	37.21±0.01*	11.07±0.11	16.83±1.73	2.09±0.01*
	Infected	29.65±0.01*	10.30±0.02	13.59±0.01	1.67±0.01*

Values are Mean±SD, * = Significant, n=3, *P=0.05*

Table 4: Sensitivity of relative growth rate, net assimilation rate and leaf area ratio per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

WAI	RGR (gg^{-1} day^{-1})		NAR (g cm^{-2} day^{-1})		LAR (cm^{-2} g^{-1})	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
4	0.0119 ± 1.53E-06	0.0069 ± 5.8E-07*	0.00099 ± 5.53E-07	0.00054 ± 5.77E-07*	13.65 ± 0.06	12.17 ± 0.06*
8	0.0074 ± 3.72E-06	0.0048 ± 1.73E-06*	0.00057 ± 0.0000	0.00039 ± 5.77E-07*	18.91 ± 0.06	13.11 ± 0.06*
12	0.0052 ± 0.000001	0.0037 ± 0.000001c*	0.00038 ± 2.08E-06	0.00028 ± 5.77E-07*	26.24 ± 0.06	17.56 ± 0.06*

Values are Mean±SD, * = Significant, n=3, *P=0.05*

Table 5: Sensitivity of relative water content and leaf nitrogen content per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection

WAI	Leaf N (%)		RWC (%)	
	Healthy	Infected	Healthy	Infected
2	68.79 ± 0.01	30.23 ± 0.01*	49.40 ± 0.02	49.38 ± 0.01
4	60.35 ± 0.01	27.76 ± 0.01*	60.47 ± 0.02	50.10 ± 0,01*
8	44.26 ± 0.02	21.65 ± 0.02*	79.98 ± 0.01	51.32 ± 0.02*
12	30.58 ± 0.01	19.24 ± 0.01*	84.61 ± 0.01	50.74 ± 0.01*

Values are Mean±SD, * = Significant, n=3, *P=0.05*

Table 6: Sensitivity of photosynthetic pigments per plant of *Amaranthus hybridus* to Telfairia mosaic virus infection (mg g^{-1} FW)

WAI	Chl a		Chl b		Chl a + chl b		Ratio of chl a/b		Carotenoids	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
2	2.67 ± 0.01	2.53 ±0.01	2.43 ± 0.01	2.23±0.02	5.10 ±0.01	4.76 ±0.01	1.18 ±0.01	1.13± 0.02	0.040±0.01	0.030±0.01*
4	4.58 ± 0.01	2.56 ±0.01*	2.73 ±0.01	2.28±0.01*	7.31 ±0.01	4.84 ±0.02*	1.68 ±0.02	1.12±0.01*	0.047 ±0.01	0.032±0 .02*
8	4.93 ± 0.01	2.67 ±0.02*	2.93 ±0.01	2.36±0.01*	7.86 ±0.02	5.03 ±0.01*	1.68 ±0.02	1.13 ±0.01*	0.059 ±0.01	0.034±0.02*
12	5.47 ± 0.02	2.69 ±0.01*	3.08 ±0.01	2.39±0.01*	8.55 ±0.02	5.08 ±0.01*	1.78 ±0.01	1.13 ±0.01*	0.078 ±0.01	0.035±0.01*

Values are Mean±SD, * = Significant, n=3, *P=0.05*

decreasing the leaf surface area for light absorption with a resultant decrease in Photosynthesis. Investigation on sensitivity of *A. hybridus* revealed significant reduction in growth and yield components following infection with TeMV. Report of shoot height reduction in the present study is in line with earlier report of decrease in height of sweet potato infected with Sweet potato feathery mottle virus (SPFMV) and Sweet potato chlorotic stunt virus (SPCSV) (Wang *et al*., 2019). Ullah *et al.* (2017) observed a similar trend of reduction in yield contributing parameters such as plant height, fresh shoot and root weight, dry shoot and root weight in susceptible tomato genotypes infected with Tomato mosaic virus (ToMV).

Relative growth rate, NAR and LAR were highly sensitive to TeMV infection depicted by reduction in contents. These findings are in agreement with reports of reduction in growth and yield parameters of *A. viridis* inoculated with TeMV (Mofunanya *et al*., 2021). Relative growth rate, NAR and LAR are physiological parameters that measure the actual growth of plants was adversely affected. Relative growth rate of a plant is the product of LAR which is leaf area per unit total plant biomass and NAR. Growth in leaf mass can result from an increase in leaf area growth or thickness. Healthy plants of *A. hybridus* had higher maximum relative growth rates than infected ones. Net assimilation rate is the increase in plant dry weight per unit leaf area and unit time. Leaf area ratio is the ratio between total leaf area and total plant weight, and RGR. The variation in these parameters due to TeMV infection affected whole plant functioning as manifested by decrease in performance. Atwell *et al.* (1999) observed that variation in LAR frequently has more direct impact on whole-plant growth than variation in NAR. The driving variable for whole plant growth, and the proportion of plant biomass invested in leaf area or leafiness have an important bearing on RGR. The sensitivity of *A. hybridus* to TeMV engendered severe reduction in leaf biomass yield. Yield loses in this study was attributed to reduction in leaf area, number of leaves produced, LFW, LDW, RGR, NAR and LAR which are indices of leaf biomass yield. Reduction in these parameters affected photosynthetic activities, altered energy production which resulted in stunted growth and poor yield depicting the link between vegetative growth and yield. *Amaranthus hybridus* constitutes a major part of the diets of the Nigerian people as the leaves and young stems are used in the preparation of soup, stew, yam and rice thereby improving the food needs of the people which are mainly starchy in nature. Reduction in physiological parameters found in this study is disturbing for growers because this vegetable is cultivated mainly for its leaves which are sole and serve as a source of income generation for rural farmers and urban growers. Reduction implies poor earning by growers whose income is anchored to the production of large quantity of leaves which are sold for money. Low quantity amount to low income earning, high quantity high income earning. *Amaranthus hybridus* is a vegetable of high economic and nutritional value widely grown and consumed in Nigeria. Plants are highly sensitive to virus infection manifested by several changes in whole plant physiology related to growth and productivity.

Other physiological parameters such as RWC, leaf nitrogen, chlorophyll *a*, chlorophyll *b* and carotenoids showed high sensitivity to TeMV manifested by decrease in contents. Sensitivity of the RWC of *A. hybridus* to TeMV led to lower amount in infected when compared to the healthy. This result is in accordance with report of lower water content in chronically infected plants (Hull, 2002). Water loss from the leaves is one of the most common physiological parameters limiting efficiency of photosynthesis and biomass productivity in plants. The vegetable is a crop with high potential for biomass productivity. Sustaining RWC at optimal levels strengthens the crop to withstand constraints in its growing environment thus contributing to good yield. The turgidity of vegetative parts is very important in achieving efficient growth and development. Decrease in RWC is a sensitive indicator to plant growth. Leaf nitrogen content was adversely affected by the sensitivity of *A. hybridus* to TeMV infection. Nitrogen is an integral component of chlorophyll. Chlorophyll molecule comprised of four atoms of nitrogen, changes in leaf chlorophyll content has a direct effect on leaf nitrogen. Telfairia mosaic virus caused a decrease in leaf chlorophyll content as well as leaf nitrogen content affecting the photosynthetic capacity of *A hybridus*. Decrease in leaf-N content of infected plants affected leaf greenness thereby affecting yield.

Due to the sensitivity of *A. hybridus* to TeMV infection, photosynthetic pigments (Chlorophyll *a*, *b*, carotenoids) were severely reduced. A similar report of reduction in photosynthetic pigments in chilli plant by Cucumber mosaic virus was found (Rahman *et al.,* 2019). Leaf chlorophyll concentration provides information about the physiological well being of the plant. These reductions became obvious due to severe mosaic symptom characteristic of TeMV. Changes in biochemical compounds such as chlorophyll *a*, *b*, carotenoids and leaf nitrogen disrupted the physiological processes of *A. hybridus*. These changes were due to virus particles multiplication upon infection of *A. hybridus*. Virus infection affects the process of photosynthesis by decreasing photosynthetic pigments which play significant role in carbon fixation. Decrease in pigments affected the light absorption ability of *A. hybridus* which is key to photosynthesis. Leaf chlorophyll level is directly related to stress physiology. Chlorophyll *a* and *b* are the two most important pigments in photosynthetic processes, and low levels of these pigments due to TeMV infection have a direct effect on the process of photosynthesis, low pigment levels drastically reduces the process, consequently, poor plant performance and low crop productivity. Leaf chlorophyll concentration measures the degree of greenness of leaves of crops. The sensitivity of *A. hybridus* chlorophyll pigment which gives green colour to leaves in this study occurred as a result of severe mosaic symptoms of TeMV on the leaves, inhibition of chlorophyll synthesis and chloroplast destruction. Carotenoids pigments play important roles in light harvesting, photosynthesis, photoprotection, pollinator attraction and phytohormone production. Carotenoids are also precursors for abscisic acid [Crozier *et al*., 2000], strigolactone synthesis, shoot branching and signaling (Yoneyama *et al.,* 2018). Reduction in leaf biomass yield in this study may be attributed to reduction in chlorophyll *a* which is the principal light absorbing pigment in photosynthesis. This is the first report of *A. hybridus* physiological changes due to TeMV infection. Owing to reduction in yield of *A. hybridus*, a vegetable of high economic value, due to its sensitivity to TeMV, control of the virus is inevitable.

5. Conclusion

Amaranthus hybridus exhibited high degree of sensitivity to TeMV infection with reduction in growth and yield, leaf nitrogen, relative water content, chlorophyll *a*, chlorophyll *b* and carotenoids. At initial period of infection, the virus did not cause any meaningful effect on all physiological parameters investigated except for RGR, NAR and photosynthetic pigments. Infected plants depicted gradual decrease in leaf area, shoot height, number of primary shoot branches, and number of leaves produced, leaf fresh and dry weight, RGR, NAR and LAR compared to healthy plants. The dry weight of shoot and fresh weight of *A. hybridus* root were less sensitive to TeMV infection. This reduction in yield is of great concern, the focus should be on the virus control to salvage *A. hybridus* from economic and nutritional loses.

6. Conflict of interest

Authors have declared that no conflict of interest exists.

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