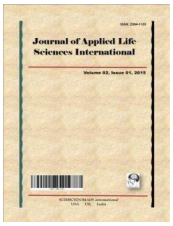

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Changes in Some Enzymes Activities of *Amaranthus viridis* L. Inoculated with *Telfairia Mosaic Virus* (TeMV)

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Authors' contributions

This work was carried out between the authors. Author AAJM designed the study, managed the literature search, handled the data and wrote the manuscript. Author ATO performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Investigations were carried out to assess changes in some enzymes activities of *Amaranthus viridis (A. viridis)* inoculated with *Telfairia mosaic virus* (TeMV).
Methodology: Symptoms of *Telfairia mosaic virus* (TeMV) are characterized by severe mosaic and leaf malformation. Healthy and inoculated leaves of *A. viridis* were assayed for changes in peroxidase (POD), polyphenol oxidase (PPO), glucose-6-phosphate dehydrogenase (G6PdH) and 6-phosphogluconate dehydrogenase (6PGdH) activities spectrophotometrically at wavelengths appropriate for each enzyme for a period of 120 days after inoculation (DAI). Activities of enzymes in TeMV inoculated and healthy plants of *A. viridis* were measured in μg^{-1} FW and U/L.
Results: Results revealed a gradual increase in activities of POD and PPO from 14 - 60 DAI, followed by a decline at 90 DAI and a subsequent increase at 120 DAI in inoculated plants while G6PdH and 6PGdH activities showed gradual increase from 14 – 30 DAI, followed by a decrease from 60 – 90 DAI and subsequent increase at 120 DAI. Activities of POD, PPO, G6PdH and 6PGdH

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at all periods of growth were significantly (P = 0.05) higher in inoculated plants compared to the healthy. Results also revealed that activities of all enzymes in U/L were significantly higher than activities in μg^{-1} FW. Fluctuation in all assayed enzymes activities was observed in inoculated samples. Highest percentage increases in enzymes activities in μg^{-1}FW for POD, PPO, G6PdH and 6PGdH were 54.3%, 45.3%, 55.3% and 44.6% respectively. Corresponding activities in U/L were 81.2%, 86.2%, 91.3% and 87.0%.

Conclusion: Activities of all enzymes were found to increase in inoculated leaves due to TeMV infection when compared to healthy leaves. Changes induced by TeMV infection led to increase and fluctuation in all enzymes activities with alterations in physiological processes of *A. viridis* climaxing to poor growth and reduction in yield.

Keywords: Enzymes activities; Amaranthus viridis; Telfairia mosaic virus (TeMV).

1. INTRODUCTION

Plants in their natural environment are constantly being confronted by different pathogenic microorganisms such as fungi, bacteria, nematodes, viroids and viruses [1,2]. In plant-virus interaction, the earliest response of the plant cell to pathogen or biotic stress is the production of reactive oxygen species (ROS) [3], which play a crucial role in pathogenesis. Oxidative stress in plant-virus combination is manifested as increase in various physiological processes [4]. Biotic stress has been associated with several alterations in plant metabolic activities. The infection of a plant by a virus results in disturbances in the host plant metabolism that are accompanied by the development of characteristics disease symptoms.

Changes in the activities of enzymes have been reported in various host-virus combinations [5], [6]. Decrease in enzymes activities have been reported in a few cases while majority of the reports pointed to increased in enzymes activities [5]. Viral infection promotes many changes in the protein profile of plants [6]. The infection of sweet oranges by Citrus tristeza virus (CTV) resulted in increased accumulation of some proteins involved with stress and defense responses against pathogens. The protein related to stress was found to accumulate after virus infection [6]. Protein is activated when the plant is submitted to pathogen attacks [7].

Higher PPO activity in lettuce plants infected by Lettuce mosaic virus (LMV) has been reported [8]. Infection of *Cucumber pepo* L. by Cucumber mosaic virus (CMV) produced a stimulatory effect on the capacity for oxidative pentose phosphate pathway G6PdH and 6PGdH activities, glycolysis (ATP and pyrophosphate-dependent phosho-kinases), anaplerotic reactions (NAD-dependent malic enzyme, phosphenol pyruvate carboxylase) and oxidative electron transport (cytochrome c. oxidase) [9]. Plum pox virus (PPV) caused a decrease in some antioxidant enzymes with increase in Class 1 ascorbate peroxidase (APX), peroxidase (POX as designated in this study) and polyphenol oxidase (PPO) enzymes in *Prunus* species [10]. [11] Studied the effect of Yellow mosaic virus (YMV) on pathogenetic related enzymes in mothbean and reported an increase in the activities of POD, PPO, phenylalanine ammonia lyase and tyrosine ammonia lyase with a decrease in catalase activity in leaves of inoculated plants. Effect of Potato virus Y on the NADP-Malic enzyme of *Nicitiana tabacum* led to increase in enzyme activity of infected leaves [12]. The concentration of DG6PdH was found to be enhanced in PVY^{NTN} at maximum symptom development. On the contrary, substrates of NADP-dependent enzymes (L- malate and DG6P) were approximately 2-4 folds respectively higher in PVY^{NTN} infected *N. tabacum* leaves; concentration of D-glucose was similar in infected and healthy plants [12]. Citrus tristeza virus (CTV) infection induced an increase in oxidative stress, leading to higher activity in antioxidant enzymes compared to the uninfected [13]. Infection of *Telfairia occidentalis* byTelfairia mosaic virus (TeMV) engendered significant increase in enzymes activities of POD, PPO, G6PdH and 6PGdH [5].

Polyphenol oxidase (EC 1.10.3.1) is an important enzyme found in most plant species [14] that catalyzes the oxidation of mono, di and polyphydric phenols into quinones [15] with the concomitant reduction of oxygen into water which results in protein complexing and the formation of brown melanin pigments. The most frequently suggested role for polyphenol oxidase (PPO) in plants has been in defense against herbivore and pathogens [14]. Plant peroxidases (EC 1.11.1.7) catalyze various reactions in plant growth and development. There have been a number of

reports confirming the involvement of peroxidases in protective plant metabolism [16]. Peroxidases are key enzymes of lignin biosynthesis and important pathogenesis-related proteins (PR-proteins). Defense responses in plants are characterized by the early accumulation of phenolic compounds at the site of infection and the slowing down of pathogen development through rapid cell death. A role for phenolics and phenol oxidizing enzymes like POD and PPO in plant resistance against viral diseases has been implicated by several investigators [2].

Glucose-6-phosphate dehydrogenase (G6PdH EC 1.1.1. 49) and 6-phophogluconate dehydrogenase (6PGdH EC 1.1.1.44) are vital enzymes of the oxidative pentose phosphate pathway (PPP) responsible for the production of NADPH. The production of NADPH in plants is necessary for biosynthesis of specific defense compounds (like phytoalexins, flavonoids, lignins, compounds active in osmosis) which is important under stress [17] or act as cofactor for antioxidative enzymes [18]. NADPH is an energy molecule useful in many pathways including cycle like glutathione-ascorbate needed in the defense against oxidative stress in plants and for the production of ATP (energy molecule utilized for cellular processes) through the cytochrome system, which moves the biosynthetic processes requiring energy [19], including the polymerization of the bases needed for viral RNA synthesis. G6PdH isozymes have been reported to be present in the cytosol and plastid [20]. Isozymes actions have also been reported to lead to the formation of ribulose-5-phosphate an essential raw material for incorporation into viral genome [21].

Amaranthus viridis L. also called slender amaranth belongs to the family Amaranthaceae. It is eaten traditionally as a vegetable in Nigeria and other parts of Africa [22] and is commonly known as '*Tete'* among the Yorubas in Nigeria. The leaves have been used in dishes such as *mas huni* in the diet of the Maldives for centuries [23]. *A. viridis* has been a vital item of Australia food since the 19th Century. In 1889 a botanist Joseph Maiden wrote: ''It is an excellent substitute for spinach, being far superior to the leaves of the white beet sold for spinach in Sydney. The seeds of *A. viridis* can be eaten as snacks or used in biscuits. Seeds can also be used in making porridge by boiling seeds in water. Unlike other amaranth the seeds of *A. viridis* can be harvested by scraping the ripe spikes between the fingers [24]. Amaranthaceae family is one of the susceptible hosts to Telfairia mosaic virus (TeMV) with characteristics symptoms of stunting growth and chlorosis etc. [25]. Viral disease is one of the main causes for decreased crop productivity worldwide [26]. In view of the importance of this vegetable in the diet of the Nigerian people, the present study seek to investigate the changes in some oxidative stress enzymes (peroxidase and polyphenol oxidase) and enzymes of the oxidative pentose phosphate pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) activities which are growth indices of *Amaranthus viridis* L. inoculated with Telfairia mosaic virus (TeMV).

2. MATERIALS AND METHODS

2.1 Virus Source and Propagation

Telfairia mosaic virus (TeMV) used in this study was the isolate described by [25]. The TeMV infected leaf tissues stored under liquid nitrogen was reactivated by triturating the leaf tissues in pre-sterilized cold pestle and mortar in sodium phosphate (Na_2HPO_4) buffer 0.03 M, pH 8.0. The inoculums was applied by conventional leaf-rub method (mechanical or sap inoculation). With cotton swab onto *Nicotiana benthamiana*, dusted with 800 mesh carborundum. The leaves were rinsed with water after inoculation and observed for symptom expression. Subsequent inoculations using the sap transmission method were carried out on *T. occidentalis* in order to propagate and maintain the virus under screen-house at 23 ± 3°C.

2.2 Seed Planting and TeMV Inoculation

Seeds were purchased from local farmers in Akparabong Town, Ikom Local Government Area of Cross River State Nigeria. The seeds were dried for two days and scattered on polyethylene bags (16 cm in diameter) filled with steam sterilized garden soil. Seeds germinated and were inoculated with the virus at three leaf stage. The inoculum was prepared from young symptomatic leaves of *T. occidentalis* and applied on *A. viridis* dusted with 800 mesh carborundum at three leaves stage by mechanical inoculation. The leaves of the inoculated seedlings were rinsed with water and allowed for symptom development while buffer was applied on the healthy plants.

2.3 Enzyme Extraction

Two grams of fresh weight of leaf tissues of inoculated and healthy plants of *A. viridis* were harvested randomly from experimental plots (at 14, 30, 60, 90 and 120 DAI) and homogenized using laboratory mortar and pestle in 10 ml of extraction buffer. The extraction buffer consisted of 100 mM mixed monobasic potassium phosphate salt (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4), to this was added 4 g of phenolic adsorbent polyvinyl polypyrrolidone (PVPP). The buffer was thoroughly stirred with the aid of a magnetic stirrer and adjusted to final pH of 7.2. Extraction was carried out at 4°C. The homogenate filtered through cheese cloth and the filtrate centrifuged at 5000 rpm for 5 minutes. The supernatant was stored on ice block in boxes and used as crude enzyme source in assaying for POD and PPO.

2.4 Enzyme Purification

To 2 ml of crude enzyme extract was added 2 ml of cold acetone. The mixture was allowed to stand for five minutes and then centrifuged at 5000 rpm for 5 minutes. The precipitate formed was resuspended in 1 ml cold assay buffer (KH_2PO_4 and K_2HPO_4) as partially purified enzyme filtrate. Four mills of this partially purified enzyme filtrate was used to commence the purification process. To this 4 ml was added 0.8 g ammonium sulphate salt and stirred with the aid of a magnetic stirrer for 10 minutes and the content poured into a test tube and centrifuged. After centrifugation the filtrate was decanted and the precipitate discarded. To the filtrate was added 2 g of ammonium sulphate and stirred for 10 minutes. The content was again poured into the test tube and centrifuged at 5000 rpm for another 5 minutes. The filtrate was decanted and the precipitate used for purification. The assay buffer 0.5 ml was then added and used in assaying POD and PPO.

2.4.1 Assay for peroxidase (POD)

Assay buffer for peroxidase activity consisted of 50 mM of mixed phosphate buffer containing 0.7 g of KH_2PO_4, 0.9 g of K_2HPO_4, pH 7.2. The assay mixture contained in a final volume of 3.3 ml, made up of 3 ml of assay buffer, 0.1 ml of 10 ml guaiacol and 0.1 ml enzyme preparation. The reaction was initiated with the addition of 0.1 ml of 10 ml H_2O_2. Absorbance readings were taken after 1 minute at 436 nm spectrophotometrically with the assay buffer as blank. The POD activity expressed was calculated using an extinction coefficient of 6.39 $mol^{-1}cm^{-1}$ for guaiacol dehydrogenation product [27].

2.4.2 Assay for polyphenol oxidase (PPO)

To 3 ml of assay buffer (50 mM mixed phosphate salts, pH 7.2) in a cuvette was added 0.1 ml of 10 mM dihydroxyphenylalanine (DOPA) and 0.1 ml enzyme preparation. The reaction was started with the addition of 0.1 ml of 10 mM H_2O_2 in a final volume of 3.3 ml. The absorbance of the mixture was measured after 1 minute at a wavelength of 470 nm [28]. The activity of PPO was calculated using an extinction coefficient of 1433 $nMcm^{-1}$ for the quinine product [29].

2.4.3 Assay for G6PdH EC1.1.1.44 and 6PGdH EC 1.1.1.49 activities

All enzyme assays were carried out spectrophotometrically at 3°C following the reduction of NADP at 340 nm as described by [30]. The assay mixtures consisted of 100 ml triethanolamine hydrochloride-NaOH. (pH 7.5), 10 ml $MgCl_2$, 10 ml NADP, 10 ml 6-phosphogluconate acid or glucose 6-phosphate and 5 ml of crude enzyme preparation. To 2 ml of assay buffer was added 0.1 ml of 0.8 mM 6-phosphogluconate or 0.1 ml of 0.83 mM glucose-6-phosphate, 0.1 ml of 12.5 mM $MgCl_2$, NADP and 0.1 ml of crude enzyme preparation added last to start the reaction in a final volume of 2.4 ml and read spectrophotometrically at 430 nm for inoculated as well as healthy plants of *A. viridis* in μg^{-1}FW and U/L. Readings were taken at 4 minutes. All G6PdH activities were corrected for the possible complicating effect of 6PGdH on the G6PdH assays using the method of [31].

2.5 Statistical Analysis

Data obtained in this study were analyzed by using the independent t-test. Results were also expressed as percentage differences and differences between means were determined at 5% probability level.

3. RESULTS

3.1 Changes in POD Activity of *Amaranthus viridis* Inoculated with Telfairia Mosaic Virus (TeMV)

Significant (P = 0.05) changes in POD activities in μg^{-1}FW and U/L are presented in Table 1. Results revealed higher POD activity of

inoculated plants at all periods of growth compared to the healthy. Peroxidase activity at 14 DAI had value of 255.17±0.0006 μg^{-1}FW for inoculated plants compared to healthy value of 186.62± 0.003 μg^{-1}FW. There was a progressive increase in POD activity of inoculated plants measured in μg^{-1}FW up to 60 DAI, followed by a decrease in activity at 90 DAI and a subsequent increase at 120 DAI. Peroxidase activity measured in U/L also revealed a similar trend of fluctuation in activity. Highest POD activity occurred at 60 DAI with highest percentage reduction of 54.3% and 81.2% for activity measured in μg^{-1}FW and U/L. Inoculated plants had initial increase in POD activity at 14 DAI of 5611.79 ± 0.0002 U/L compared to value of 3345.8 ± 0.0003 U/L for healthy plants. Fluctuation in POD activities measured in both μg^{-1}FW and U/L were observed with activity in U/L being higher than activity in μg^{-1}FW. Values of POD activities obtained for inoculated plants irrespective of the period of development were significantly higher (P = 0.05) compared with the healthy.

3.2 Changes in PPO Activities in μg^{-1}FW and U/L of *Amaranthus viridis* Inoculated with Telfairia Mosaic Virus (TeMV)

Results revealed a significant (P =0.05) gradual rise in PPO activity in μg^{-1}FW from 14 DAI with mean value increase of 2.557 ± 0.0003 μg^{-1}FW for inoculated compared to healthy value of 2.110 ± 0.0002 μg^{-1} FW up to 60 DAI with highest activity value of 3.201 ± 0.0001 and 2.203 ± 0.0003 μg^{-1}FW for inoculated and healthy plant samples. This was followed by a decline in activity at 90 DAI of inoculated plant sample with value of 3.001 ± 0.0001 μg^{-1} FW and a subsequent increase at 120 DAI with value of 3.119 ± 0.0001 μg^{-1} FW compared to healthy plants value of 2.201 ± 0.0006 μg^{-1} FW. PPO activity in U/L showed a similar trend of fluctuation in activity measured in μg^{-1}FW and U/L with percentage reductions at 14, 30, 60, 90 and 120 DAI of 67.8%, 72.9%, 86.2%, 70.6% and 73.2% respectively (Table 2). Reduction in PPO activity was higher in U/L than in μg^{-1} FW.

3.3 Changes in 6PGdH Activities in μg^{-1} FW and U/L of *Amaranthus viridis* Inoculated with Telfairia Mosaic Virus (TeMV)

Results of changes induced by TeMV on 6PGdH activities measured in μg^{-1}FW and U/L are presented in Table 3. For inoculated plant, 6PGdH activities in μg^{-1}FW and U/L exhibited significant (P = 05) increases at all stages of development compared to healthy samples. There was increase in 6PGdH activities in μg^{-1}FW and U/L from 14 to 30 DAI, followed by a decline from 60 to 90 DAI and a gradual increase at 120 DAI. Changes in 6PGdH activities in μg^{-1}FW at 30 DAI had significant (P =0.05) increase in value of 4.10 ± 0.006 μg^{-1}FW compared to mean value for healthy plants of 2.64± 0.006 μg^{-1}FW. Activity of 6PGdH in μg^{-1} FW at 60 and 90 DAI did not differ significantly in inoculated plants. Changes in 6PGdH activity at later stage of growth (120 DAI) revealed a significant increase in inoculated plants with value of 3.57 ± 0.003 μg^{-1} FW compared to value of 2.65 ±0.003 μg^{-1} FW for healthy plants. Results of 6PGdH activity in U/L presented a similar pattern. Fluctuation in the activity of 6PGdH in U/L revealed percentage increase of 67.1%, 72.9%, 81.2%, 75.2% and 79.8% for 14, 30, 60, 90 and 120 DAI.

3.4 Changes in G6PdH Activities in μg^{-1} FW and U/L of *Amaranthus viridis* L. Inoculated with Telfairia Mosaic Virus (TeMV)

Results revealed a general increase in G6PdH activity of inoculated plants when compared to the healthy plants. Results also revealed more increase in enzyme activity in U/L than in μg^{-1}FW. Inoculated plants showed a rise in G6PdH activity in μg^{-1}FW from 14 to 30 DAI with a decline in activity from 60 to 90 DAI. There was a gradual increase in G6PdH activity at a later period of growth (120 DAI). Highest enzyme activity was observed at 30 DAI with value for inoculated plants of 70.56 ± 0.01 μg^{-1}FW compared to value of 48.79 ± 0.006 μg^{-1}FW for healthy plants. At later period of growth a rise in enzyme activity of 70.50 ± 0.01 μg^{-1}FW was observed for inoculated plants compared to healthy plants activity of 50.64 ± 0.006 μg^{-1}FW. Activity in U/L followed a similar trend with highest activity observed at 30 DAI and lowest activity at 90 DAI. Fluctuation in G6PdH activity in U/L revealed percentage reduction in activity of 60.6%, 74.8%, 87.0%, 55.7% and 62.3% at 14, 30, 60, 90 and 120 DAI respectively (Table 4).

4. DISCUSSION

Changes in the activities of some (POD, PPO, G6PdH and 6PGdH) enzymes of *Amaranthus*

viridis inoculated with TeMV were investigated over a 120 days after inoculation. Telfairia mosaic virus (TeMV) infection of *A. viridis* engendered significant increase in enzymes activities of POD and PPO in comparison to healthy plants. Results revealed significant changes with increase in the activities of these enzymes for inoculated plants compared to the healthy. Peroxidase and polyphenol oxidase which are phenol-oxidizing enzymes have been reported to be associated with diseases [32]. [33] reported a significant rise in peroxidases activity in cucumber plants infected with Cucumber mosaic virus which coincided with virus symptoms emergence. In *Cucurbita moschata* infected with Yellow vein mosaic virus, substantial increase in the activities of superoxide dismutases, ascorbate peroxidase, guaiacol peroxidase and catalase was observed when compared to healthy control plants [34]. [2] reported enhanced activities of esterase, polyphenol oxidase and superoxide dismutase in Mesta plants infected with Yellow vein mosaic virus. A significant change was observed for Glutathione reductase, catalase and ascorbate peroxidase level in early stage of infection with begomovirus. A comparable analysis of GSH and GSSG content in infected and control plants of different ages indicated that plants are under oxidative or nitrosative stress [35]. Infection of *Telfairia occidentalis* by TeMV caused increase in POD and PPO activities [5]. Changes in POD activity has been found to be correlated to many physiological and morphological events; symptom severity. The increase in POD and PPO activities observed in *A. hybridus* plant infected with TeMV may be imputed to disease severity emerging from the prevention of oxidation of phenolics into quinones by PPO and peroxides detoxification by POD as virus symptoms depicted on the host plant became more severe with prolonged infection. Increase in the activity of PPO and accumulation of oxidized polyphenol products are common physiological changes reported in virus infected plants [36]. Polyphenol oxidases are widely considered as vital in the initial stage of plant defense after membrane damage provoking the release of phenols and quinones [37], which are lethal to pathogens [38], due to lignifications, suberization and polymerization of hydroxyl-proline-rich glycoproteins, regulation of cell wall and wound healing [39].

This investigation also revealed significant increase in the activities of G6PdH and 6PGdH which are enzymes of the oxidative pentose phosphate pathway for inoculated plants when compared to the healthy. This result is in agreement with earlier observation of increase in G6PdH and 6PGdH activities in marrow plants infected with Cucumber mosaic virus [9], in tobacco plants infected with Potato virus Y [40], in PVY and TMV infected tobacco plants [41], and in *Telfairia occidentalis* infected with Telfairia mosaic virus (TeMV) [5]. Increase in G6PdH and 6PGdH activities in this study may be attributed to attempt by these enzymes in *A. hybridus* inoculated plants to meet the increasing demand for energy for viral genome replication and coat protein synthesis and to compensate for decrease in photosynthesis, glycolysis, increase in respiration and reduced sugar level characteristic of virus infection of plants. G6PdH and 6PGdH are vital enzymes in the oxidative PPP through which NADPH is produced. G6PdH is a crucial enzyme for the maintenance of redox potential in cell leading to the production NADPH through the oxidative pentose phosphate pathway which is vital in glutathione reduction. According to [42], reduced glutathione protects the cell from oxidative damage by destroying hydrogen peroxide and peroxidase free radicals.

Findings of this study also revealed fluctuations in all enzymes activities of *A. viridis* inoculated with TeMV. Results revealed a trend of a gradual rise in enzymes activities from 14-60 DAI and a decline in activities at 90 DAI and a subsequent rise at 120 DAI was observed for POD and PPO. Fluctuations in these enzymes activities are threatening because plants vital functions are anchored on these enzymes. Fluctuation in activities of these enzymes may be attributed to the struggle by the virus to overcome the host defense. Similarly, G6PdH and 6PGdH enzymes showed an initial increase in activities from 14-30 DAI, followed by a decline in activities from 60-90 DAI and a subsequent rise at 120 DAI when compared to the healthy plants. The subsequent rise in the activities of these enzymes for inoculated plants could be ascribed to TeMV breaking through the host protein defense. In less virulent attacks, the plant may show capacity for recovery some days after inoculation [4] but in severe virus infection, it is difficult.

Table 1. Changes in peroxidase (POD) activities of *Amaranthus viridis* inoculated with Telfairia mosaic virus (TeMV)

DAI	µg-1FW			U/L		
	Inoculated	Healthy	(%) Difference	Inoculated	Healthy	(%) Difference
14	255.17 ±0.0006*	186.62± 0.003	36.7	5611.79 ± 0.0002*	3345.8 ± 0.0003	67.1
30	279.79 ±0.0006*	190.45± 0.003	46.9	6346.50 ±0.0004*	3671.11 ±0.0003	72.9
60	310.89 ±0.0002*	201.26 ±0.002	54.3	6652.47 ± 0.0003*	3671.19 ±0.0002	81.2
90	300.01 ±0.0001*	201.27± 0.002	49.1	6431.51 ±0.0006*	3671.18 ±0.0003	75.2
120	301.09 ±0.0001*	201.27 ±0.002	49.6	6601.22 ±0.0006*	3671.18 ±0.0002	79.8

*DAI = Days after inoculation, Values are mean ± SD, N = 3 readings, * Significant (P = 0.05). Percentage difference was obtained by expressing the difference between the value for healthy and inoculated as percentage of the healthy*

Table 2. Changes in polyphenol oxidase (PPO) activities of *Amaranthus viridis* inoculated with Telfairia mosaic virus (TeMV)

DAI	µg-1FW			U/L		
	Inoculated	Healthy	(%) Difference	Inoculated	Healthy	(%) Difference
14	2.557 ± 0.0003*	2.110 ± 0.0002	21.2	49.90 ± 0.004*	29.73 ± 0.003	67.8
30	2.831 ± 0.0003*	2.202 ± 0.0002	28.6	60.01 ± 0.001*	34.69 ± 0.004	72.9
60	3.201 ± 0.0001*	2.203 ± 0.0003	45.3	64.61 ± 0.001*	34.70 ± 0.004	86.2
90	3.001 ± 0.0001*	2.201 ± 0.0006	36.4	59.21 ± 0.003*	34.71 ± 0.002	70.6
120	3.119 ± 0.0001*	2.201 ± 0.0006	41.7	63.12 ± 0.001*	34.72 ± 0.002	73.2

*DAI = Days after inoculation, Values are mean ± SD, N = 3 readings, * Significant (P =0.05). Percentage difference was obtained by expressing the difference between the value for healthy and inoculated as percentage of the healthy*

Table 3. Changes in 6 phosphogluconate dehydrogenase (6PGdH) activities of *Amaranthus viridis L.* inoculated with Telfairia mosaic virus (TeMV)

DAI	µg-1FW			U/L		
	Inoculated	Healthy	(%) Difference	Inoculated	Healthy	(%) Difference
14	3.10 ± 0.001*	2.20 ±0.006	40.9	123.20± 0.006*	69.50 ±0.001	77.3
30	4.10 ± 0.006*	2.64 ± 0.006	55.3	135.09 ±0.001 *	70.63 ± 0.001	91.3
60	3.45 ± 0.006*	2.66 ±0.003	29.7	125.30± 0.001*	70.70± 0.001	77.2
90	3.40 ± 0.003*	2.65± 0.003	28.3	116.16± 0.006*	70.60± 0.006	64.5
120	3.57 ± 0.003*	2.65 ±0.003	34.7	116.40 ±0.003*	70.59 ±0.006	65.0

*DAI = Days after inoculation, Values are mean ± SD, N = 3 readings, * Significant (P = 0.05). Percentage difference was obtained by expressing the difference between the value for healthy and inoculated as percentage of the healthy*

Table 4. Changes in glucose 6 phosphate dehydrogenase (G6PdH) activities of *Amaranthus viridis* inoculated with Telfairia mosaic virus (TeMV)

DAI	µg-1FW			U/L		
	Inoculated	Healthy	(%) Difference	Inoculated	Healthy	(%) Difference
14	63.59 ±0.006*	45.71.55± 0.006	39.1	1.59 ± 0.006*	0.99 ± 0.006	60.6
30	70.56 ± 0.01*	48.79 ± 0.006	44.6	2.15 ± 0.01*	1.23 ± 0.01	74.8
60	70.53 ± 0.01*	50.73 ± 0.01	38.5	2.30 ± 0.006*	1.23 ± 0.01	87.0
90	69.74 ± 0.01*	50.65 ± 0.01	37.3	1.90 ± 0.01*	1.22 ± 0.01	55.7
120	70.50 ± 0.01*	50.64 ± 0.006	39.2	1.98 ± 0.006*	1.22 ± 0.01	62.3.3

*DAI = Days after inoculation, Values are mean ± SD, N = 3 readings, * Significant (P =0.05). Percentage difference was obtained by expressing the difference between the value for healthy and inoculated as percentage of the healthy.*

Hull [36] reported that where necrosis does not occur for many host-virus combinations, there is a rise in the rate of respiration, which begin before symptoms appear and continue for a period of time as disease develops. Several changes in the metabolism of diseased plant accompany increase in respiration after infection. Thus, the activity or concentration of several enzymes of the respiration pathways seems to be increased, accumulation and oxidation of phenolic compounds, increased activation of the pentose phosphate pathway which is the main source of phenolic compounds [36]. Increase in respiration due to virus infection could also be explained as a result of increase in metabolism. Virus infection of plant places a high energy demand to enable the infected plants carry on their activities. The energy required for these processes is derived from ATP produced through respiration. The more ATP is utilized the more ADP is produced stimulating respiration. It is also possible that plants because of infection, utilizes ATP energy less efficiently than a healthy plant and due to waste of part of the energy, an increase in respiration is induced and the resulting greater amount of energy enables the plant cells to carry out their accelerated processes [36]. The significant higher increase in G6PdH and 6PGdH enzymatic activities in inoculated *A. viridis* caused by TeMV infection in μg^{-1} FW and U/L when compared to the healthy controls in this study suggests increased respiration. Similarly, [9] reported that infection resulted in magnificent increases in the respiratory capacity and substantial alteration in metabolism in *Cucurbita pepo* infected with CMV. The alteration in growth, glucose utilization and acid secretion correlated with enzymes activities of G6PdH and 6PGdH and pyruvate kinase. This increase in respiration and alteration in metabolism resulted in higher demand for energy which is limiting due to infection thus, poor growth and development of inoculated plants compared to the healthy ones. The alteration in growth, glucose utilization and acid secretion correlated with enzymes activities of G6PdH and 6PGdH and pyruvate kinase.

Because of the ability of PPO and POD to drive the oxidation and condensation of lignin precursors, it has been suggested by [43] that PPO might be responsible primarily for the polymerization of monolignols into olignols whereas POD would be more likely to be involved in catalytic reactions resulting from olignols to highly condensed lignin. In higher plants, PPO activity is ubiquitious, as they are involved in phenol metabolism and in defense mechanism against pathogen [44]. Many reports have identified PPO to play a role in the polymerization of monolignols into olignols, the precursor molecules of lignin. The higher activities of POD, PPO, G6PdH and 6PGdH observed for inoculated or diseased plants alludes or suggests a probable mechanism of overcoming the stress due to TeMV infection of *Amaranthus viridis*. The hyperactive profile of PPO observed in diseased plants is usually associated with an improvement in the host defense mechanism [44], but in this investigation the host defense system in *A. viridis* plants appeared to have failed totally despite the enhanced activities of the enzymes observed for inoculated plants.

5. CONCLUSION

This study revealed significant increase in POD, PPO, G6PdH and 6PGdH enzymes activities due to TeMV infection of *A. hybridus* at all post inoculated periods compared to the healthy plants. There were significant changes in the activities of these enzymes in inoculated plants depicted by increase and fluctuation in activities. Results also revealed higher activities in U/L compared to μg^{-1} FW. These changes induced by TeMV infection had profound effect on the *A. viridis* growth pattern.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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