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SDS-PAGE Band Patterns of Partially Purified Peroxidase Isozymes Extracted From Telfairia Mosaic Virus (Temv) Infected And Healthy Plant Leaves of *Telfairia Occidentalis* (Hook F.)

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Abstract: *This study was carried out to separate Telfairia occidentalis peroxidase isozymes extracted from leaves of Telfairia mosaic virus (TeMV) infected and healthy plants by SDS-PAGE and compare their banding patterns for difference. Telfairia occidentalis plants were grown under green house condition and arranged in a completely randomized design prior to inoculation. Randomly selected plants were inoculated with TeMV. Peroxidase isozymes extracted from healthy and infected plants were separated by SDS-PAGE, using 12.5% resolving gel buffered at pH 8.8 and 5% stacking gel in tris glycine buffered at pH 6.8. The isozymes profiles of healthy and infected plants were qualitatively analyzed for presence or absence of bands and quantitatively using Rf values. Analysis of the peroxidase isozyme profiles revealed that both healthy and infected T. occidentalis plants have seven identical bands each with Rf values 0.09, 0.30, 0.35, 0.43, 0.55, 0.68, 0.83 and molecular weights of 66.07, 36.31, 30.20, 23.44, 16.60, 11.75 and 7.08 respectively. The results suggest that SDS-PAGE of peroxidase enzymes cannot be used for detection of TeMV infection of T. occidentalis.*

Keywords: *Band patterns, Peroxidase isozyme, Telfairia mosaic virus (TeMV), Telfairia occidentalis.*

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I. Introduction

Telfairia occidentalis Hooker fil is a member of Cucurbitaceae family commonly called fluted pumpkin. Telfairia occidentalis is a crop of economic importance grown across the lowland humid tropics in West Africa, Nigeria, Chana and Sierra Leone being the major producers. Fluted pumpkin is a highly nutritious leafy vegetable native to West Africa [1]. It is one of the most important vegetables grown in Southern Nigeria mainly for its leaves which constitute an important component in the diet of the people. The leaves of fluted pumpkin which can be harvested at all stages of growth [2] are used in the preparation of popular soups ‘’edikang ikong’’, a much sought-after decicacy in Cross River and Akwa Ibom States and ‘’Ofe ugu’’ in the Igbo-speaking States [3]. *Telfairia occidentalis* is rich in essential amino acids, vitamins and mineral nutrients [4], [3], [5] The nutritional advantage of *Telfairia* plant is a clear indication of its agronomic superiority over many plant protein sources. The leaves of *T. occidentalis* are also used as protein supplement in broiler starter diets [6]. The immature seeds are cooked or roasted or fermented and eaten as slurry [7]. The mature seeds contain non drying oil that can be used in margarine production, as cooking oil, for making paints and varnishes [8].

Viral diseases are among the main causes of decreased crop productivity worldwide [9]. The production of *Telfairia occidentalis* is limited by a number of diseases, the most significant of which are of viral aetiology [10]. Three viruses inducing mosaic and mosaic-like symptoms have been reported on fluted pumpkin in Nigeria. The reported viruses are; a Y-strain of Cucumber mosaic virus (CMV-Y) [11], a strain of Pepper veinal mottle virus (PVMV) [12], [1] and Telfairia mosaic virus (TeMV) [13], [14]. Infected leaves exhibited mosaic, severe leaf malformation and distortion and reduction in leaf size. A survey carried out between 1987 and 1988 by [1] showed that TeMV infection was the most prevalent of the three viruses, occurring in 12 major fluted pumpkin producing states in Southern Nigeria with an incidence of 5-10%. Telfairia mosaic virus (TeMV) is a limiting factor for the production of *T. occidentalis* a nutritious leafy vegetable widely consumed in the southern parts of Nigeria. Plants respond to invasion by pathogens with various biochemical and genetic changes [15].

Morphological and physiological changes occur in plants as a result of virus infection [16], [17]. Increased amount of protein has been reported in spring wheat infected by WSMV [18]. Increase in free amino

acid proline and disease-related proteins in host plants have been reported by [19]. Telfairia mosaic virus (TeMV) infection of *Telfairia occidentalis* caused increase in enzyme activities of peroxidase, polyphenol oxidase, glucose-6-phosphate and 6-phosphogluconate dehydrogenase [20]. Infection of *Cucurbita moschata* by a Nigerian strain of Moroccan watermelon mosaic virus (MWMV): Lagenaria breviflora isolate caused mosaic, chlorosis and leaf malformation as well as decrease and increase in physiological parameters with alterations in vital metabolic processes [21]. Plants encountering biotic stress such as viral infection generally react with changes in their protein profile or develop a secondary response which involves the production of Reactive Oxygen Species (ROS) [22], [23]. To combat and reduce the damage caused to cells by ROS, plants use a set of enzymes and antioxidant substances such as ascorbate [23].

.Plant peroxidases (EC 1.11.1.7) with different isoforms catalyze various reactions in plant growth and development [24]. There have been a number of reports confirming the involvement of peroxidases in protective plant metabolism [25]. Peroxidase (POX) is one of the first enzymes produced by infected plant cells to provide fast defense against plant pathogens [26], [27]. Peroxidases are key enzymes of lignin biosynthesis and important pathogenesis-related proteins (PR-proteins). Electrophoresis of peroxidase isozymes is a potential tool for screening and early detection of TeMV infection of *T. occidentalis* plants. This study examines the effect of TeMV virus infection on peroxidase isozyme composition of *Telfairia occidentalis*. The objective is to carry out SDS-PAGE of peroxidase extracted from the leaves of infected and healthy plants and compare the banding patterns to see if they differ.

II. Materials And Methods

2.1 Seed collection

Fruits of *T. occidentalis* from which seeds were obtained were purchased from local farmers in Akparabong, Ikom Local Government Area, Cross River State, Nigeria. The seeds were removed and sorted to select seeds of uniform size. The selected seeds were sundried for two days to enhance germinability and thereafter sown in steam sterilized fertile garden soil in 16 cm diameter polyethylene bags. The germinated seeds (seedlings) were staked to enhance leaf productivity.

2.2 Virus source, preparation of inoculum and virus propagation

The virus used in this research was provided by Dr A. J. Vetten of the Federal Biological Centre for Agriculture and Forestry (BBA) Braunschweig, Germany, in infected dried leaf material kept under liquid nitrogen. The virus was reactivated by homogenizing the leaf tissues in presterilized cold mortar and pestle in sodium sulphate (Na_2HPO_4) buffer 0.03 M, pH 8.0. The inoculum was then applied by conventional leaf rub method or sap inoculation with cotton swab onto *Nicotiana benthamiana*, dusted with an abrasive (carborundum, 800 mesh). The leaves of the inoculated plants were then rinsed with water and left for symptom expression. Subsequent inoculation of *T. occidentalis* in order to propagate and maintain the virus under green house condition at 25 ± 3^oC was carried out by sap inoculation.

2.3 Plant Inoculation and experimental design

Fifteen experimental plants were arranged in three rows of five plants each. Eight randomly selected plants were inoculated with the virus and the remaining seven inoculated with the buffer only to serve as control. The experimental set up was monitored for symptoms development such as mosaic, severe leaf malformation and distortion characteristic of TeMV infected *T. occidentalis*.

2.4 Enzyme extraction

Two gram of fresh leaves was weighed (Ohaus C. S. 5000, U.S.A). The weighed leaf tissue of the healthy and infected *T. occidentalis* obtained from experimental plots were homogenized using mortar and pestle in 10 ml of extraction buffer. Extraction buffer of 100 mM mixed phosphates: monobasic potassium phosphate and dibasic potassium phosphate (KH_2PO4 and K_2HPO_4) containing 1% polyvinyl polypyrolidone (PPP) and adjusted to a final pH 7.0 was used. Extraction was carried out at 4^oC. The homogenate was filtered through cheese cloth and the filtrate centrifuged (Model 0406-2) at 5,000 rpm for 5 minutes. The supernatant was stored as crude enzyme source [28].

2.5 Electrophoresis of partially purified crude enzyme extracts

To 2 ml of the crude enzyme extract was added 2 ml of cold acetone. The mixture was allowed to stand for five minutes and then centrifuged at 5,000 rpm for five minutes. The precipitate was resuspended in 1 ml cold assay buffer and fractionated by discontinuous sodium dodecyl sulphate, polyacrylamide gel electrophoresis (SDS-PAGE), using 12.5% resolving gel buffered at pH 8.8 and 5% stacking gel in tris glycine buffered at pH 6.8, following the protocol of [29] on a Biometra Electrophoresis unit (Model 010-100). The wells in which samples were **loaded were numbered 1 to 8** and the sample run in each well was noted (Plate

1). One well to the left in which protein standards from Sigma Chemical Company was run was noted M (Plate 1). Partially purified peroxidase extract from leaves of healthy and infected *T. occidentalis* plants were loaded in Lanes 1 and 4 respectively.

2.6 Confirmation of peroxidase activity

Since enzymes or proteins are denatured in SDS-PAGE and are therefore, not catalytically active, peroxidase was demonstrated by staining gel under non-denaturing condition with o-dianisidine and hydrogen peroxidase [30].

III. Results

Table 2: Data computation from plate 1 for standard graph

Protein molecular markers	Molecular weight in Kilodaltons	Log of molecular weight	Migration distance of dyefront (cm)	Migration distance of protein markers (cm)	Relative mobility (Rf) values
			4.00		
Aprotinin Bovine lung	6.5	0.81		3.1	0.78
Trypsin inhibitor soybean	20.0	1.30		2.2	0.55
Trypsinogen Bovine Pancreas	24.0	1.38		1.7	0.43
Glyceraldehyde 3 Phosphate	29.0	1.46		1.5	0.38
Dehydrogenase, Rabbit muscle	36.0	1.56		1.2	0.30

The peroxidase isozyme banding patterns obtained following SDS-PAGE of the partially purified enzyme extracts from leaves of healthy and Telfairia mosaic virus infected plants are shown in Plate 1 (Lanes 1 and 4 respectively). Plate 2 is the demonstration of the presence of peroxidase enzyme in the foliar enzyme extract from healthy and infected *T. occidentalis* plants (Lanes 1 and 4 respectively). Table 1 shows computation of data from Plate 1 with the logarithm of the molecular weights of protein markers, the migration distance of the dye front, the migration distance of each marker and their relative mobilities (Rf values).

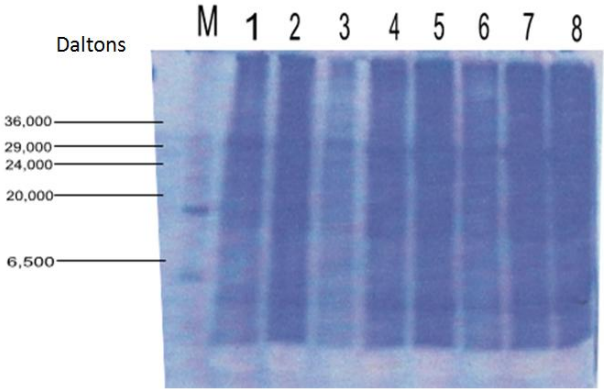

Plate 1: Sodium dodecyl sulphate polyacrylamide gel electrophoresis isozyme band patterns.

Lane 1: Isozyme pattern of peroxidase extracted from healthy plants
Lane 4: Isozyme pattern of peroxidase extracted from infected plants
M – Lane in which the molecular weight marker was loaded

A graph of the logarithm of molecular weights of the protein markers against their relative mobilities was used as the standard graph from which the molecular weights of unknown peroxidase isozymes of healthy and infected *T. occidentalis* foliar extracts were derived, using their Rf values (Table 2).

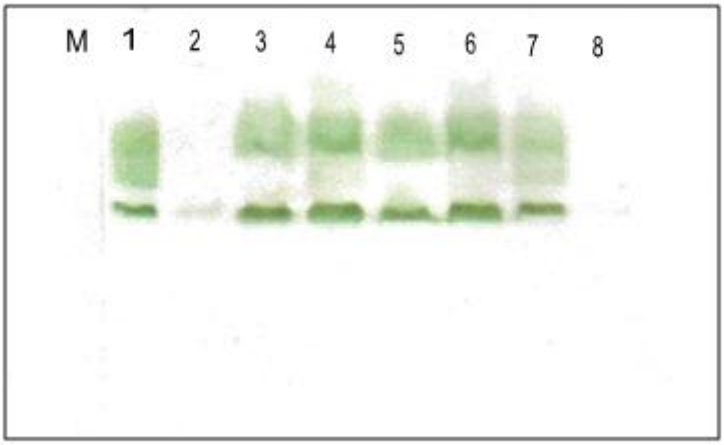

PLATE 2: O-dianisidine confirmation of peroxidase activity in native polyacrylamide gel electrophoresis (PAGE) of *Telfairia occidentalis* enzyme extracts**.**

Table 2: Data computation of unknown peroxidase isozymes extracted from healthy and infected leaves of *Telfairia occidentalis* inoculated with Telfairia mosaic virus (TeMV) (Plate 1, Lane 1 and 4)

Unknown isozyme band	Migration distance of band (cm)		Rf value	Estimated log of molecular weight	Estimated molecular weight (KDa)
	Healthy	**Infected**			
B_1	0.3	0.3	0.09	1.82	66.07
B_2	1.2	1.2	0.30	1.56	36.31
B_3	1.4	1.4	0.35	1.48	30.20
B_4	1.7	1.7	0.43	1.37	23.44
B_5	2.2	2.2	0.55	1.22	16.60
B_6	2.7	2.7	0.68	1.07	11.75
B_7	3.3	3.3	0.83	0.85	7.08

The SDS-PAGE gel analysis revealed similar protein profiles for healthy (control) and TeMV infected leaves (Plate 1, Lanes 1 and 4). The isozyme band pattern was analyzed qualitatively by observing for the presence or absence of bands in the protein profile for the infected compared to that of the control, and also analyzed quantitatively using the Rf values. Both healthy and infected *T. occidentalis* peroxidase isozyme profiles had the same number of bands (seven) (Plate 1; Lanes 1 and 4) with Rf values of 0.09, 0.30, 0.35, 0.43, 0.55, 0.68 and 0.83 and molecular weights of 66.07, 36.31, 30.20, 23.44, 16.60, 11.75 and 7.08 kilodaltons respectively. The o-dianisidine test using native PAGE (Plate 2) meant for confirmation of peroxidase revealed differences in the thickness of protein bands of infected plants compared to the healthy. The band for healthy (Lane 1) was thicker than the band for infected plants (Lane 4).

IV. Discussion

Peroxidases are antioxidant enzymes involved in plant defense response to pathogen attack [31], [32], [33], [34], [35]. When plants are exposed to pathogen attack (abiotic stress) the production of Reactive Oxygen Species (ROS) increases and cause significant damage to cells. Peroxidase enzymes are part of the antioxidants in plants that can detoxify ROS [36]. Increased peroxidase activity has been reported in many plants exposed to external biotic stresses [37]. Telfairia mosaic virus (TeMV) infection reduces the market value of *Telfairia occidentalis* leaves resulting in economic loss to the farmer [17].

In this study SDS-PAGE of peroxidase enzyme extracted from healthy and TeMV infected *Telfairia occidentalis* plants was carried out and the isozyme band patterns were compared to see if they differ. Zymogram of SDS-PAGE revealed similar isozyme band patterns of healthy and TeMV infected plants. However, confirmatory test for peroxidase revealed differences in thickness of bands of infected plants compared to the healthy. The bands formed in this case represented several isozymes stacked together because of poor protein separation in native PAGE. [19] reported that isozyme patterns of acid phosphatase, polyphenol oxidase and superoxide dismutase from healthy and diseased Mesta plants infected by Yellow vein mosaic virus produced similar types of band patterns.

Different banding patterns of peroxidase extracts of healthy and virus infected plants separated by SDS-PAGE have been reported [38], [34], [39]. Why similar peroxidase isozyme profiles of healthy and

infected were obtained in this study is not understood. Since any protein can take up the Coomasie Brillian Blue stain used, peroxidase activity was confirmed under non denaturing condition by carrying out native PAGE and staining with o-dianisidine. The separation of isozymes by SDS-PAGE has some limitations [40]; incomplete denaturation, unusual amino acid sequence and/or presence of non-protein residues can affect the mobility resulting in error. For this reason, molecular weight estimates by this method is best referred to as apparent molecular weight [40]. Perhaps any of the limiting factors obscured any difference that should have been detected.

V. Conclusion

Results of SDS-PAGE in this study showed that peroxidase isozymes band patterns of healthy and TeMV-infected *T. occidentalis* did not differ. However, o-dianisidine confirmatory test for peroxidase showed that healthy plants had thicker overall protein bands than the infected plants. Based on the result SDS-PAGE in this study, it is concluded that SDS-PAGE cannot be used to detect TeMV infection of *Telfairia occidentalis*.

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