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Comparative Analysis of SDS-PAGE Isozyme Band Pattern of Crude Polyphenol Oxidase Extracts From Leaves of Healthy And Telfairia Mosaic Virus (Temv) Infected Telfairia Occidentalis (Hooker Fil.)

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Abstract: *The most prevalent type of viral infection of Telfairia occidentalis in Nigeria is Telfairia mosaic virus (TeMV). Infected leaves exhibit mosaic and mosaic-like symptoms, severe leaf malformation, leaf distortion and reduced leaf size. Farmers suffer economic losses if effort is not made to reduce the incidence. This study investigated the possibility of detecting TeMV infection of T. occidentalis by SDS-PAGE of polyphenol oxidase isozymes. Fifteen test plants of T. occidentalis were grown under green house condition. Eight randomly selected plants were inoculated with TeMV and seven were left as control. Crude polyphenol oxidase enzyme extract from healthy and infected plants were fractionated by SDS-PAGE. Analysis of the polyphenol oxidase isozyme profiles revealed five similar bands each for infected and healthy plants. The bands were at Rf 0.30, 0.43, 0.55, 0.65 and 0.83 and their estimated molecular weights were 36.31, 24.00, 16.60, 12.30 and 6.92 kilodaltons respectively. Though the isozymes bands of the healthy plants were thicker, this was not considered sufficient evidence to establish that SDS-PAGE of polyphenol oxidase can be used to detect TeMV infection of T. occidentalis because distortion at one end of the gel during processing may be responsible for the difference in thickness.*

Keywords: *Polyphenol oxidase, SDS-PAGE, Telfairia occidentalis, Telfairia mosaic virus (TeMV).*

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I. Introduction

Telfairia occidentalis Hooker fil (family Cucurbitaceae) commonly called fluted pumpkin is a highly nutritious leafy vegetable native to West Africa [1]. It is rich in essential amino acids, vitamins and mineral nutrients ([2], [3], [4] and is widely cultivated in Southern Nigeria, mainly for its leaves which are used for making popular soups (like ‘’edikang ikong’’, ‘’Ofe ugu’’). The leaves of this vegetable have high nutritional, medical and industrial values thus, plays an important role in traditional medical practice and local diets, improves households income generation, broaden food base as it supplies the body with essential mineral nutrients [5]. Fluted pumpkin is an important economic crop; apart from the leaves, the fermented seeds are used in the production of local custard ‘’Ogiri ugu’’. The seeds are also used in cookie formulations and marmalade manufacturing [6]. The seed cotyledons are processed into seasonings, infants weaning foods and flour supplements. Seeds have lactation promoting properties and are in high demand by nursing mothers. 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] The production of fluted pumpkin is limited by a number of diseases, the most important of which is of viral aetiology [9]. Three viruses inducing mosaic and mosaic-like symptoms have been reported on fluted pumpkin in Nigeria. These are Y- strain of Cucumber mosaic virus (CMV-Y) [10], a strain of Pepper veinal mottle virus (PVMV)[11], [1] and Telfairia mosaic virus (TeMV) [12]. [13]. Infected leaves exhibited mosaic, severe leaf malformation and distortion and reduction in leaf size. A survey conducted 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%.Biochemical and physiological changes occur in plants as a result of virus infection [14], [15]. Reports of virus infection on plants protein content, enzymes activities and isozymes band patterns are inconsistent. [16], [17] reported increase in protein in alfalfa and ash gourd infected by Alfalfa mosaic virus (AMV) and Papaya ring spot virus (PRSV) respectively. [17] reported decrease in N and in host protein content in ash gourd due to infection by Bottle gourd mosaic virus (BgMV) and Watermelon mosaic virus-2 (WMV-2). [18] reported higher activity of POD, PPO, phenylalanine ammonia lyase and tyrosine ammonia lyase in lettuce infected by Lettuce mosaic virus (LMV) and in mothbean infected by Yellow

mosaic virus (YMV) with a decrease in catalase activity. [19] also reported an increase in peroxidase (POD), polyphenol oxidase (PPO), glucose-6-phosphate dehydrogenase (G6PdH) and 6-phoshogluconate dehydrogenase (6PGdH) activities in *Amaranthus viridis* infected by Telfairia mosaic virus (TeMV). Isozyme band patterns for acid phospatase, polyphenol oxidase and superoxide dismutase from infected and healthy Mesta plants produced similar types of band pattern. Catalase isozyme band pattern of diseased Mesta plant was different from that of healthy plant with a new band observed in diseased while some bands were more pronounced in healthy material but missing in the diseased plant. Peroxidase isozyme profile revealed the disappearance of some bands in diseased plants which were present in their controls. The band profile for esterase revealed as extra band in diseased plants and observed other hyperactive bands in diseased plants indicating higher activity when compared to their respective healthy plants [20]. Polyphenol oxidase (EC 1.10.3.1) an enzyme found in most plant species [21] catalyze the oxidation of mono, di and polyphydric phenols into quinones [22] 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 defence against herbivore and pathogens [20], [21]. [23] observed increase in the density and number of peroxidase and polyphenol oxidase isozymes in Zucchini squash (*Curbita pepo* cv. Eskandarani) plants exhibiting systemic acquired resistance (SAR) against Zucchini yellow mosaic virus (ZYMV). Reports like this suggest the possibility of changes in polyphenol oxidase isozymes composition of *Telfairia occidentalis* in response to TeMV infection. Therefore, electrophoresis of polyphenol oxidase enzyme is a potential tool for screening and early detection of TeMV infection of *T. occidentalis* plants. This study examines the possibility of using SDS-PAGE of polyphenol oxidase extracted from the leaves of healthy and infected *T. occidentalis* plants for diagnosing TeMV infection. The objective work is to carry out SDS-PAGE of polyphenol oxidase extracted from healthy and TeMV infected plants and compare the isozyme band patterns to see if they differ.

II. Materials And Methods

2.1 Seed collection

Seeds of *T. occidentalis* used in this study were purchased from local farmers in Akparabong, Ikom Local Government Area of Cross River State, Nigeria. The seeds were sorted for uniformity of size. The selected seeds were sundried for two days to enhance germinability and thereafter sown on steam sterilized fertile garden soil in 16 cm diameter polyethylene bags. The germinated seeds (seedlings) were staked to promote adequate leaf production

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2.2 Virus source and virus propagation

The Telfairia mosaic virus (TeMV) used in this study was the very isolate described by [13]. The virus isolate was provided by Dr A. J. Vetten of the Federal Biological Centre for Agriculture and Forestry (BBA) Braunschweig, Germany, in infected dried leaf material stored under liquid nitrogen. The virus was re-activated by triturating 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 (mechanical or sap inoculation) with cotton swab onto *Nicotiana benthamiana*, predusted with carborundum (800 mesh). The inoculated leaves were then rinsed with water and left for symptom expression. Subsequent inoculation using the sap transmission method was carried out on *T. occidentalis* in order to propagate and maintain the virus under green house condition at 25 ± 3^oC.

2.3 Plant Inoculation and experimental design

A total of fifteen plants used in this study were arranged in three rows of five plants each. Eight randomly selected plants were inoculated with the virus and the remaining seven inoculated with buffer only, to serve as control. The experimental set up was monitored for symptoms expression that included, mosaic, severe leaf malformation and distortion characteristic of TeMV infected *T. occidentalis*.

2.4 Enzyme extraction

Two grams fresh weight (Ohaus C. S. 5000, U.S.A) of leaf tissue of the healthy and infected *T. occidentalis* obtained from experimental plants were separately homogenized using mortar and pestle in 10 ml of extraction buffer. Extraction buffer consisted of 100 mM mixed phosphates; monobasic potassium phosphate and dibasic potassium phosphate (KH_2PO4 and K_2HPO_4) containing 1% polyvinyl polypyrolidone (PVPP) and adjusted to a final pH of 7.0. Extraction was carried out at 4^oC. The homogenate was filtered through cheese cloth and the filtrate centrifuged at 5,000 g for 5 minutes (model 0406-2). The supernatant was stored as crude enzyme source [24]. The crude enzyme extract was 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 [25] on a Biometra

Electrophoresis unit (Model 010-100). The crude enzyme extract sample was diluted with sample buffer in a ratio of 1:4 and heated at 95^{o}C for four minutes to denature the proteins. Using a micropipettor, the wells at the top of the stacking gel numbered 1-8 were loaded with 10 microlitres of denatured samples (Plate 1). A well to the left labeled M (Plate 1) was loaded with protein standard from Sigma Chemical Company (Table 1). Crude extract of polyphenol oxidase from leaves of TeMV infected and healthy *T. occidentalis* plants were loaded in lanes 3 and 8 respectively. The following samples; partially purified peroxidase extract from healthy plants, partially purified catalase extract from infected plants, partially purified peroxidase extract from infected plants, partially purified catalase extract from healthy plants, partially purified polyphenol oxidase extract from healthy plants and crude peroxidase extract from infected plants were run in lanes 1, 2, 4, 5, 6 and 7 respectively.

III. Results

Table 1: Molecular weights of proteins in commercial molecular weight marker

Proteins	**Molecular weight in Daltons**
Aprotinin, Bovine lung	6,500
Trypsin inhibitor, Soybean	20,000
Trypsinogen, Bovine Pancrease	24,000
Glyceraldehyde 3 Phosphate	29,000
Dehydrogenase Rabbit Muscle	36,000

The zymogram of polyphenol oxidase obtained following SDS-PAGE showed that both healthy and infected plants had similar patterns of five bands each (Plate 1 Lanes 3 and 8). In both lanes, the bands were at Rf 0.30, 0.43, 0.55, 0.65 and 0.83. Figure 1 is a graph of the logarithm of the molecular weights of the protein markers against their relative mobilities (Rf) values. This provided the standard graph from which the apparent molecular weights of unknown polyphenol oxidase isozymes were derived, using their Rf values. The apparent molecular weight of the five polyphenol oxidase isozymes of both healthy and infected plants were 36.31, 24.00, 16.60, 12.30, and 6.92 respectively (Table 3). The isozymes bands of the healthy plants were thicker compared to the infected plants. The polyphenol oxidase isozyme banding patterns obtained following SDS-PAGE of crude enzyme extracts from leaves of healthy and TeMV infected plants are shown in Lanes 3 and 8 respectively (Plate 1). Table 2 shows computation of data from Plate 1 with the logarithm of the molecular weights of the protein markers, the migration distance of the dye front, the migration distance of each marker and their relative mobility (Rf) values.

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

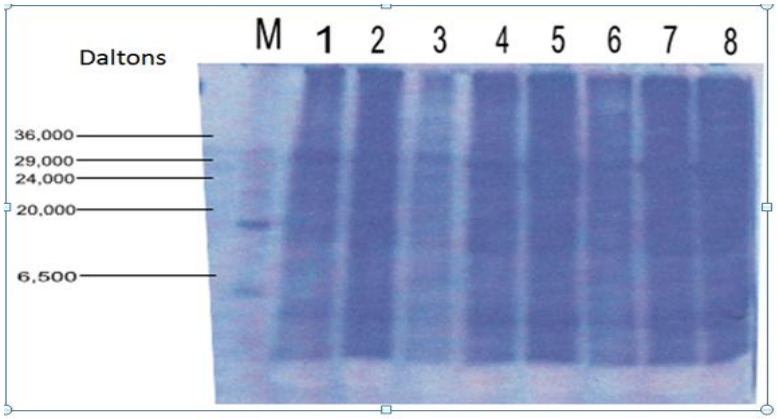

Plate 1: Sodium dodecyl sulphate polyacrylamide gel electrophoresis isozyme band patterns.

Lane 8: Isozyme pattern of crude polyphenol oxidase extract from healthy plants
Lane 3: Isozyme pattern of crude polyphenol oxidase extract from infected plants
M – Lane in which the molecular weight marker was loaded

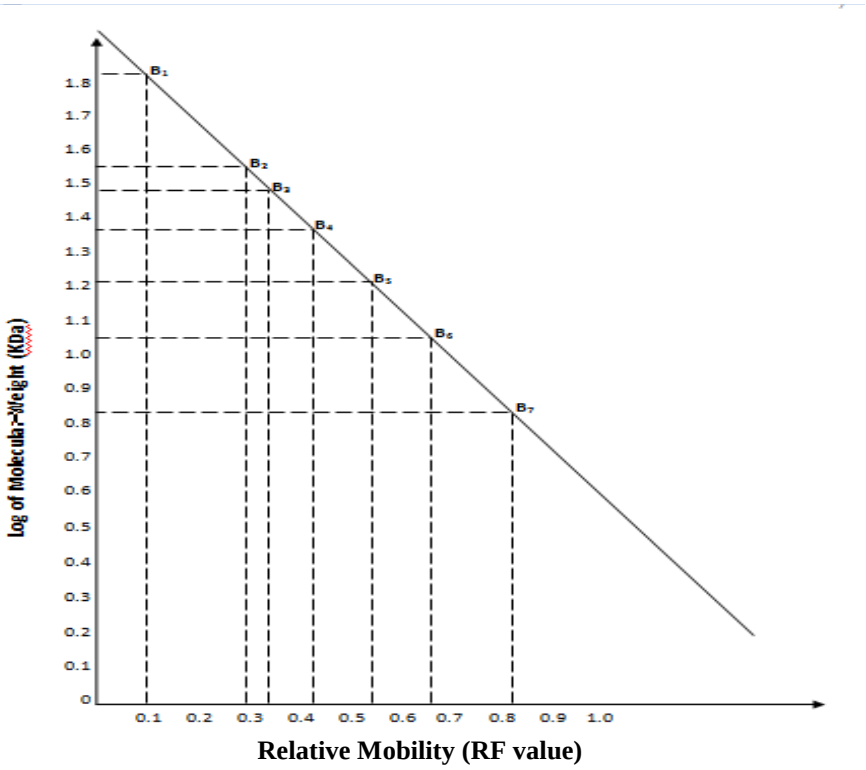

Fig. 1:Ploto frelative mobility value (x-axis) versus the Log of molecular weight (y-axis) of poyp henol oxidase isozymes

Table 3: Data computation of unknown polyphenol oxidase isozymes extracted from healthy and Telfairia mosaic virus (TeMV) infected leaves of *Telfairia occidentalis* (Plate 1, Lanes 8 and 3)

Unknown isozyme band	Migration distance of band (cm)		Rf value	Estimated log of molecular weight	Estimated molecular weight (KDa)
	Healthy	**Infected**			
B_1	1.2	1.2	0.30	1.56	36.31
B_2	1.7	1.7	0.43	1.38	24.00
B_3	2.2	2.2	0.55	1.22	16.60
B_4	2.6	2.6	0.65	1.09	12.30
B_5	3.3	3.3	0.83	0.84	6.92

IV. Discussion

Environmental stresses of biotic and abiotic nature cause characteristic changes in the physiology and metabolic processes of higher plants [26]. Infection by pathogens can cause substantial biochemical alterations leading to harmful effects on plant health. Viral diseases may cause retardation in plant growth with reduction in yield [27], [15], [28]. Telfairia mosaic virus infection of *Telfairia occidentalis* causes considerable losses to farmers in Southern Nigeria. There have been reports suggesting the involvement of polyphenol oxidase enzyme in plant defense against viral infection [29], [23]. In line with the growing interest in the possible use of electrophoresis to detect virus infection of plants, the present investigation was carried out to compare the polyphenol oxidase SDS-PAGE isozyme band patterns of healthy and TeMV infected plants. Results revealed similar patterns of five polyphenol oxidase isozyme bands for both healthy and TeMV infected plants. It was also observed that the isozyme bands of the healthy plants were thicker compared to the infected plants. This result is partly in agreement with that of [20] who reported that polyphenol oxidase isozyme of virus infected and healthy Mesta plants produced similar types of band patterns, though a marked increase in activity of polyphenol oxidase was observed in infected plants. In contrast, [30] found that isozyme bands of rice suffering from Tungro disease caused by double infection of Rice Tungro Bacilliform Virus (RTBV) and Rice Tungro

Sphiracle Virus (RTSV) appeared thicker compared to the bands of healthy plants.It is not understood why in this investigation polyphenol oxidase of healthy plants produced thicker bands. Differences in intensity of staining, reflects relative abundance of individual isozyme [31]. If polyphenol oxidase is involved in defense mechanism of *T. occidentalis* against TeMV it is expected that its activity would increase during infection, therefore, the isozyme bands of infected should have been thicker. Other possible causes of thicker bands include; incomplete denaturation of the enzyme in the sample, presence of a predominant poypeptide, or overloading of protein in the well [[31]. In the present study, each sample was heated at 95^{o}C for four minutes to denature the proteins and a micropipette was used to load equal volumes of 10 microlitres in each well. The thicker isozyme bands of healthy *T. occidentalis* plant may also be due to distortion at the lane 8 end of the gel during processing. The use of protein gels (SDS-PAGE) to estimate the molecular weight of isozymes has limitations [31]. Incomplete denaturation, unusual amino acid sequence and/or presence of non-protein residues can affect mobility resulting in error in the estimated protein weights. Therefore, molecular weight estimates of proteins by this method are best referred to as apparent molecular weight [31].

V. Conclusion

In view of these limitations, the difference in thickness of polyphenol oxidase isozyme bands of healthy and infected *T. occidentalis* was not considered sufficient evidence to establish that SDS-PAGE can be used for detection of TeMV infection.

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