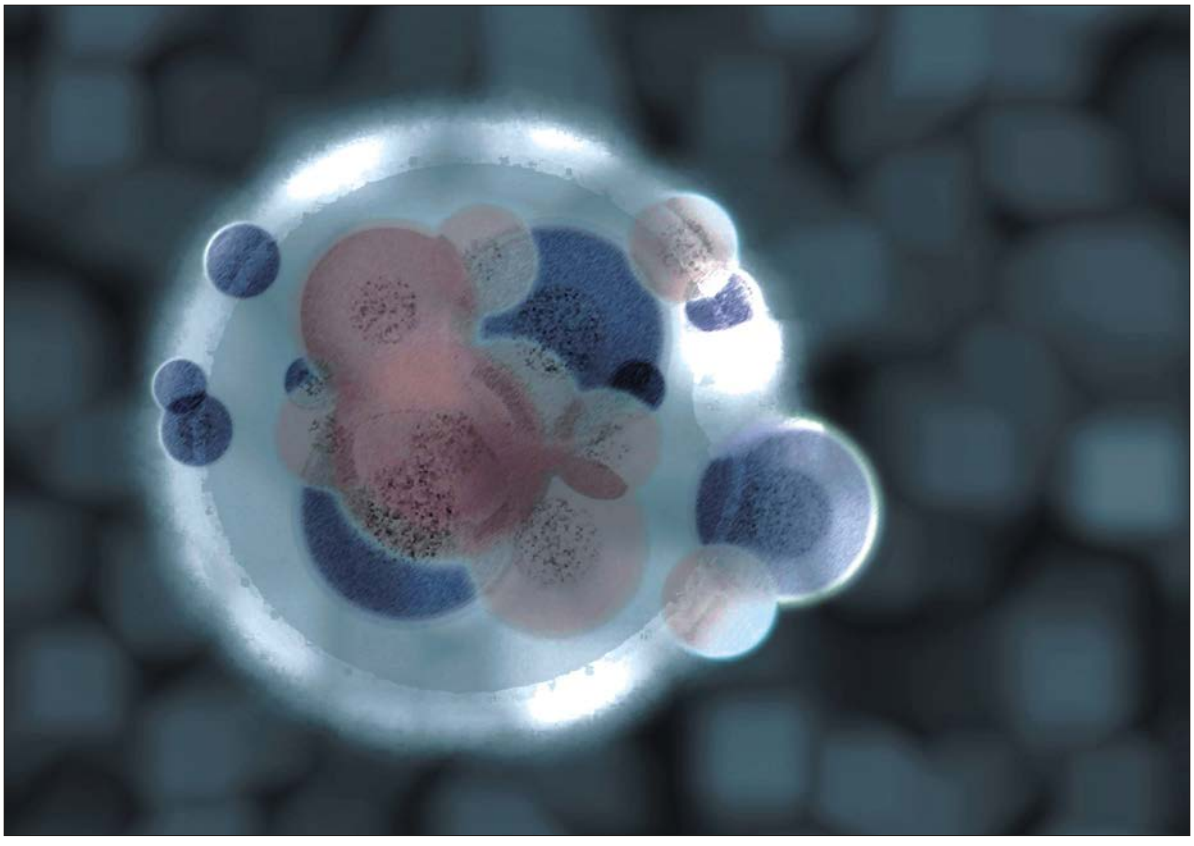

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 6 (5): 916-920, 2006
ISSN 1727-3048

Sugar and Amino Acid Contents of Fruit and Foliar Tissues from Two Cultivars of Plantain (*Musa paradisiaca*) Susceptible and Resistant to Cigar-end Rot Disease Caused by *Verticillium theobromae*

[1]C.L. Igeleke and [2]D.K.G. Ayanru
[1]Department of Basic Sciences, Benson Idahosa University, P.M.B. 1100, Benin City, Nigeria
[2]Department of Microbiology, University of Benin, P.M.B. 1154, Benin City, Nigeria

Abstract: Floral and fruit tissues of two cultivars of plantain (*Musa paradisiacal* L.), P100-F (susceptible) and P200-1 (resistant to cigar-end rot disease), grown side by side in a field plot in Benin City, Nigeria, were analysed for soluble and tissue-bound amino acids, total carbohydrate and crude protein using paper chromatography and micro Kjeldahl methods. Diminished carbohydrate and protein concentrations and intensity spotting of alamine and leucine were found in fruit tissue digest of the susceptible plantain cultivar (P100-F) as compared with values in digests from the resistant cultivar (P200-1). The relevance of these results in relation to the management of cigar-end rot disease of plantain is discussed.

Key words: Amino acids, total carbohydrate, crude protein, susceptible, resistant, plantain fruits, cigar-end rot disease

INTRODUCTION

The relevance of metabolites in the susceptibility or resistance of host plants to disease incidence and severity has been highlighted by some workers. Inman (1962) found a close relationship between host carbohydrate levels and growth and differentiation of rust in bean plants. Increased sugars in infected wheat tissues has been reported by Gerwitz and Durbin (1960). Other investigators have reported a decrease in the sugar content of rust-infected tissues (Krog *et al*., 1961). Ayanru (1970) also reported that analysis of root extracts of oats showed that susceptible seedling consistently leached greater quantities of total carbohydrates than resistant seedlings.

Mechanisms by which plant exudates may influence disease have been discussed (Schroth and Hilderbrand, 1964). Some of these mechanisms have linked inoculum potential with the nutritional status of a micro-organism. Garrett's concept of inoculum potential is based largely on the influence of nutrients and plant exudates on micro-organisms (Garrett, 1960). He defined inoculum potential as the energy of growth of a pathogen available for infection or an increase in the number of infecting units or both.

Reports on the relevance of sugars and proteins as sources of energy and structural materials in the elaboration of protoplasm suggest that carbohydrate and protein transformations within host tissues are significant in parasite development (Inman, 1962). Plantain is known to have considerable nutritional value, most cultivars being good sources of carbohydrates (Swennen, 1990). They are also climacteric fruits, producing some quantities of ethylene that sets off a sharp increase in respiration soon after harvest, undergoing some other changes which include the conversion of starch to sugar, a softening of the pulp and a change in skin colour from some shade of green to yellow (Asiedu, 1987; Fermin, 1991; Collin and Dalnic, 1991). In ripening behaviour of Senorita banana fruits from the shooting stage to maturity and senescence, Munasque and Mendoza (1990) observed that starch accumulated with increasing age. It declines towards maturity as total sugars accumulate towards the mature state. Pulp soluble solids and titratable acidity increase gradually with advancing maturity. Such physico-chemical changes are likely to pre-dispose tissues to microbial degradation.

Amino acids are believed to play important role in plant resistance mechanisms and various changes have been shown in diseased plants. Benedict and Hilderbrand (1958) reported that resistance to soybean stem canker may increase with an increase in amino acid content. Other reports have implicated increase of amino acid contents in the susceptibility of host plants. Ayanru (1970) reported that abundant amounts of alanine, aspartic acid, glutamic acid, serine and threonine were found in root extracts of oat susceptible to *Helminthosporium victoriae* compared to amounts in extracts of a resistant variety.

Corresponding Author: C.L. Igeleke, Department of Basic Sciences, Benson Idahosa University, P.M.B. 1100, Benin City, Nigeria

Information is scanty on the role of amino acids in the susceptibility or resistance of plantain to disease incidence and development. Steward *et al.* (1960) reported on factors which affect nitrogenous compounds of banana fruits. Anno and Lambert (1985) carried out soluble amino acid determination in plantain rhizome of cultivars corne 1 (false horn) to identify the most proteinic amino acids. They identified 23 amino acids with glutamic acid being most predominant followed by aspartic acid and alanine. Other amino acids identified include threonine, serine, leusine and lysine. The relationship between amino acid constituents and cigar-end rot disease development in plantain needs to be investigated. This paper reports on the sugar and amino acid contents of a cigar end rot disease susceptible and resistant varieties of plantain.

MATERIALS AND METHODS

Two plantain (*Musa paradisiaca* L.) cultivars were used in this study. The first was a cigar-end rot disease highly susceptible false horn cultivar designated as P100-F. The second cultivar was an IITA plantain accession, Tmp X 5511 - 2 which is a hybrid of Obino l' Ewai X Calcutta 4 (Emoghene, 1996). This was resistant to cigar-end rot disease and is designated as P200 -1.

Floral and fruit tissues of the two cultivars, growing side by side in a field plot in Benin City, were analysed for total carbohydrate and crude protein at the Biochemistry Department of the University of Benin, Benin City, Nigeria. Tissues investigated were the bracts (flowers), mature lamina (fully expanded), immature fruit peel and pulp (4 - 7 day old) and mature, (2 - month - old) fruit peel and pulp. The peel was excised from the pulp using a scapel. The samples were pre-treated according to the methods of Jones and Steyn (1973). They were washed in Teepol, a demineralised water containing a 0.2% sodium hexametaphosphate solution, to remove surface dirt and rinsed thrice with distilled water. The samples were wrapped with whatman No. 1 filter paper, put in clean muslin bags and dried for 48 h at 65°C in a forced draught Gallenkamp (British) oven. The samples were powdered using a Gallenkamp laboratory micro hammer mill and stored in labeled glass bottles at 4°C in a refrigerator for chemical analysis.

Determination of total carbohydrate was carried out by an adaptation of the method of Dubois *et al.* (1956). A solution of each sample was prepared by grinding 10.0 g in a mortar with a pestle and the aid of acid washed sand. Few drops of distilled water were added to make a slurry. The slurry was diluted with distilled water and filtered through a muslin cloth into a 100 mL volumetric flask. The volume of the filtrate was made up to 100 mL with distilled water. About 1.0 mL of each sample solution was pipetted into test tubes in triplicates, each containing 1.0 mL of 5% phenol solution and 5.0 mL concentrated sulphuric acid. The mixture was stirred, allowed to stand for 30 min and read on a spectrophotometer (Junior Coleman, Model 6/20 Spec II, Britain) at 485 μm.

For the determination of nitrogen and crude protein, the Kjeldahl method of digestion was used and the nitrogen in solution assayed in a Technicon Autoanalyser (Technicon Instruments Company, Basingstoke, UK). About 0.2 g of the finely ground sample was weighed into a pyrex test tube in triplicates and 2.0 g of the Kjeldahl catalyst tablet (Na_2SO_4, $CuSO_4$ Seleniuni dioxide) was added, followed by 2 mL of concentrated sulphuric acid. The samples in the test tubes were digested in a fume cupboard until the solution was clear. A blank sample containing only the catalyst and acid was prepared as the control. The digested samples were cooled, diluted to 100 mL with distilled water and the N in the digest determined using a Techincon autoanalyser. Percentage crude protein was determined by multiplying the value of the N in each sample by a converting factor of 6.25 (Deutscher, 1990)

Determination of soluble and tissue-bound amino acid contents in the two test plantain cultivars was carried out using a unidirectional paper chromatography according to the method described by Smith and Feinberg (1977). The tissues analysed were the flowers, mature lamina, immature fruit peel and pulp.

For free amino acid assay, a slurry was prepared from 1.0 g of each sample as described for carbohydrate samples. A 10 mL solution was stirred in a Gallenkamp magnetic stirrer at 1000 rpm for 24 h and concentrated to about 2 mL in a water bath set at 70°C. Using a capillary tube, about 0.01 mL of each sample solution was spotted 2.5 cm apart at different origins on a ruled line of a Whatman No. 1 filter paper to detect free amino acids. Tissue-bound amino acids were assayed for by suspending 1.0 g of each sample in 10 mL distilled water and hydrolysing the sample by the addition of 10 mL of 2N HCL. The mixture was incubated for 4 h at 100°C and allowed to cool. With the aid of capillary tube, about 0.01 μL of each hydrolysed sample solution was spotted at different origins of a paper chromatogram for amino acids detection.

Butanol-glacial-acetic acid-water (40:10:10,v: v: v), solvent was put in a chromatographic tank and allowed to ascend for about 2 h. Standard amino acids were spotted alternatively with the hydrolysed tissue samples at different origins and run in the chromatogram developed with 2% ninhydrin solution as the locating reagent. The

chromatograms were dipped in a tray containing the ninhydrin solution and heated in an oven at 195°C for two minutes. The spots were outlined with pencil immediately the colours were formed. Spotting intensities were scored as + + + (intense), + + (moderate), + (trace) and - (no spotting). Identification of the constituent amino acids was carried out by comparing the spot intensities of tissue hydrolysates with those obtained from the standard amino acids.

To quantify the amount of amino acids in the plantain tissues, the immature fruit peel and pulp of the two test cultivars were assayed. About 1.0 mL of each hydrolysate was added to 0.9 mL distilled water and 1.0 mL of ninhydrin reagent in a test tube. The contents of the tubes were mixed and each tube transferred into boiling water in a beaker placed in a water bath at 100°C for 20 min. The solution was allowed to cool and 6 mL of 50% isopropanol was added and read at a wavelength of 570 μm in the spectrophotometer. A standard calibration curve was prepared with known amino acids and the quantities of the amino acids contained in the samples calculated from the optical densities and expressed as percentages of dry weight of sample.

RESULTS

Significantly diminished ($p \leq 0.05$) concentrations of carbohydrate, crude protein and nitrogen were found in tissues of the false horn plantain P100-F (susceptible) as compared with those in tissues of the 11TA, P200-I (resistant) cultivar (Table 1), using the t-test statistical analysis. The highest carbohydrate and protein concentrations were in fruit pulp samples, relative to other tissues. Concentrations of carbohydrate and protein were significantly enhanced ($p \leq 0.05$) in peel and pulp of the mature as compared with similar tissues of immature samples (Table 2). Carbohydrate content of mature pulp tissue was over 600% and that of protein over 60% higher than of immature ones.

Table 1: Carbohydrate, protein and nitrogen contents of tissues of P100-F and P200-I plantain cultivars

	Plantain cultivar		
Plantain tissue	P100-F	P200-I	Difference[a] (%)
	Carbohydrate (%)		
Bract	0.29[b]	0.349	-20.34**
Lamina	0.298	0.319	-7.05**
Peel (4-7 day old)	0.306	0.334	-9.15**
Pulp (4-7 day old)	0.370	0.540	-5.04**
	Nitrogen (%)		
Bract	0.018	0.025	-38.89*
Lamina	0.13	0.016	-23.07*
Peel (4-7 day old)	0.017	0.020	-17.65*
Pulp (4-7 day old)	0.120	0.140	-16.67**
	Protein (%)[c]		
Bract	0.113	0.156	-38.05**
Lamina	0.081	0.156	-23.46**
Peel (4-7 day old)	0.106	0.125	-17.92**
Pulp (4-7 day old)	0.750	0.875	-16.67**

[a]Difference (%) = P100-F-P200-I/P100-F × 100; differences with * or ** are significant at the 5 or 1% probability levels, respectively. [b]Means of 3 replications. [c] Protein (%) = Nitrogen (%) × 6.25

Table 2: Comparative concentration of carbohydrate and protein in tissues of the susceptible plantain cultivar (P100-F) of different maturity categories

	Plantain tissue		
Nutrient constituent	Immature	Mature	Difference[a] (%)
Peel (%)			
Carbohydrate	0.306±0.002[b]	0.343±0.002	-12.09**
Protein	0.106±0.002	0.118±0.0008	-11.32*
Pulp (%)			
Carbohydrate	3.370±0.029	25.00±0.469	-641.84**

[a]Difference (%) = Immature–Mature/Immature × 100; differences with * or ** are significant at the 5 or 1% probability levels, respectively. [b] Means of three replications

Table 3: Intensity ratings of the amino acids identified in tissues of P100-F and P200-I plantain cultivars

	Plantain tissue[a]							
	P100-F				P200-I			
Amino acid	B	L	Pe	Pu	B	L	Pe	Pu
Alanine	++	++	++	++[b]	+++	+++	+++	+++
Leucine	+	+	++	+	+	++	+++	+

[a]Plantain tissue: B = Bract, L = Lamina, Pe = Peel, Pu = Pulp, [b]+++ = Intense spotting, ++ = Moderate, t = Trace and - = No spotting

Table 4: Comparative amino acid concentrations in tissues of P100-F and P200-I plantain cultivars

	Amino acid (%)					
	P100-F		P200-I (%)		Difference[a]	
Plantain tissue	Ala.[b]	Leu.	Ala.[b]	Leu.	Ala.[b]	Leu.
Immature peel	0.139[c]	0.036	0.360	0.047	-158.99**	-30.56**
Immature pulp	0.158	0.0061	0.368	0.019	-132.91**	-211.48**

[a]Difference (%) = P100-F-P200-I/P100-F × 100; differences with ** are significant at $p \leq 0.01$, [b]Ala = Alanine, Leu = Leucine, [c]Means of 3 replicates

Two ninhydrin - positive spots were identified in the digest of the test plantain cultivars for all four tissues assayed. These spots were identified as alanine and leucine, based on comparison with spotted standard amino acids (Table 3). The spotting intensity for alanine in all four tissues, viz; bract, lamina, immature fruit peel and pulp were more in tissue digests from the resistant (P200-I), when compared with that of the susceptible (P100-F) cultivar. For leucine, the spotting intensity was highest in the peel of P200-I and occurred only as trace in bracts, pulp and lamina tissue of P100-F. In the digests of the two cultivars, alanine values were consistently higher than leucine, while the values of both amino acids were higher ($p \leq 0.01$) in the P200-I than P100-F (Table 4), using the t-test statistical analysis.

DISCUSSION

Highly significantly ($p \leq 0.01$) diminished carbohydrate, crude protein and nitrogen were contained in tissues of the local false horn, P100-F (susceptible), as compared to those of the 11TA accession, P200-I

(resistant). This is similar to the findings of Ayanru (1987) who observed diminished levels of total carbohydrates and metabolizable energy contents in mealy bug infested as compared to uninfested cassava tissues. Ayanru (1970) observed also that root extracts of susceptible oat seedlings consistently leached greater quantities of total carbohydrates than resistant seedlings, while Krog *et al.* (1961) reported a decrease in the sugar content of rust-infected wheat tissues. Inman (1962) implied a close relationship between host carbohydrate levels and growth and differentiation of parasites. He recognized the significance of sugars and proteins as sources of energy and structural material in parasite development. These differences in nutritional status may partly account for observed susceptibility and resistance between the two test plantain cultivars.

Differential concentrations ($p \leq 0.01$) of carbohydrates and proteins were also observed in fruit peel and pulp tissues of the susceptible cultivar (P100-F) of different maturity categories. This finding is of significance and requires further investigation to ascertain the relationship between disease development and nutritional status at different developmental stages. Various physical, chemical and physiological changes have been reported in the developmental stages of plantain (Asiedu, 1987; Collin and Dalnic, 1991; Fermin, 1991), cigar-end rot disease incidence being reported to occur early on immature fruits in the field (Pasberg-Gauhl and Gauhl, 1996).

Alanine and leucine were the amino acids identified from the tissue digests of the two test plantain tissues. The spotting intensity of these two amino acids was more in tissue digest from the P200-I (resistant) when compared with that of the P100-F (susceptible) cultivar. Quantitatively, the values of the two amino acids were also higher ($p \leq 0.01$) in the tissues of P200-I than those of P100-F. These results are similar to those of Burton and DeZeeuw (1961) who reported marked reduction of asparagine and glutamine in severely diseased tissues of scab-infested cucumber foliage. Similar observations have been reported by Benedict and Hilderbrand (1958) and Kuc *et al.* (1959) on stem canker of soybeans and scab infection of apples, respectively. In contrast, however, Ayanru (1970) and Doney *et al.* (1970) implicated elevated amino acid contents in the susceptibility of host plants.

Amino acids are important in a plant's resistance mechanisms (Burton and DeZeeuw, 1961). They play significant roles in nitrogen metabolism (Miller, 1955). However, only limited information is available on the role of amino acids in susceptibility or resistance of plantain to disease incidence and development. Factors which affect nitrogenous compounds in relation to flowering, fructification and growth of plantain have been studied (Steward *et al.*, 1960). Anno and Lambert (1985) carried out soluble amino acid determination in a plantain rhizome to identify the most proteinic amino acids. The essence of the elevated quantities of alanine and leucine reported in the resistant cultivar (P200-I) as compared with the susceptible (P100-F) one in this study, as well as their relevance in the susceptibility and resistance to *V. theobromae* between the two test cultivars needs to be ascertained in further investigations.

REFERENCES

Anno, A. and C. Lambert, 1985. Identification et determination quantitative d'acides amines chez le banamier plantain (cultivar corne 1), In: International Cooperation for Effective Plantain and Banana Research. Proceedings of the third meeting of the International Association for Research on Plantain and Banana (IARPB) held in Abidjan. Cte d'Ivoire, 27-31 May 1985, pp: 65-71.

Asiedu, J.J., 1987. Physiochemical changes in plantain (*Musa paradisiaca*) during ripening and the effect of degree of ripeness on drying. Trop. Sci., 27: 249-260.

Ayanru, D.K.G., 1970. The effects of heterogenous oat populations on the epiphytotic development of Victoria blight. Ph.D. Thesis, IowaStateUniversity of Science and Technology, Ames, Iowa, pp: 193.

Ayanru, D.K.G., 1987. Effects of mealy-bug (*Phenacoccus manihoti)*infestationon on cassava yield components and plant tissue. Der Tropenlandwirt, Zeitschrift fur die landwirtschaft in den Tropen and Subtropen, 88: 5-10.

Benedict, W.C. and A.A. Hilderbrand, 1958. The application of chromatographic methods to a study of the susceptibility of soybeans stem canker. Can. J. Plant Sci., 38:155-163.

Burton, C.L. and D.J. Dezeeuw, 1961. Free amino acid constituents of healthy and scab-infected cucumber foliage. Phytopathology, 51: 776-777.

Collin, M.N. and R. Dalnic, 1991. Evolution of several physio-chemical criteria of plantain (cv. Orishele) during ripening. Fruit, 46: 13-17.

Deutscher, M., 1990. Guide to Protein Purification. Academic Press Inc., London and New York.

Doney, D.L., J.M. Fife, and E.D. Whitney, 1970. The effect of the sugar beat nematode *Heterodera schachtii* on the free amino acids in resistant and susceptible *Beta* species. Phytopathology, 60: 1727-1729.

Dubois, M., A.K. Gilles, J.K. Mamilton, A.P. Rebers, and F. Smith, 1956. Colorimetric method for determination of sugar and related substances. Anal. Chem., 28: 350-356.

Emoghene, A.O., 1996. Strains of Mycosphaerella fijiensis Morelet associated with black leaf streak disease of plantain and banana (*Musa* sp.) in Southern Nigeria and studies on biological control of the disease. Ph.D Thesis. University of Benin, Benin City, pp: 164.

Fermin, A., 1991. Chemical and physical changes in plantain (*Musa paradisiaca)* during ripening. Trop. Sci., 31: 183-187.

Garrett, S.D., 1960. Inoculum Potential, In: Plant Pathology: An Advanced Treatise (Vol. III). Diamond, A.E. and J.C. Horsefall (Eds.), Academic Press, Inc. New York, 3: 23-56.

Gerwitz, D.L. and R.D. Durbin, 1960. Some metabolic changes in wheat due to stem rust infection and different temperatures. Phytopathology, 50: 636 (abstract).

Inman, R.E., 1962. Disease development, disease intensity and carbohydrate level in rusted bean plants. Phytopathology, 52: 1207-1211.

Jones, J.B. and W.J.A. Steyn, 1973. Sampling, handling and analysing of plant samples, In: Soil Testing and Plant Analysis. Soil Science Society of America Inc. Madison, Winscosin, USA., pp: 25-48.

Krog, N.E., D. Letourneau and H. HART, 1961. The sugar content of wheat Leaves infected with stem rust. Phytopathology, 51: 75-77.

Kuc, J., E. Barnes, A. Daftisios and F.B. Williams, 1959. The effect of amino acids on susceptibility of apple varieties to scab. Phytopathology, 49: 313-315.

Miller, E.V., 1955. The Chemistry of Plants. Reinhold Publishing Co., New York, pp: 348.

Munasque, V.S. and D.B. Mendoza, 1990. Developmental physiology and ripening behaviour of Senorita banana (*Musa* sp. L.) fruits. Food J., 5: 152-57.

Pasberg-Gauhl, C. and F. Gauhl, 1996. Musa Research in the Plant Health Management Division at IITA: Activities at the High Rainfall Station, Onne in Nigeria, In: Plantain and Banana: Production and Research in West and Central Africa. Ortiz, R. and M.O. Akoroda (Eds.), Proceedings of a Regional Workshop Held in Port- Harcourt, Rivers State, Nigeria. Sept. 1995. IITA Publishers, Ibadan, pp: 7-14.

Schroth, M.N. and D.C. Hilderbrand, 1964. Influence of plant exudates on root infecting fungi. Ann. Rev. Phytopathol., 2: 101-132.

Smith, I. and J.G. Feinberg, 1977. Paper and Thin Layer Chromatography and Electrophoresis (2nd Edn.). Shandon Southern Products Ltd., Runcorn, England, pp: 233.

Steward, F.C., A.C. Hulme, S.R. Frieberd, M.P. Hegarty, J.K. Pollard, R. Rabason and R.A. Bar, 1960. Physiological investigations on the Banana plant. II- Factors which affect nitrogen compounds of fruits. Ann. Bot., 24: 118-146.

Swennen, R., 1990. Plantain Cultivation under West African Conditions. reference manual. International Institute of Tropical Agriculture (I.I.T.A.) Am. Printing Group Co. Ltd., Thailand, pp: 24.