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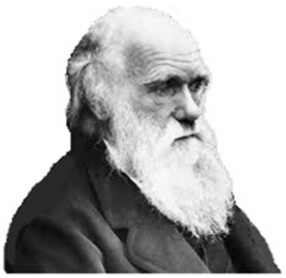

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Anatomical Features of Lignocellulosic Tissues from Underground Rhizomes of *Thaumatococcus daniellii* (Benn.) Benth. in a Rainforest Zone of Nigeria

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ABSTRACT

Published information about anatomical studies of the lignocellulosic material derived from the rhizomes of *Thaumatococcus daniellii* is almost non-existent, thereby necessitating this current study. This is aimed at examining microscopically, the anatomical features of the cell wall structure of this plant's rhizomes. Consequently, rhizomes differentiated into sections, based on the mean height of stalks growing on each section, were harvested in the arboretum of the Department of Forest Resources and Wildlife Management, University of Benin campus, Benin City, Nigeria, washed thoroughly with clean water to remove sand from the surface, placed in ethanol, taken to the laboratory and further fixed in increasingly concentrated ethanol for the periods ranging from 1 to 3 hours. Using a microtome, thin slices of tissues 30 μm thick were obtained from the different sections along the rhizomes in the cross-sectional transverse and longitudinal planes, stained, mounted on glass slides and dried. Observation of tissues mounted on glass slides, including capturing of photomicrographs, were carried out by employing a photomicroscope at 40× magnification. On analysing the different photomicrographs, we saw that the anatomical features of the cell wall for lignocellulosic tissues from the different parts of the rhizome were similar in preponderance and shape in each of the planes. It is expected that the outcome of this research will contribute to the series of background information needed in utilisation and for further researches that will improve knowledge concerning this lignocellulosic material.

***Keywords*:** *Thaumatococcus daniellii*, cell wall tissues, photo-impression, transverse / longitudinal planes, rhizomes

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1. INTRODUCTION

Thaumatococcus daniellii is a plant that widely grows in the hot, humid tropical rain forest and coastal zone of West Africa, with usefulness in a plethora of local applications (Yeboah *et al*, 2003; Ekpe and Ottou, 2006). These applications range from the use of the leaves for wrapping and boiling food to the extraction of an intensely sweet, non-toxic and heat stable protein (thaumatin) from its arils for sweetening or as the taste modifier in beverages, desserts, chewing gums, and pet foods. The earlier cited examples, of course, do not foreclose other uses, such as weaving with the stalks (petioles), as fetish plant, for medicinal purposes, among others. This valuable plant is a rhizomatous, perennial and monocotyledonous herb that propagates itself by rhizomes and can grow up to 2.5 or 3 m in height depending on the age and environment of the plant (Yeboah, 2003; Bartoszewski, 2003; Szwacka, 2002; Inglett, 1968; Adesina, 1977; Wel, 1978).

The plant can be part of the undergrowth of forest trees and has been found to also grow profusely in parts of forest with open canopy. Nevertheless, much of the stalks, for instance, are still poorly utilised in Nigeria even as some studies, such as Oluwadare and Sotannde, (2006), Ogunsanwo *et al*., (2012), and Sotannde and Oluwadare, (2014) have shown that it has appreciable quantity of crude fibres which is an indication of high quantity of cellulose when it was evaluated for its potential as a source of raw material for pulp and paper. If this is the case for the stalks, it should not be unexpected that the plant's underground rhizomes are not currently utilised for applications, such as source of lignocellulosic fibre. The reason for this is not far from the various technical challenges and lack of knowledge on rhizome's properties like cell wall anatomy. Consequent upon the non-utilisation of this plant's rhizome as fibre source, stemming from poor knowledge regarding its properties is the need for studies that will assist in surmounting this challenge. This is particularly important in this country where the demand for lignocellulosic material is increasing, following the reduction in size and quality of forests (Erakhrumen, 2011; 2012; and 2014). In line with the foregoing, this research, which is aimed at characterising the lignocellulosic cell wall anatomy for the underground rhizome of *T. daniellii* was conducted through photomicroscopy of tissues from the cross-sectional transverse and longitudinal portions, in order to provide baseline information needed for understanding its anatomical structure, especially in this part of the world where this kind of study has not been previously carried out.

2. MATERIALS AND METHODS

2. 1. Sample collection

The rhizomes of *T. daniellii* were obtained from the arboretum of the Department of Forest Resources and Wildlife Management, University of Benin, Benin City, Nigeria, located on the southern part of Edo State, Nigeria. This part of Edo State is located between latitude 06°15' N – 06°27' N and longitude 05°30' E – 05°40' E. The topography is flat with gentle slope. The area has an annual rainfall of between 1,500 and 2,000 mm with an average temperature of 25 °C in the rainy season and 28 °C in the dry season. It is part of a low lying plain covered with porous sand that rises gently north–eastward, with soils derived from sand stones and shades and very recent deposits susceptible to leaching (Egbe *et al*., 1989; Kalu and Anigbere, 2011).

2. 2. Specimen Preparation and Microscopic Analyses

The rhizomes were harvested in December 2016. They were categorised into three, based on the mean height of stalks growing on each section of the rhizome as at the time they were harvested. Stalks growing on each section of the rhizome were first measured for their mean heights. Difference in the mean stalk's height was used to distinguish one section of the rhizome from the other since all the plants grew vigorously under the same canopy cover in the arboretum, however, their actual ages were not determined. The part of rhizome having stalks with the shortest mean length growing on it was categorised as Rhizome I, while that with stalks having the mean height falling in between the shortest and the longest length growing on it were categorised as Rhizome II, those with the longest mean stalk height were categorised as Rhizome III. Three different samples of rhizomes were harvested from each of the category making a total of nine.

The harvested rhizomes were thoroughly washed with a clean water to completely remove sand from its surface, properly coded for identification and placed in concentrated ethanol on the field to aid the process of tissue dehydration. Two properly coded tissue specimens, of the same size, were obtained from the rhizomes, on the transverse and longitudinal planes, for the purpose of comparing photomicrographs obtained on the same plane. In total, thirty-six samples (*i.e.* eighteen samples each on the transverse and longitudinal planes) were obtained for microscopic analyses. The tissues sampled from the different locations were further immersed in ethanol solutions of increasing concentrations from 70% until 100% (*i.e.* water free alcohol) for a period ranging from 1 to 3 hours, to ensure water in the tissue was gradually replaced by the alcohol.

Using a manual rotatory microtome, thin slices of tissues of 30 μm thick were obtained from the rhizomes in the transverse and longitudinal planes. Afterwards, these thin slices of tissues were stained using stains with crystal violet and methyl red colours, mounted on glass slides with DPX medium and allowed to dry. Observations of the cell wall tissues were carried out using a nine mega-pixels Amscope 2.0 photomicroscope at 40× magnification to view and obtain the photomicrographs of the slides' content for comparison. The photomicrographs were obtained with this photomicroscope at the laboratory of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

3. RESULTS

Plates 1 to **12** are the pictorial representations of the photomicrographs obtained from the microscopic examinations of the different lignocellulosic tissues from the sampled sections of the rhizomes. Plates 1, 2, and 3 are the first set of photomicrographs for the tissues from the transverse plane on Rhizomes I, II, and III, respectively, while plates 4, 5, and 6 are the second set of photomicrographs for the tissues from transverse plane on Rhizomes I, II, and III, respectively. Plates 7, 8, and 9 are the first set of photomicrographs for the tissues from the longitudinal planes on Rhizome I, II, and III, respectively, while plates 10, 11, and 12 are the second set of photomicrographs for the tissues from the longitudinal planes on Rhizomes I, II, and III, respectively.

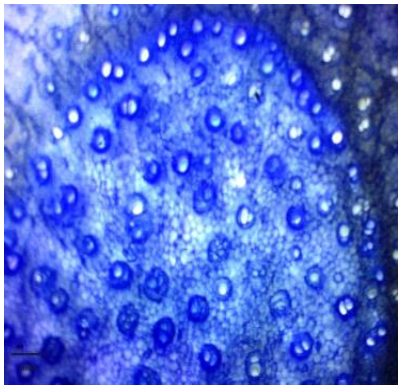

Plate 1. First photomicrograph of lignocellulosic tissue obtained from the transverse plane for Rhizome I

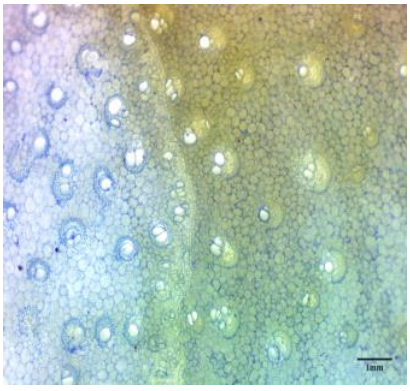

Plate 2. First photomicrograph of lignocellulosic tissue obtained from the transverse plane for Rhizome II

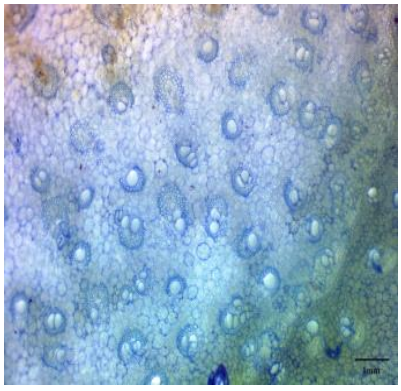

Plate 3. First photomicrograph of lignocellulosic tissue obtained from the transverse plane for Rhizome III

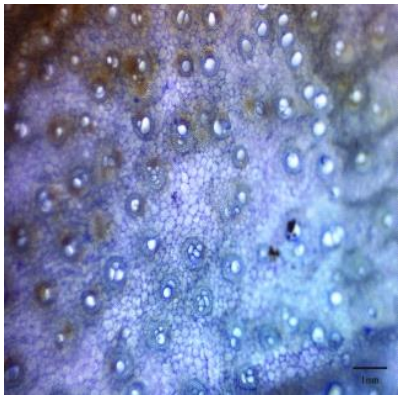

Plate 4. Second photomicrograph of lignocellulosic tissue obtained from the transverse plane for Rhizome I

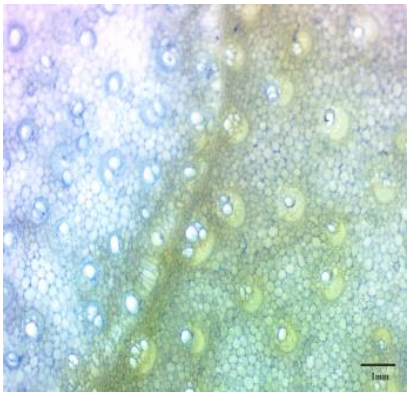

Plate 5. Second photomicrograph of lignocellulosic tissue obtained from the transverse plane for Rhizome II

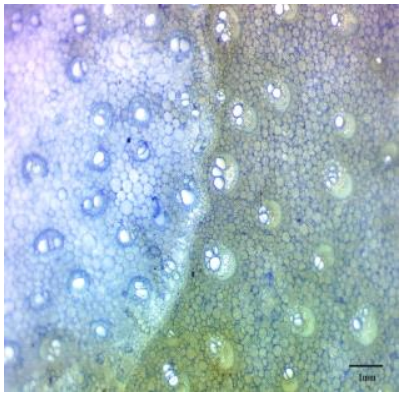

Plate 6. Second photomicrograph of lignocellulosic tissue obtained from the transverse plane for Rhizome III

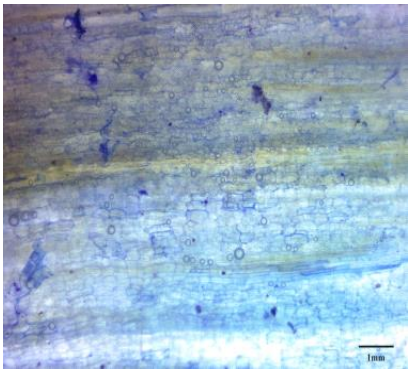

Plate 7. First Photomicrograph of lignocellulosic tissue obtained from the longitudinal plane for Rhizome I

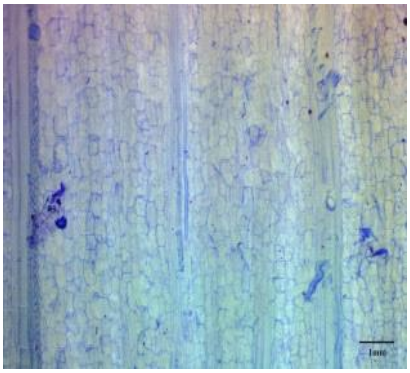

Plate 8. First photomicrograph of lignocellulosic tissue obtained from the longitudinal plane for Rhizome II

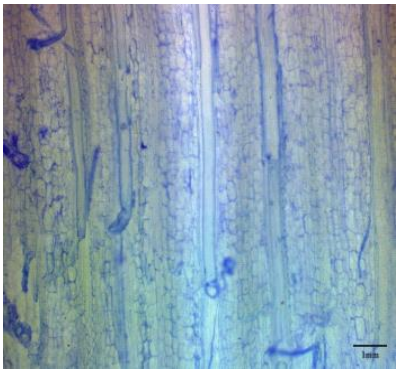

Plate 9. First photomicrograph of lignocellulosic tissue obtained from the longitudinal plane for Rhizome III

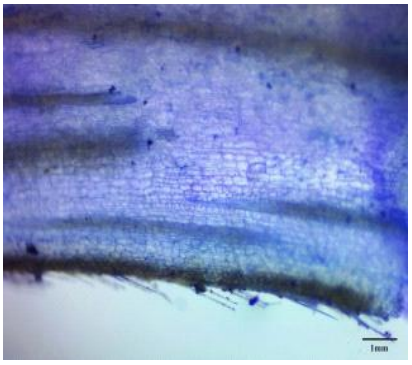

Plate 10. Second photomicrograph of lignocellulosic tissue obtained from the longitudinal plane for Rhizome I

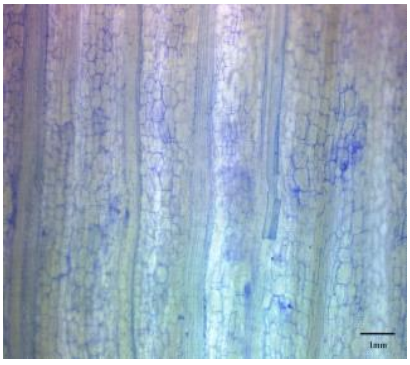

Plate 11. Second photomicrograph of lignocellulosic tissue obtained from the longitudinal plane for Rhizome II

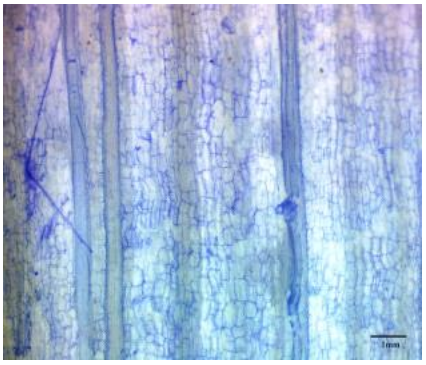

Plate 12. Second photomicrograph of lignocellulosic tissue obtained from the longitudinal plane for Rhizome III

4. DISCUSSION

The photomicrographs obtained for the lignocellulosic tissues sampled on the cross-sectional transverse and longitudinal planes showed that the cell wall constituents are in variable sizes and shapes. Nonetheless, it could be seen on the transverse plane that cell wall fibres are predominantly oval in shape while they could be seen as strands on the longitudinal plane. This is as a result of the way the tissues were oriented and sourced on the two planes. Observations comparable to these (although not on underground rhizomes) had been noted for some other non-wood fibre plants (Gritsch *et al*., 2004; Abdul Khalil *et al*., 2010; Kaur and Dutt, 2013). This observation, on the two planes, appeared similar for the lignocellulosic tissues obtained from Rhizome I, II, and III. This implies that the preponderance, sizes, and shapes of these cells appeared similar along the rhizome.

In the same manner, it was noticed that when the first and second photomicrographs obtained for lignocellulosic tissues sourced from the same section of the rhizome were compared, they appeared similar in preponderance and shapes of constituent materials under the microscope. This observation of similarity in the first and second photomicrographs obtained for lignocellulosic tissues sourced from the same part of the rhizome was also noted on the transverse and longitudinal planes. In line with these results, it can be assumed that the anatomical presentation by the second photomicrograph is an affirmation of the first one obtained for lignocellulosic tissues sourced from the same part of the rhizome, thereby, implying that what was seen under the microscope is the true situation, concerning the rhizomes' lignocellulosic matrix at the different sections along its length.

In addition, the cell wall anatomy presented by these photomicrographs on both planes, gave an impression of one that was similar to those of wood and non-wood lignocellulosic cell wall where vessels are grounded and embedded within the fibrous tissue (Ashori *et al*., 2011; Abdul Khalil *et al*., 2010; Kaur and Dutt, 2013). However, this assertion is not intended to gloss over the fact that cell walls for non-wood fibre are noted to be different from those of wood irrespective of the relative ease with which they can be explained to be similar in line with their variable but comparable cellulosic, hemicellulosic, and lignin content (Abdul Khalil *et al*., 2006; Abdul Khalil *et al*., 2008). Consequently, the simplistic deduction that can be made, as a

result of this similarity noted earlier, is that this underground rhizome may be useful in some applications for which lignocellulosic fibre from wood are used.

Furthermore, the photomicrographic view of the cell wall, for the transverse and longitudinal planes, also revealed somewhat similar anatomical presentations along the rhizome. What this likely indicates, based on lignocellulosic cell wall photomicrography, is that the rhizome in all sections of its length might possess similar preponderance and shapes of fibre. This observation also appears not to be dependent on the size and ages of the stalks growing on the different sections of the rhizome, although it is noteworthy that stalks' ages were not determined in this study. Nevertheless, the motive behind sampling lignocellulosic tissues along the rhizome, where the stalks with different mean height were growing, was to see if there are anatomical differences, based on photomicrographs, in the constituents of rhizome's cell wall related to stalk's tenderness and age.

For the avoidance of doubt and as stated earlier, there appeared not to be difference, anatomically, in the cellular constituents along the length of the underground rhizomes in terms of occurrence and shape of fibre cells, based on the photomicrographs. What can be inferred from the foregoing is that maturation and cellular characteristics of this plant's rhizome is unlikely to be influenced by the petioles growing on it. It can therefore be deduced, from utilisation standpoint, that every part of the rhizome can be randomly harvested. These assertions cannot be substantiated here as they are beyond the scope of this study. However, it will be worthwhile to carry out further research with a view to resolving these present uncertainties identified earlier including others that can assist in maximal and sustainable utilisation of this lignocellulosic material.

5. CONCLUSION

Based on the results of the photomicrography of lignocellulosic tissues obtained from the different parts of the rhizomes in the transverse and longitudinal planes, it could be stated that this part of *Thaumatococcus daniellii* may possess some of the cell wall characteristics required to be considered a potential alternative to lingocellulosic fibres from woody plants in some applications. Therefore, insights from this study have provided a basis for further research into the improvement of the knowledge base with respect to this lignocellulosic material.

References

[1] Abdul Khalil, H.P.S., Ireana Yusra, A.F., Bhat, A.H., and Jawaid, M. (2010). Cell Wall Ultrastructure, Anatomy, Lignin Distribution, and Chemical Composition of Malaysian Cultivated Kenaf Fibre. *Industrial Crops and Products*, 31: 113–121.

[2] Abdul Khalil, H.P.S., Siti Alwani, M., and Mohd Omar, A.K., (2006). Chemical Composition, Anatomy, Lignin Distribution, and Cell Wall Structure of Malaysian Plant Fibers. *Bioresources*, 1: 220–232.

[3] Abdul Khalil, H.P.S., Siti Alwani, M., Ridzuan, R., Kamarudin, H., and Khairul, A. (2008). Chemical Composition, Morphological Characteristics, and Cell Wall Structure

of Malaysian Oil Palm Fibers. *Polymer-Plastics Technology and Engineering*, 47: 273–280.

[4] Ashori, A., Hamzeh, Y., and Amani, F.J. (2011). Lemon Balm (*Melissa officinalis*) Stalk: Chemical Composition and Fiber Morphology. *Journal of Polymers and the Environment*, 19(1): 297–300.

[5] Egbe, N.E., Ayodele, E.A., and Obatotu, C.R. (1989). Soil Nutrition of Cocoa, Kola, Cashew and Trees: Progress in Tree Crop Research (2nd Edition): Ibadan, Nigeria. Pp. 28–30.

[6] Ekpe, K.E. and Ottou, R. (2006). Benefiting from Conservation: How the Collection of *Thaumatococcus daniellii* Fruits in Ghana is emerging as a Forest Industry. *Sylvanet*, 19(1): 2–4.

[7] Erakhrumen, A.A. (2011). Recent Trends in Global and Regional Forest Cover Changes: Continuously Increasing Forest Size and Quality are Crucial Steps toward Environmental Sustainability. In: Forestry in the Context of the Millennium Development Goals (L. Popoola, K. Ogunsanwo and F. Idumah, eds). Proceedings of the 34th Annual Conference of the Forestry Association of Nigeria held in Osogbo, Nigeria, from the 5th to 10th December, 2011, volume 1: 476–496.

[8] Erakhrumen, A.A. (2012). Recent Estimations of Net Forest Cover Changes in Nigeria and their likely Implications on Efforts toward Carbon Emission Reduction. In: De-reservation, Encroachment and Deforestation: Implications for the Future of Nigerian Forest Estate and Carbon Emission Reduction. (J.C. Onyekwelu, B.O. Agbeja, V.A.J. Adekunle, G.A. Lameed, P.O. Adesoye and A.O. Omole, eds). Proceedings of the Third Biennial National Conference of the Forests and Forest Products Society held in Ibadan, Nigeria, from the 3rd to 6th April, 2012, pp 582–590.

[9] Erakhrumen, A.A. (2014). Declining Tropical Forests: A Challenge to Sustainability of Forest-Dependent Industries and Climatic Stability. In: Forests and Forest Products: Key to Sustainable Livelihood (M.O. Adedire, J.C., Onyekwelu, D.O. Oke, V.A.J. Adekunle, O.A. Jayeola and A.O. Oladoye, eds). Proceedings of the Fourth Biennial National Conference of the Forests and Forest Products Society held in Abeokuta, Nigeria, from the 22nd to 26th April, 2014, pp 26–34.

[10] Gritsch, C.S., Kleist, G., and Murphy, R. (2004). Developmental Changes in Cell Wall Structure of Phloem Fibres of the Bamboo *Dendrocalamus asper*. *Annals of Botany*, 94: 497–505.

[11] Kalu, C. and Anigbere, R.F. (2011). Social Benefits of Non-Timber Forest Products (NTFPS): An Assessment of Employment Generation from NTFPS Enterprises in Benin Metropolis, Edo State, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*, 7(2): 30–35.

[12] Kaur, H. and Dutt, D. (2013). Anatomical, Morphological and Chemical Characterization of Lignocellulosic By-Products of Lemon and Sofia Grasses Obtained after Recuperation of Essential Oils by Steam Distillation. *Cellulose Chemistry and Technology*, 47(1-2): 83–94.

[13] Ogunsanwo, O.Y., Adedeji, G.A., and Ajibabi, A.S. (2012). Pulping Potential of *Thaumatococcus daniellii* (Benn) Benth. in Omo and Oban Forest Reserve of Nigeria. *International Journal of Science and Nature*, 3(2): 580–585.

[14] Oluwadare, A.O. and Sotannde, O.A. (2006). Variation of the Fibre Dimensions in the Stalks of Miraculous Berry (*Thaumatococcus danielli* Benth). *Production Agricultural Technology*, 2(1): 85–90.

[15] Sotannde, O.A. and Oluwadare, A.O. (2014). Fibre and Elemental Contents of *Thaumatococcus daniellii* Stalk and its Implications as a Non-Wood Fibre Source. *International Journal of Applied Science and Technology*, 4(1): 178–185.

[16] Yeboah S.O., Hilger T.H., and Kroschel J. (2003). *Thaumatococcus daniellii* (Benn.) Benth. – A Natural Sweetener from the Rain Forest Zone in West Africa with Potential for Income Generation in Small Scale Farming. A Paper Presented at Deutscher Tropentag hosted by Georg-August-Universität Göttingen from the 8th to 10th October, 2003. Technological and Institutional Innovations for Sustainable Rural Development. 11 pages.

[17] G. Bartoszewski, A. Niedziela, M. Szwacka, and K. Niemirowicz-Szczytt. Modification of tomato taste in transgenic plants carrying a thaumatin gene from *Thaumatococcus daniellii* Benth. *Plant Breeding* Volume 122, Issue 4, August 2003, Pages 347-351

[18] Maria Szwacka, Magdalena Krzymowska, Anita Osuch, Magdalena Ewa Kowalczyk, and Stefan Malepszy. Variable properties of transgenic cucumber plants containing the thaumatin II gene from *Thaumatococcus daniellii*. *Acta Physiologiae Plantarum* June 2002, Volume 24, Issue 2, pp 173–185

[19] G. E. Inglett and Joann F. May. Tropical plants with unusual taste properties *Economic Botany* October 1968, Volume 22, Issue 4, pp 326–331

[20] Simon K. Adesina and John D. Higginbotham. Studies on a novel polysaccharide gel from the fruit of *Thaumatococcus daniellii* (Benth). *Carbohydrate Research* Volume 59, Issue 2, December 1977, Pages 517-524

[21] H. van Der Wel, A. Wiersma, and J.N. Brouwer. Tritium labelling of Thaumatin I, a sweet-tasting protein from Thaumatococcus daniellii benth, by reductive methylation. *Journal of Labelled Compounds and Radiopharmaceuticals* Volume14, Issue5, 1978, Pages 735-740.