COMPARATIVE STUDY OF THAUMATIN PROTEIN FROM *Thaumatococcus daniellii* GROWN AT DIFFERENT GROWTH ENVIRONMENTS

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ABSTRACT

Introduction: This study was carried out to investigate thaumatin profile in *Thaumatococcus daniellii* fruit cultivated in different growth condition. **Methods:** Three different samples used were *T. daniellii* that grown naturally, *T. daniellii* treated with low fertilizer and *T. daniellii* treated with high fertilizer. The aril part of the fruit contains intense sweetener protein that can substitute sugar and other artificial sweetener was analaysed by Bradford assay and SDS-PAGE for determination of protein content and differences in protein expression repectively. **Results:** The total protein concentration of each sample calculated using Bradford assay were 0.367 ± 0.007 µg/µl, 0.343 ± 0.014 µg/µl and 0.426 ± 0.027 µg/µl for *T. danielli* grown at natural condition (uncontrolled environment), *T. daniellii* treated with low fertilizer and *T. daniellii* treated with high fertilizer respectively. All *T. daniellii* samples showed the presence of thaumatin as shown by the SDS-PAGE image. Only a single band appeared on the SDS-PAGE at the molecular weight of 22 kDa which is the actual size for thaumatin. The band intensity of *T. danielli* treated with high fertilizer appeared to be highest compared to the other two samples showing high in protein expression. **Conclusion:** This finding will be useful for other researchers to find out more about this plant for thaumatin production with the best growth condition.

KEYWORDS: *Thaumatoccocus danielli*, **t**haumatin, Bradford assay, SDS-PAGE, protein expression

INTRODUCTION

Since ancient times, human really appreciate the savory taste of sweetness in food and drinks. This behavior has led to adverse health effect such as diabetes, obesity and dental cavity problems as most human only care about sweet food (Lustig, Schmidt, & Brindis, 2012). Therefore, artificial sweeteners have been developed to counteract these problems. Artificial sweeteners are said to have low calories and capable to mimic the sweet taste of sugar. However, there a lot of research done recently to show the controversies regarding the health risk due to ingesting artificial sweetener in food (Swithers, 2013). Nowadays, people are more interested in natural sweetener which are taken from plant such as thaumatin protein from *Thaumatococcus daniellii* fruit.

Originated from coastal areas and tropical rain forests of West Africa, *T. daniellii* or also known as ketamfe is a rhizomatous plant. The most abundance place to find this plant is in Nigeria, Ghana and Cote d'Ivoire (Chinedu et al., 2014). *T. daniellii* contributes a lot to the economy in West Africa especially in rural area via selling its fruits, leaves, stalks and rhizomes (Arowosoge & Popoola, 2002). The aril of *T. daniellii* contains a very sweet, heat-stable and safe protein called thaumatin. This protein commonly used for taste enhancer in desserts, beverages, pet foods and chewing gums (Yeboah, Hilger & Krochcel, 2002).

According to De Vos et al. (1985), thaumatin is said to be 3000 times sweeter than sucrose on weight basis. The properties of heat stable and acidic tolerant of this protein makes it a very impressive sweetener for pharmaceutical application. Moreover, thaumatin can also be used flavor enhancement and masking the bitterness and astringency especially in medicines. Other than that, thaumatin is a highly soluble in water and stable in freeze-dried form (De Vos et al., 1985). Therefore, these properties of thaumatin make it easier for scientists to extract the protein.

One of the concerns about *T. daniellii* is the environmental factors affecting the content of thaumatin. According to Waliszewski et al. (2005), cultivators of this plant have determined that best soil for growth of *T. daniellii* is the pure soil. Other factors contributing to growth of *T. daniellii* are frequency of rainfall, usage of fertilizers, shading and human activity including hunting, cultivation and insecticide application (Sweetman, 2014). There are only a few researches that have been done on environmental effect contributing to the content of thaumatin. Hence, the aim of this study is to compare the thaumatin content and expression in *T. daniellii* fruit in natural and controlled environment. Thaumatin protein from both sources were extracted, quantified using Brafford assay and analyzed using SDS PAGE.

MATERIAL AND METHODS

Sample collection and preparation

Aril part of fruit of *T. daniellii* was used as a starting material for thaumatin extraction. Two samples from fruits of *T. daniellii* grown in controlled condition (high and low fertilizers respectively) and one sample from fruits of *T. daniellii* grown in natural environment were used. The *T. daniellii* samples grown in controlled condition were obtained from Universiti Teknologi MARA (UiTM) Shah Alam while *T. daniellii* sample grown in natural environment was obtained from Botanical Garden in Tawau, Sabah. High irradiance ranged between 30 to 50% was given to the two samples grown in controlled condition. Besides that, two samples grown in controlled condition were also treated with complete blue and green nitrogen (N), phosphorus (P) and potassium (K) or NPK at the rates of 5 g and 10 g respectively for low and high fertilizers condition.

Thaumatin Extraction

The method for thaumatin extraction was done using simple conventional technique demonstrated by Wel and Loeve (1972) with some modifications. Firstly, the aril of the fruit was mechanically cut from above the seed using a small blade. The amount of aril taken from each sample was weighted and recorded. After that, the aril was freeze-dried for 1 to 2 days. Next, the arils were grinded into fine powder by using mortar and pestle. Distilled water was added to the powder with ratio of 4 mg

powder in 1 ml distilled water. The mixture solution was transferred into 50 ml falcon tube and kept at -20 °C prior to further analysis.

Protein Quantification

Standard Bradford assay was used for protein quantification for each extracted thaumatin sample and Bovine Serum Albumin (BSA) was used as the standard. The absorbance for respective BSA concentrations and thaumatin were measured using spectrophotometer at 595 nm.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The 12 % resolving gel of Tris-HCl buffers with pH 8.8 and 4 % stacking gel of Tris-HCl buffers with pH 6.8 was used to separate thaumatin extracted from *T. daniellii* that grow at different growth conditions. The electrophoresis was ran at 60 mA and at voltage of 110 V for 2 hours. Then the gel was removed from the cassette and was stained with staining solution of Coomasie Brilliant Blue R250 with gradual shaking for overnight. Then, the gel was destained using destaining solution with gradual shaking. Finally, the gel was viewed by using densitometer GS-800.

RESULTS AND DISCUSSION

Protein Quantification

The concentration of the protein in *T. daniellii* varied between samples as presented in Table 1. *T. daniellii* treated with high fertilizer has the highest protein concentration (0.426 ± 0.027 μg/μl), whereas *T. daniellii* treated with low fertilizer has the lowest protein concentration (0.343 ± 0.014 μg/μl). Meanwhile, naturally grown *T. daniellii* has protein concentration of 0.367 ± 0.007 μg/μl.

Table 1 Protein concentration of different *T. daniellii* samples.

Growth Condition of Sample	Protein Concentration (μg/μl)
Natural grown	0.367 ± 0.007
Low fertilizer treatment	0.343 ± 0.014
High fertilizer treatment	0.426 ± 0.027

From the protein quantification result, *T. daniellii* treated with high fertilizer was expected to have higher protein content than naturally grown *T. daniellii* as blue and green NPK fertilizer contain one of the most important nutrients which is nitrogen. Nitrogen is an important component of nucleic acids which responsible for protein synthesis in plant (Heidari & Mohammad, 2012). According to Song et al., (2012), application of nitrogen fertilization affectively increases the protein content in rice grain. Besides, protein content in soybean seed also increases with increase dosages of NPK (Perkasa, Utomo & Widiatmoko, 2016). NPK fertilizer also help to improve fruiting habits, hasten maturity as well as increasing disease resistance and protein levels in seeds of eggplant (Nafiu et al., 2011).

However, *T. daniellii* treated with low fertilizer has lower protein content compared to naturally grown *T. daniellii* sample. This might happen due to possible protein degradation in the sample. However, protein samples that being degraded would still appear in assay (Kinoshita et. al, 2009). Lastly, every protein has its own optimum pH to stabilize for its metabolism, storage and activity. That is why a protein could denature and become inactivated as well as losing their main function outside permissible pH range (Robinson, 2015). Therefore, it is very important in any study or experiment to maintain the pH of a protein solution.

Irradiance also play important role in influencing protein content in *T. daniellii* samples. 30 to 50% irradiance means that only 30 to 50% sunlight will reach will the plant. With this condition, the plant will have 50 to 70% more shade than naturally grown *T. daniellii*. According to Blair, Alcaniz and Harrell (1983), crude protein content in plant and leaves will increase as shade deepened.

Moreover, long-term presence in high irradiance can results in loss of light-harvesting pigment proteins compared to leaves exposed to low irradiance (Anderson & Osmond, 1987). This might directly or indirectly affect the protein content in *T. daniellii* samples. This situation also depends on plant species as most plant can acclimate to different irradiance condition by altering some protein and enzyme complexes.

Protein Profiling

In this experiment, SDS-PAGE was used to separate the protein based on their molecular weight and size. Based from SDS-PAGE result, all three *T. daniellii* samples have their bands appear at the size of 22 kDa which is similar to the actual molecular weight of thaumatin as shown in Fig 1 as indicated by arrow. It could be clearly seen that *T. daniellii* treated with high fertilizer has the highest band intensity compared to *T. daniellii* grown in natural environment and *T. daniellii* treated with low fertilizer. Thus, it can be concluded that the best condition to grow *T. daniellii* plant is with treatment of high fertilizer which the thaumatin will be expressed more in order to get more thaumatin yield.

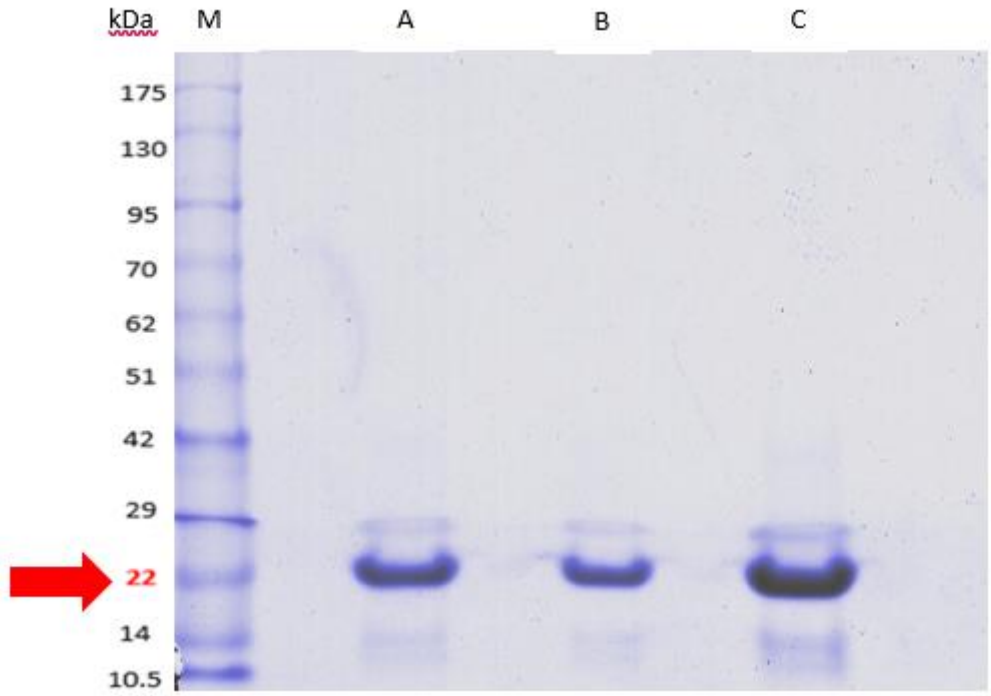

Figure 1. SDS-PAGE profiles of thaumatin proteins from *T. daniellii* grown in different environment. M, Protein marker; A, *T. daniellii* grown in natural environment, B= *T. daniellii* grown in controlled condition (low fertilizer), C= *T. daniellii* grown in controlled condition (high fertilizer).

According to Lu et al. (2005), higher or greater amount of nitrogen treatment will increase expression of various genes involved in protein metabolism. The reason for *T. daniellii* grown in high fertilizer condition to have highest band intensity might be due to increased protein expression. Protein expression can be affected by the amount and form of fertilizer used. Using fertilizer that contain high amount of organic nitrogen, protein expression in maize can be manipulated to reduce environmental pollution without decreasing crop yield (Guo et al., 2015). This clearly showed that good fertilizer usage and management can affect the protein expression of plant.

CONCLUSIONS

In conclusion, all extracted protein of *T. daniellii* samples grown in different environment were estimated to contain thaumatin, indicated by the appearance of the polypeptide bands at the size of 22 kDa. Other than that, *T. daniellii* treated with high fertilizer has the highest protein concentration and protein expression compared to the other two tested samples, *T. daniellii* grown in natural environment and *T. daniellii* treated with low fertilizer.

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