EFFECTS OF LIGHT SOURCES AND DRYING METHODS ON PLANT GROWTH AND STEVIOL GLYCOSIDES CONTENT OF STEVIA (Stevia rebaudiana Bertoni)

MOHD AZIZ BIN RASHID

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EFFECTS OF LIGHT SOURCES AND DRYING METHODS ON PLANT GROWTH AND STEVIOL GLYCOSIDES CONTENT OF STEVIA (Stevia rebaudiana Bertoni)

By

MOHD AZIZ BIN RASHID

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2017
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of
the requirement for the degree of Master of Science

EFFECTS OF LIGHT SOURCES AND DRYING METHODS ON PLANT
GROWTH AND STEVIOL GLYCOSIDES CONTENT OF STEVIA
(Stevia rebaudiana Bertoni)

By

MOHD AZIZ BIN RASHID

June 2017

Chairman : Phebe Ding, PhD
Faculty : Agriculture

Stevia (Stevia rebaudiana Bertoni) has received great attention with the rise in demand
for low-sugar food and beverage additives, and natural alternative to sugarcane sugar
and artificial sweeteners. The leaves produce intensively sweet steviol glycosides
(mainly stevioside and rebaudioside A). Stevia is an obligate short-day plant with a
critical daylength of 13 h. Daylength in Malaysia which is less than 13 h causes stevia
to flower early, resulting in a low leaf yield and a low percentage of sweetener content.

In the first experiment, the effects of night interruption technique using incandescent,
fluorescent cool day light (C.D.L.), fluorescent warm white (W.W.), light-emitting
diode (LED) C.D.L. and LED W.W. for 60 min daily to lengthen vegetative phase, on
plant biomass and steviol glycoside content of stevia were investigated. The study was
carried out using a nested design with a 6 x 4 factorial arrangements of six light sources
and four growing weeks after night interruption initiated (week 2, 4, 6 and 8) with four
replications. Results showed that night interruption technique extended vegetative
phase from 20 days (control) to 120 days; thus allowing accumulation of plant biomass
and steviol glycosides content of stevia were investigated. The study was
carried out using a nested design with a 6 x 4 factorial arrangements of six light sources
and four growing weeks after night interruption initiated (week 2, 4, 6 and 8) with four
replications. Results showed that night interruption technique extended vegetative
phase from 20 days (control) to 120 days; thus allowing accumulation of plant biomass
and steviol glycosides content. Leaf biomass and steviol glycosides of all light sources
treated plants increased significantly as compared to control plants especially in week 6
and 8. Fluorescent W.W. can be considered as the best light source for night
interruption technique of stevia as total steviol glycosides content per plant increased
by 190-270% as compared to other light sources. The second experiment was
conducted to determine the effects of several drying methods on steviol glycosides
content of stevia leaves. Six drying method treatments were arranged in a completely
randomized design (CRD) with four replications. Stevioside content was not
significantly affected by the drying of sun, air, oven and freeze at different temperature
to obtain pre-determined moisture content (MC). However, rebaudioside A content was
significantly reduced by 3.38% under oven drying at 70 °C to obtain 10% MC. The
reduction indicated that thermal degradation of rebaudioside A occurred at higher
temperature. In conclusion, sun drying is the easiest and cheapest technique to dry
stevia leaves without causing significant reduction of stevioside and rebaudioside A.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN SUMBER CAHAYA DAN KAEDAH PENGERINGAN KEPADA PERTUMBUHAN POKOK DAN KANDUNGAN GLIKOSIDA STEVIOL STEVIA (Stevia rebaudiana Bertoni)

Oleh

MOHD AZIZ BIN RASHID

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terma berlaku pada suhu tinggi. Kesimpulannya, pengeringan matahari adalah teknik paling mudah dan murah untuk mengerinkan daun stevia tanpa menyebabkan pengurangan signifikan kepada steviosida dan rebaudiosida A.
ACKNOWLEDGEMENTS

First of all, I would like to express my utmost appreciation to my supervisory committee chairman, Associate Professor Dr. Phebe Ding for the opportunity to conduct this study and for the guidance, advice, encouragements, critical and profound suggestions imparted throughout the course of the study. My appreciation also goes to my supervisory committee member, Associate Professor Dr. Siti Aishah bt Hassan for her assistance throughout my research. Special thanks and appreciation also goes to my former mentor Dr. Abd Jamil bin Zakaria (Universiti Sultan Zainal Abidin) and Dato' Dr. Mohamad Zabawi b. Abd Ghani (Malaysian Agricultural Research and Development Institute) for their constructive criticism, suggestions and support. I also would like to thank the Malaysian Agricultural Research and Development Institute (MARDI) for allowing me to use their research facilities such as rainshelter, laboratory and research instruments at MARDI Headquarters Serdang, Selangor. Special thanks to my former research assistant, Hj Md Noh Hj Ismail for the kind help and assistance. My heartiest appreciation goes to my beloved parents, Hj. Rashid bin Mamat and Hjh. Hasnah bt Abdullah, my sisters and brothers for their support and encouragement. Last but not least, my appreciation goes to my friends, Mr. Mohd Najib Mohd Arif, Mr. Khairudin Che Tak, Mr. Mohd Fadli Hassan, Mr. Mir Nizam Abd. Suki, Mr. Mohd Asyraf Kassim, Mr. Azuan Amron, Madam Norziana Zina Zawawi and Madam Maya Izar Khaidizar for their help, support and motivation throughout my study.
I certify that a Thesis Examination Committee has met on 14 June 2017 to conduct the final examination of Mohd Aziz bin Rashid on his thesis entitled "Effects of Light Sources and Drying Methods on Plant Growth and Steviol Glycosides Content of Stevia (Stevia rebaudiana Bertoni)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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Effects of drying methods on total steviol glycosides content per leaf dry weight. Error bars represent standard error of the means. Means with the same letter are not significantly different by LSD at $P \leq 0.05$. 

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<td>AGR</td>
<td>Absolute growth rate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>AOP</td>
<td>Antioxidant properties</td>
</tr>
<tr>
<td>C.D.L.</td>
<td>Cool day light</td>
</tr>
<tr>
<td>CRD</td>
<td>Completely Randomized Design</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DMRT</td>
<td>Duncan’s Multiple Range Test</td>
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<td>ELSD</td>
<td>Evaporative Light Scattering Detector</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>Fm</td>
<td>Maximum fluorescence</td>
</tr>
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<td>Fo</td>
<td>Initial fluorescence</td>
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<tr>
<td>Fv</td>
<td>Variable fluorescence</td>
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<tr>
<td>GA</td>
<td>Gibberellins</td>
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<td>GRAS</td>
<td>Generally Recognized as Safe</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<td>IBA</td>
<td>Indole-3-butyric acid</td>
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<td>LAI</td>
<td>Leaf Area Index</td>
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<td>LAR</td>
<td>Leaf area ratio</td>
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<td>LED</td>
<td>Light-emitting diode</td>
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<td>LD</td>
<td>Long-day</td>
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<td>LSD</td>
<td>Least Significant Differences</td>
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<td>MARDI</td>
<td>Malaysian Agricultural Research and Development Institute</td>
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<td>MC</td>
<td>Moisture content</td>
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<tr>
<td>NPK</td>
<td>Nitrogen, phosphorus and potassium</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>NS</td>
<td>Non-significant</td>
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<tr>
<td>P</td>
<td>Probability</td>
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<td>PAR</td>
<td>Photosynthetically Active Radiation</td>
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<td>Pfr</td>
<td>Phytochrome far red</td>
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<td>ppm</td>
<td>parts per million</td>
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<tr>
<td>Pr</td>
<td>Phytochrome red</td>
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<td>rpm</td>
<td>revolutions per minute</td>
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<td>RT</td>
<td>Retention time</td>
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<td>r</td>
<td>Pearson’s correlation coefficients</td>
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<td>SAS</td>
<td>Statistical Analysis System</td>
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<td>S.E.</td>
<td>Standard error</td>
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<td>SLA</td>
<td>Specific leaf area</td>
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<td>SD</td>
<td>Short-day</td>
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<td>TPC</td>
<td>Total phenolic content</td>
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<tr>
<td>U.S.A.</td>
<td>United States of America</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra-violet</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>W.W.</td>
<td>Warm white</td>
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<tr>
<td>µl</td>
<td>Microliter</td>
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CHAPTER 1

INTRODUCTION

1.1 Background

Stevia has received great attention with the rise in demand for low-carbohydrate, low-sugar food and beverage additives. Leaves of this plant produce a zero-calorie, non-nutritive, high potency sweetener with a sweetening value which is 150-450 times (by weight) higher than sucrose (Kinghorn, 1987). The sweeteners, containing steviol glycosides can be used as a natural alternative sweetener to other synthetic sweeteners such as aspartame, saccharine or acesulfame-K that constantly being associated with health concerns (Puri et al., 2011). Steviol glycosides are considered safe for consumption with no major contradictions, warnings and side effects reported (Ferri et al., 2006; Geuns, 2000).

Stevia was first introduced into Malaysia in the mid-1970’s but failed to turn into a commercial crop mainly due to lack of suitable varieties, crop management and production technology (Ghawas et al., 2009). All variety of stevia introduced from other producing countries are photoperiod-sensitive. Daylength in Malaysia (which is less than 13 h) causes stevia to flower early, resulting in a low leaf yield per harvest and a low percentage of sweetener content. Early flowering also causes frequent harvesting and this leads to higher labour requirement and increases in production cost (Tan et al., 2008). The plants were harvested approximately every month (with lower leaf yield) as compared to 3-5 times annually in countries with longer daylength.

The use of classic incandescent lamps (rich in far-red light) as night interruption delayed the flowering and induced stevioside content of stevia (Zaidan et al., 1980) but it was considered not economical due to long hours of exposure required and less energy-efficiency. Commercial bulbs such as fluorescent lamps and light-emitting diode (LED) are more energy efficient, however its effect on plant biomass and flowering of stevia is still lacking. In this study, several types of commercial bulbs were used to impose night interruption in stevia plants and its effects to increase leaf biomass, delay flowering and induce steviol glycosides contents were determined.

Several drying methods are used to dry stevia leaves but the most preferable and practiced method is by sun drying (Samsudin and Aziz, 2013; Gates, 1997). However, sun drying will probably reduce the steviol glycosides content in the leaves. Abou-Arab et al. (2010) found that sun drying for 48 h caused 0.81% reduction of stevioside in stevia leaves. According to Steve Marsden of Herbal Advantage Inc., very little steviol glycoside will be lost if sun-drying for 8 h or less (Richard, 1996). However, proper experiment and scientific data are required to confirm the significance of steviol glycosides reduction by drying methods. The study on the effects of drying methods on steviol glycosides content is still lacking. In the second study, the effects of several drying methods on steviol glycosides content were determined.
1.2 Objectives

The objectives of the study were:

1. To determine the effects of different light sources as night interruption on vegetative and reproductive growth, and steviol glycosides content of stevia.
2. To determine the effects of several drying methods on steviol glycosides content of stevia.

1.3 Significance of study

The demand for stevia is growing due to increase cases of Diabetes mellitus, cardiovascular diseases, hypertension, obesity and other health problems among Malaysians, as well as concern over the safety of chemical sweeteners. Sweeteners from stevia plant are natural, zero-calorie, has a low glycemic index, can reduce blood sugar level and safe for consumption. However, the photoperiod-sensitive in stevia causes the plants to flower early that leads to low leaf yield and low sweetener content, thus preventing the commercial and large-scale production under local conditions. Previously, the study using light sources were merely to investigate the photoperiodism characteristics in stevia and not meant for agronomic purposes and technique for large scale production.

The technique of night interruption using commercial light sources in this study can be used to manipulate photoperiodism of stevia. The success of this technique will prevent early flowering, increase plant growth, leaf yield and sweetener content of stevia. Short duration of light exposure as night interruption will be cost effective and more environment friendly as compared to other technique such as day lengthening by continuous long hours of light exposure. Therefore, the findings of this study will give many benefits for local farmers and producers as well as researchers. The farmers and stevia producers can adopt this technique to overcome the agronomic problem and plant physiological challenges; thus will increase the production of stevia in Malaysia. The results from second study, which is the effects of drying methods on sweetener content, will benefit stevia producers in maintaining good quality of stevia at post harvest stage.
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