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Effects of Light Sources and Drying Methods on Plant Growth and Steviol Glycoside Content of *Stevia rebaudiana* Bertoni

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ABSTRACT

Stevia (*Stevia rebaudiana* Bertoni) has received great attention with the rise in demand for low-sugar food and beverage additives, and natural alternative to cane sugar and artificial sweeteners. The leaves produce intensively sweet steviol glycosides (mainly stevioside and rebaudioside A). Stevia has been reported as a short-day plant with a critical daylength of 13 h. Daylength less than 13 h causes stevia to flower early, resulting in a low leaf biomass yield and percentage of sweetener content. The effects of night interruption treatment using six light sources for 60 min daily with the aim to lengthen vegetative phase, increase plant biomass and steviol glycoside content of stevia were investigated. Night interruption was shown to extend 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 after treatment initiated. Fluorescent and light-emitting diode (LED) were energy-efficient and effective as light source for night interruption. Fluorescent warm white showed the highest increase in total steviol glycosides content per plant by 190-270% most probably because it contained the highest red light at 614 nm as compared to other light sources. Stevioside content was not significantly affected by drying methods but rebaudioside A content was significantly reduced by 3.38% under oven drying at 70ºC. The reduction indicated that thermal degradation of rebaudioside A has occurred at higher temperature.

Keywords: Light-emitting diode; photoperiodism; rebaudioside A; *Stevia rebaudiana* Bertoni; sun drying.

INTRODUCTION

Stevia rebaudiana Bertoni is a herbaceous perennial plant of the Asteraceae family, native to Paraguay and borderlands of Brazil and Argentina (Soejarto, 2002). It is one of 154 members of the genus Stevia and one of the only two genera that produce sweet steviol glycosides. Steviol glycosides are zero-calorie and high potency sweeteners with sweetening value of 150 to 450 times (by weight) higher than sucrose (Kinghorn, 1987). Around 33 steviol glycosides are known to occur in the leaves of stevia and nine are sweet glycosides (Ohta et al., 2010). The main sweet steviol glycosides are stevioside and rebaudioside A. Stevioside traditionally makes up the majority of the sweetener (60 to 70%) and it is also responsible for the bitter aftertaste (DuBois, 2000). Rebaudioside A is usually present at 30 to 40% and of particular interest because it has the most desirable flavour profile with the sweetest taste (Dacome et al., 2005). Steviol glycosides can be used as a natural alternative sweetener for other synthetic sweeteners such as aspartame, saccharine or acesulfame-K that are constantly being associated with health concerns (Puri et al., 2011). Steviol glycosides are considered safe for consumption without major contradictions, warnings and side effects reported (Ferri et al., 2006). The World Health Organization concluded that steviol glycosides have shown

no adverse effects when taken at recommended doses (Benford et al., 2009).

Photoperiodism is the ability of an organism to detect day length (Taiz and Zeiger, 2002). Plants that flower when the day length is shorter than critical day length are classified as short-day (SD) plants such as chrysanthemum*, Nicotiana tabacum*, *Glycine max*, *Perilla frutescens* and *Ipomoea nil* (Hopkins and Huner, 2008; Kim et al., 2011). Stevia is classified as an SD plant with a critical day length of about 13 to14 h (Kang and Lee, 1981; Zaidan et al., 1980). Malaysia is situated near to equatorial with latitude 1-6° N and the day length is 11.80 ± 0.40 h throughout the year. The short-day length causes stevia to flower early, resulting in a low leaf biomass per harvest and a low percentage of steviol glycoside content (Ghawas et al., 2009; Tan et al., 2008). Steviol glycosides and leaf biomass were shown to decrease after the onset of flowering (Bondarev et al., 2003; Ceunen and Geuns, 2013). The highest steviol glycoside content was found in the leaves and leaves were harvested as yield.

Photoperiod is often manipulated to induce or prevent flowering in many photoperiodic species (Blanchard and Runkle, 2009). Flowering of SD plants can be prevented or delayed by providing day-extension (lengthening day length) or night interruption lighting (Blanchard and Runkle, 2009; Taiz and Zeiger, 2002). Night interruption with a brief light is effective in preventing flowering in many SD plants, including *Xanthium* sp. and *Pharbitis* sp. (Taiz and Zeiger, 2002). 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 required long hours of exposure and less energy efficiency. Commercial bulbs such as fluorescent lamps and light-emitting diode (LED) are more energy efficient, however their effect on plant biomass and flowering of stevia is still lacking.

Several drying methods are used to dry stevia leaves but commercially, sun drying is the most preferable and practiced method (Samsudin and Aziz, 2013). Abou-Arab et al. (2010) found that sun drying for 48 h caused 0.81% reduction of stevioside in stevia leaves. However, according to Steve Marsden of Herbal Advantage Inc., very little steviol glycoside will be lost if sun drying is for 8 h or less (Richard, 1996).

MATERIALS AND METHODS

Plant preparation and growing methods

This study was conducted at Malaysian Agricultural Research and Development Institute (MARDI) Serdang, Malaysia under a 6 m × 30 m rain shelter. Stevia plants (accession MR 012 from Canada) were prepared using vegetative propagation from shoot cuttings. The cuttings (7 to 10 cm length with 3 nodes of paired leaves) were inserted into rooting media of peat moss in the plug trays under a rain shelter with 70% shade area for rooting simulation. Four weeks later, healthy rooted plantlets (10 to 15 cm in height) were transplanted into 20 cm height and 27 diameter pots under a rain shelter without any shade. Media used was the combination of mineral soil, burnt paddy husk, coco-peat and sand at 5:2:2:1. Fertilisation, watering and other standard agronomic practices were based on planting manual from MARDI with some modifications (Ghawas et al., 2009). Initially, 3 g of organic fertiliser was applied per plant with the rate of 0.5 tonne/ha. Each plant received compound nitrogen, phosphorus and potassium (NPK) green (15:15:15) with the rate of 1.0 tonne/ha every 2 weeks throughout the study. Watering was done daily or once in 2 days depending on moisture condition of the media. The upper parts of new shoots were cut using a pair of secateurs every 2 weeks, leaving two pairs of leaf nodes at lower shoot to avoid flower bud formation from apical bud.

Night interruption treatment

Night interruption treatment was started 3 months after transplanting using 5 types of artificial light sources (incandescent, fluorescent cool day light (C.D.L.), fluorescent warm white (W.W.), light-emitting diode (LED) C.D.L. and LED W.W. at 100 Watts/m^2 incandescent equivalent. The distance between plant canopy

and light sources was 1.5 m. Control plants were not given any artificial lighting. A total of 288 experimental plants were chosen for this study (48 plants per treatment with 12 plants per replication and repeated four times). Prior to this study, all plants were in vegetative phase. On the first day of night interruption initiation, shoot cutting was done for all plants, leaving two pairs of leaves for each shoot to allow the axillary buds to grow into new shoots. Night interruption treatment was applied to the plants by exposing them to the artificial lights for 60 min at 1.00 am for 8 growing weeks (Armizatul et al., 2009). The measurement of photon flux density of red, far red, red/far red ratio and photon flux density were done using a light meter (Field Scout, Spectrum Technologies, Inc., USA). Light spectrum distribution was determined using a portable absolute irradiance spectrometer (JAZ-ULM-200, Ocean Optics, Inc., USA).

Vegetative growth assessment

Vegetative growth assessments were done at 2 weeks intervals for 8 weeks. In each assessment, 72 plants were harvested (12 plants for each treatment with 3 plants per replication). The vegetative growth parameters were determined following the methods described by Hunt (2003). Total leaf area of stevia was determined using a leaf area meter (LI-3100, LICOR Inc., USA). Plant parts were then dried to constant weight at 80^{o}C for 72 h in a drying oven (Model 100-800, Memmert, Germany). The dried weight was measured using a semi-micro analytical digital balance (GR-200, A&D Company Limited, Japan). The biomass of fresh leaves used for steviol glycoside determinations and drying methods treatment was added to the total leaf dry weight assessment. The leaf biomass was calculated based on average stevia leaf moisture content of 82.0%.

Steviol glycoside determination

Five grammes of mature and completely expanded leaves from each plant (3 plants per replication) were chosen and frozen at -78°C overnight in an ultra-low temperature freezer (MDF-U32V, Sanyo, Japan). Leave samples were then lyophilised at -79°C for 72 h in a freeze dryer (BT4KZL-105, VirTis, USA). The leaves were then ground using a grinder (IKA 2871000, IKA, Germany) and filtered through 1.0 mm (18 mesh) sieve into fine powder. The ground leaf samples were then packed in sealed polyethylene bags and stored at 4 ± 1^{o}C in a refrigerator for 4 weeks before the analysis of steviol glycoside content (Abou-Arab, 2010). Steviol glycosides determined in this analysis were stevioside and rebaudioside A content. This analysis was based on Ceunen et al. (2012), Abou-Arab et al. (2010) and Woelwer-Rieck et al. (2010) with some modifications. Ground dried stevia leaves (0.1 g) were added to 10 mL of 80% methanol and incubated in a water bath at 80°C for 20 min. Samples were then centrifuged for 10 min at 6,000 g and supernatant was collected. These extraction processes were replicated thrice. Collected supernatant was dried in a vacuum evaporator at 80°C for 5 h and dissolved in 2 mL deionized water. The analysis of steviol glycoside content was done using HPLC system (The Waters 2695 Alliance, Waters Corporation, USA) with Waters 2424 Evaporative Light Scattering Detector (ELSD) and C_{18} column (4.6 mm × 250 mm). Gradient mobile phase was set using acetonitrile/water at 30 and 20% (v/v) for 5 and 10 min, respectively. Flow rate was set at 1.0 mL/min with 35°C column temperature. The samples (20 µL) were injected into the HPLC system. For quantification, standards of stevioside and rebaudioside A (Sigma-Aldrich, USA) were used.

Drying methods treatment

Before the drying treatment started, the initial moisture content (MC) of stevia leaves was determined following method described by AOAC (1990). Twenty grammes of fresh leaves were selected and dried in a drying oven at 105°C for 24 h. $Weight_1$ was the initial leaf fresh weight before drying and $Weight_2$ was the final weight after drying completed. The MC was calculated as the following equation and expressed in percentage.

$$\text{Percentage of MC} = [(\text{Weight}_1 - \text{Weight}_2) / \text{Weight}_1 \times 100\%]$$

The initial MC of stevia leaves was determined at 82.0%. Drying treatment was done by randomly selecting 80 g of mature and completely expanded leaves for each drying method. The drying methods used were freeze drying (as control), sun drying, air drying at 25°C, oven drying at 40°C and oven drying at 70°C. For sun, air and oven drying, leave samples were distributed uniformly as a single layer on 20 cm × 30 cm trays. Leave samples were weighed at 1 h intervals until it reached the weight with the pre-determined MC of 10 ± 2%. Air drying was done by drying the leaves samples in a room with temperature of 25 ± 2°C. Oven drying was done using two ovens (Beschickung Loading, Model 100-800, Memmert, Germany) with temperature set at 40 and 70°C. The drying duration required for each drying treatment to achieve pre-determined MC percentage was recorded. Dried leaves from all drying methods were ground and filtered into fine powder before subjected to steviol glycoside content determination.

Statistical analysis

The night interruption treatment study was arranged in a nested design with 6 × 4 factorial (6 light sources × 4 growing weeks after night interruption initiated) with four replications. For drying methods treatment, the study was a completely randomised design with five drying methods and four replications. The data were analysed by Analysis of variance (ANOVA) using statistical analysis system (SAS) version 9.3 (SAS Institute, Inc., Cary NC, USA) and means separation was carried out using Duncan's multiple range test (DMRT) at 5% level to determine significant differences among treatments. Pearson correlation analysis was carried out to determine the relationship between total steviol glycoside content in stevia leaves and leaf dry weight of stevia.

RESULTS AND DISCUSSION

Characteristics of light sources

The light produced by incandescent contained the highest red and far red light at 660 and 730 nm, respectively compared to other light sources used in the study (Table 1). Fluorescent W.W. showed the highest peak of red light at 614 nm wavelength with 37.2 μW/cm^2/nm absolute irradiance (Figure 1).

Table 1. Photon flux density of red, far red, red/far red ratio and photon flux density of different light bulbs used in night interruption treatment (mean ± S.E.)

Treatment	Red light at 660 nm (μmol/m^2/s)	Far red at 730 nm (μmol/m^2/s)	Red/Far red ratio	Photon flux density (μmol/m^2/s)
Incandescent	42.43 ± 2.31	56.71 ± 2.18	0.75 ± 0.03	368 ± 18
Fluorescent cool day light (C.D.L.)	5.50 ± 0.34	1.83 ± 0.17	3.08 ± 0.20	270 ± 12
Fluorescent warm white (W.W.)	7.83 ± 0.31	2.00 ± 0.13	3.92 ± 0.15	235 ± 15
LED C.D.L.	23.00 ± 1.90	4.25 ± 0.41	5.50 ± 0.28	541 ± 29
LED W.W.	39.57 ± 2.29	7.71 ± 0.47	5.15 ± 0.12	466 ± 25

LED = light-emitting diode, n=7

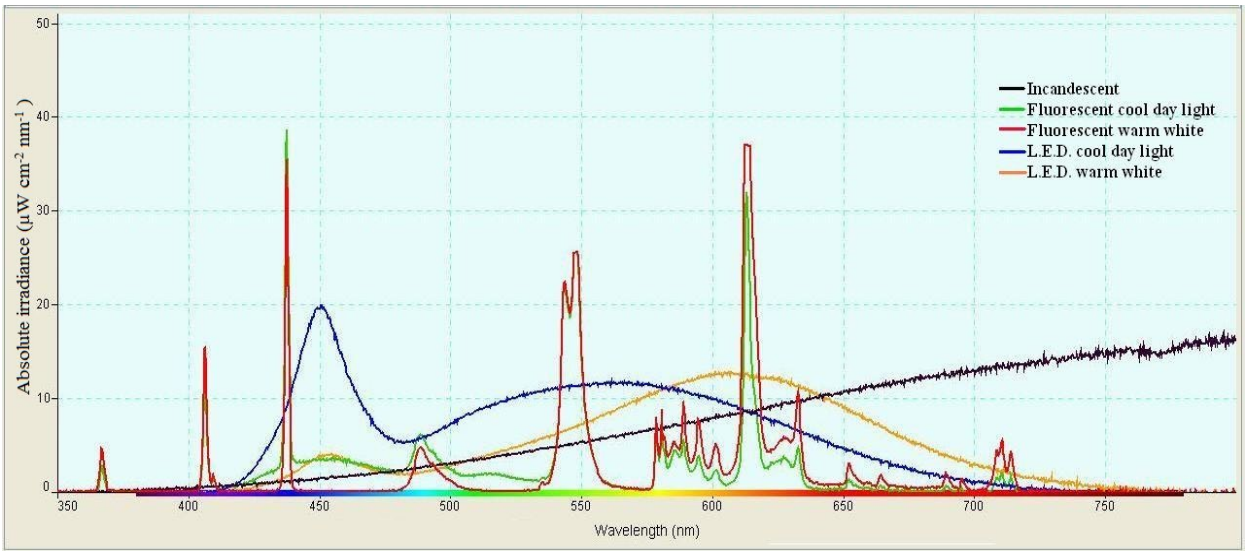

Figure 1. Absolute irradiance of light spectrum analysis of different light bulbs used for night interruption treatment on stevia

Night interruption treatment on flowering inhibition in stevia

Flower bud formation of control plants was observed after 20 days of night interruption treatment initiation. Early flowering indicated that day length of Malaysia which is shorter than 13 h of critical day length of stevia induced the flower initiation in stevia. As a result, vegetative phase of stevia plants transited to reproductive phase in less than 4 weeks after shoot cutting on the first day of night interruption treatment. Ceunen and Geuns (2013) reported that initial flower bud formation was formed 38 days after shoot cutting under short day length conditions (greenhouse study in Belgium). Under natural cultivation in southern or northern hemisphere during summer, the flowering of stevia should occur between 54 to 104 days after transplanting, depending on the day length sensitivity of the varieties (Madan et al., 2010).

Plants grown under night interruption using different light sources in this study remained in vegetative phase throughout 8 weeks of growth (Figure 2). Initial flower bud formation for plants treated with light sources was only observed about 120 days after night interruption initiated. Night interruption treatment successfully prevented flower emergence and flowering of stevia for an extended period compared to control plants under natural short-day length conditions. Supplementing 1 h of artificial light during dark period at night basically created a modified long-day (LD) conditions, thus preventing flower initiation of stevia. Ceunen and Geuns (2013) had demonstrated the use of 1 h red light from LED strip as night interruption maintained stevia plants in vegetative phase for extended period.

Flowering of SD plants is determined primarily by the duration of darkness, so any dark period greater than the critical day length will cause flowering of SD plants (Taiz and Zeiger, 2002). Night interruption technique divided long dark period of night time into two shorter periods, thus creating relatively longer day length period. Night interruption created a modified LD conditions that cancelled the effects of dark period. Vince-Prue and Canham (1983) explained that night interruption technique breaks a long dark period to deliver photoperiodic lighting, resulting in modified LD conditions in SD species. Commercially, this technique was also widely used to prevent flowering in chrysanthemum (SD plant) by lengthening day length for 4 to 5 h using incandescent and fluorescent tube at very low levels of intensity (Higuchi et al., 2012). Night interruption using artificial light sources at night mediates phytochrome-regulated response, which inhibits precocious flowering under SD conditions (Ceunen et al., 2012).

In this study, all light sources inhibited early flowering of stevia plants and successfully extended the period of vegetative phase. This was probably due to the light sources used which contained red light between 590 to 750 nm wavelength (Table 1 and Figure 1). The function of red light in phytochrome

signalling mechanism especially in SD plants has been widely studied (Hopkins and Huner, 2008; Taiz and Zeiger, 2002). The red light was absorbed by physiologically inactivating the phytocrome P_r form during dark period and converting it to the active P_{fr} form that triggered the signal to inhibit flowering. Fluorescent cool day light (C.D.L.), fluorescent W.W., LED C.D.L and LED W.W. contained higher level of red light (at 660 nm) compared to far red light (730 nm), thus explaining the conversion of physiologically inactive P_r form of phytochrome to the active P_{fr} form that inhibited flowering. Incandescent light produced higher level of far red light compared to red light. Far red light supposedly converted physiologically active P_{fr} form back to the inactive phytochrome P_r form and should induce flowering. However, the flowering of plants under incandescent light source was still inhibited. This indicated that red light of incandescent at lower level compared to far red light probably converted phytochrome P_r to P_{fr} and managed to maintain its active form. Zaidan et al. (1980) and Valio and Rocha (1977) concluded that by providing incandescent light at night (by lengthening day length), stevia plants managed to extend its vegetative growth, a result that was likely caused by the small amount of red light present in the light.

Figure 2. Comparative growth and flowering of *Stevia rebaudiana* Bertoni plants after 8 weeks of night interruption treatment using different types of commercial light bulbs. The control plants were grown without night interruption treatment and early flowering occurred while plants grown with night interruption remained in vegetative growth.

Night interruption treatment on vegetative growth

There was no significant interaction between light sources and growing weeks after initiation of night interruption on the height of stevia (Table 2). However, fluorescent W.W. showed significantly higher plant height compared to other light sources. There were significant differences ($p \leq 0.05$) observed between the interaction of light sources and growing weeks after initiation of night interruption on leaf dry weight, total plant dry weight, leaf area and total leaf area. In weeks 6 and 8, leaf dry weight and total leaf area of plants treated with night interruption were significantly higher than control (Figures 3 and 4). In week 6, light treatment increased leaf dry weight between 97.7 to 134.1% compared to control. Meanwhile, fluorescent W.W. significantly increased 178.71% total leaf area compared to control. In week 8, fluorescent W.W. showed significantly higher leaf dry weight compared to all light treatments and 221.57% higher than control.

Higher leaf dry weight of light treated plants in weeks 6 and 8 was due to the plants remaining in vegetative stage thus allowing more accumulation of leaf biomass. Ceunen et al. (2012) found higher accumulation of leaf dry mass in plants under SD conditions with night interruption using red LED strip compared to plants under controlled SD conditions. LD conditions have been shown to increase leaf area and leaf dry weight compared to SD condition (Metivier and Viana, 1979). Ren et al. (2011) reported that stevia under extended vegetative stage showed higher vegetative growth with increased leaf dry weight.

Fluorescent W.W. treated plants showed better vegetative growth performance compared to the other treatments probably due to its light characteristic. Although fluorescent W.W. contained lower red light at 660 nm compared to other light bulbs (Table 1), it contained higher red light at 614 nm (Figure 1). Ceunen et al. (2012) reported that specialised red LED strips with a wavelength of 631 nm was also effective in sustaining vegetative growth, increasing vegetative leaf biomass production and accumulation of steviol glycosides of stevia. These results showed that red light was effective in stimulating phytochrome response at a range of wavelength and not at specific wavelengths. Taiz and Zeiger (2002) also explained that although red light was most effective as a night interruption with a maximum effectiveness near 660 nm in the SD plant of *Pharbitis nil* but in other SD plant such as *Xanthium strumarum*, night interruption was most effective for red light in the range of 620 to 640 nm.

Night interruption on steviol glycoside content

There was no significant interaction between light sources and growing weeks after night interruption initiation on rebaudioside A per leaf dry weight (Table 2). There were significant interactions between light sources and growing weeks on stevioside per leaf dry weight, total steviol glycosides per leaf dry weight and total steviol glycosides per plant.

Table 2. Effects of light sources and growing weeks after night interruption initiation on plant growth and steviol glycoside (SG) content of *Stevia rebaudiana* Bertoni

Factor	Plant height (cm)	Leaf DW (g)	Total plant DW (g)	Total leaf area (cm^2)	Rebau-dioside A per leaf DW (mg/g)	Stevioside per leaf DW (mg/g)	Total SG per leaf DW (mg/g)	Total SG per plant (mg/ plant)
Light sources (L)								
Control	42.88 d[z]	4.27 d	16.35 c	849.7 c	31.86 a	92.95 c	124.81 b	501.91 c
Incandescent	47.22 b	9.25 bc	21.18 b	1840.5 b	33.41 a	107.73 ab	141.14 a	1166.96 b
Fluorescent C.D.L.	47.41 b	9.76 b	22.33 b	1944.0 b	32.90 a	103.36 b	136.25 a	1212.51 b
Fluorescent W.W.	52.19 a	11.05 a	24.87 a	2365.7 a	32.03 a	107.81 ab	139.83 a	1424.00 a
LED C.D.L.	44.97 c	9.34 bc	21.49 b	1926.7 b	31.80 a	106.93 ab	138.73 a	1186.33 b
LED W.W.	47.69 b	8.67 c	20.55 b	1743.7 b	31.83 a	109.78 a	141.61 a	1148.06 b
Growing weeks (W)								
2	26.50 d	1.88 d	4.63 d	465.17 d	31.15 b	94.10 b	125.26 c	235.73 d
4	39.25 c	4.73 c	10.97 c	1220.71 c	31.16 b	98.76 b	129.92 c	615.67 c
6	48.03 b	8.52 b	19.96 b	1669.62 b	32.32 ab	107.49 a	139.81 b	1204.42 b
8	55.54 a	12.54 a	31.19 a	2510.71 a	33.69 a	112.18 a	145.87 a	1849.29 a
Interaction								
L x W	NS	**	*	**	NS	**	*	**

[z] Means followed by the same letter in the same column and factor are not significantly different by DMRT at $p \leq 0.05$. NS, non-significant difference at $p > 0.05$. Significant difference at * $p \leq 0.05$ or ** $p \leq 0.01$. DW = dry weight.

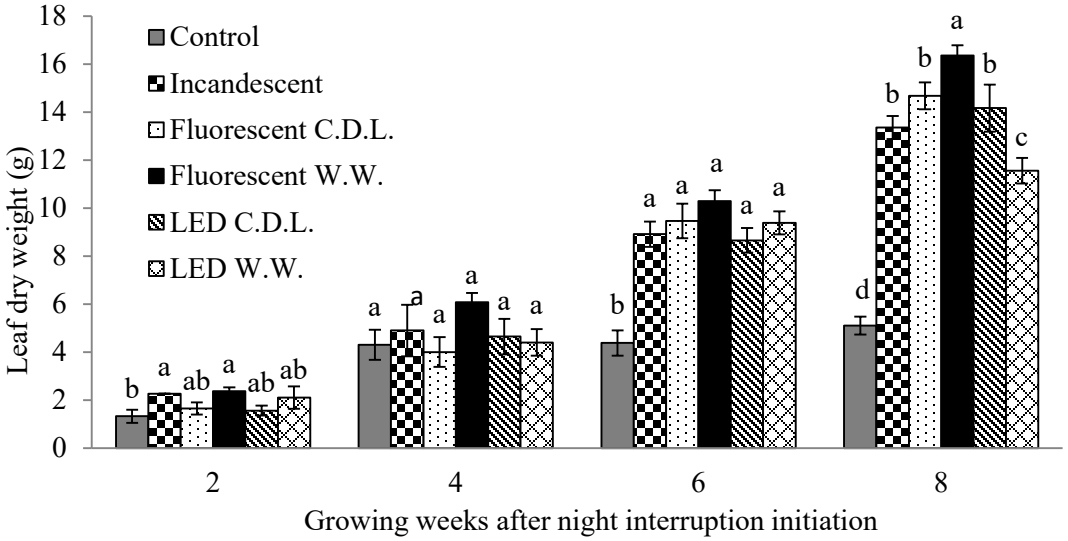

Figure 3. The effects of light sources after night interruption initiation on leaf dry weight of stevia. Error bars represent standard error of the means. Means followed by the same letter in the same week are not significantly different by DMRT at $p \leq 0.05$.

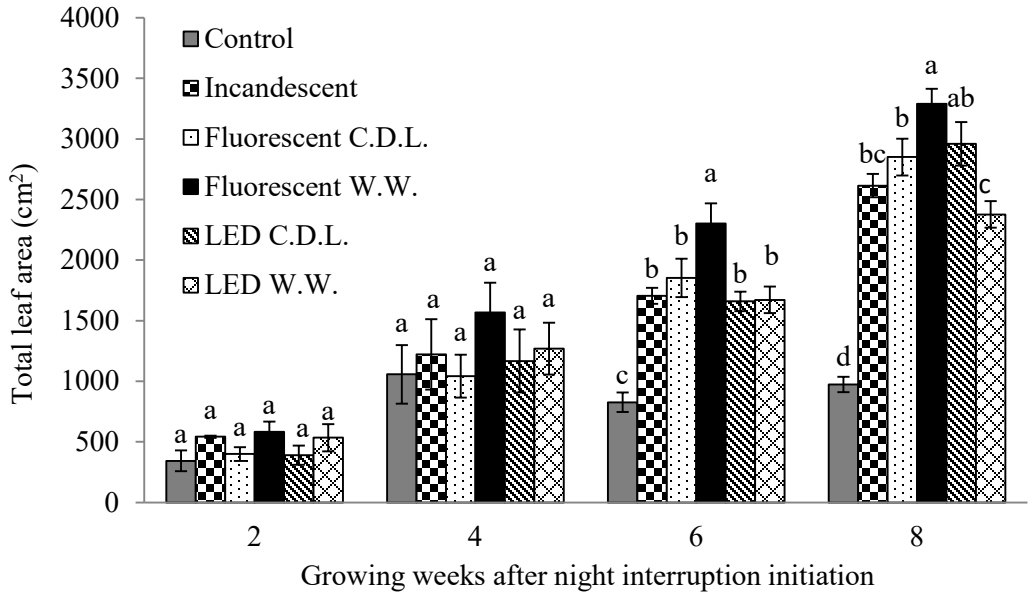

Figure 4. The effects of light sources after night interruption initiation on leaf area of stevia. Error bars represent standard error of the means. Means followed by the same letter in the same week are not significantly different by DMRT at $p \leq 0.05$.

In weeks 6 and 8, plants treated with all light sources showed significantly higher total steviol glycosides content than control (Figure 5). Night interruption treatment significantly increased total steviol glycoside content in stevia compared to control by 13.8 to 19.2 and 15.6 to 28.2% in weeks 6 and 8, respectively. Fluorescent W.W. showed significantly higher total steviol glycoside content per plant throughout 8 weeks of growth compared to other light sources treatment and control (Figure 6). Total steviol glycoside content per plant in fluorescent W.W. treated plants was 78.6, 48.2, 179.8 and 272.6% higher

than control in weeks 2, 4, 6 and 8, respectively. By weeks 6 and 8, all night interruption treated plants showed significantly higher total steviol glycoside content per plant compared to control.

Higher total steviol glycosides per leaf dry weight were observed for all light treated plants than control plants in weeks 6 and 8 (Figure 5). This was due to the retention of vegetative phase in treated plants while control plants had already flowered (reproductive phase). This indicated that flowering of stevia caused reduction in steviol glycoside content. Brandle and Rosa (1992) also explained that flowering is the main phenological factor affecting the steviol glycoside content in stevia, which is induced by day length. Zaidan et al. (1980) reported that stevia under extended vegetative phase of 16 h LD conditions showed higher stevioside content per dry leaf compared to SD conditions. The increase in steviol glycoside content using night interruption technique was also reported by Ceunen et al. (2012). Plants under extended vegetative phase using 1 h of red specialised LED light at night and 8 h photoperiod of fluorescent lamps showed higher percentage of steviol glycosides per leaf dry weight compared to plants without night interruption. Under SD conditions without night interruption, steviol glycosides per leaf dry weight was 5.88% but it increased to 9.13% under modified LD conditions after 50 days of night interruption treatment.

A strong and positive relationship (r = 0.87) was observed between total steviol glycoside content in stevia leaves and leaf dry weight (Figure 7). This indicated that the higher content of total steviol glycoside content per plant shown by fluorescent W.W. treated plants were significantly contributed by the higher leaf dry weight per plant. Metivier and Viana (1979) also found that the accumulation of steviol glycosides was positively correlated with leaf dry weight. Stevia plants grew under LD conditions showed increased leaf dry weight and stevioside content up to 50% compared to plants under SD condition. The decrease of steviol glycosides after the onset of flowering has been attributed to the decrease of leaf biomass due to reproductive development and a transport of steviol glycosides to reproductive organs (Ceunen and Geuns, 2013; Bondarev et al., 2003).

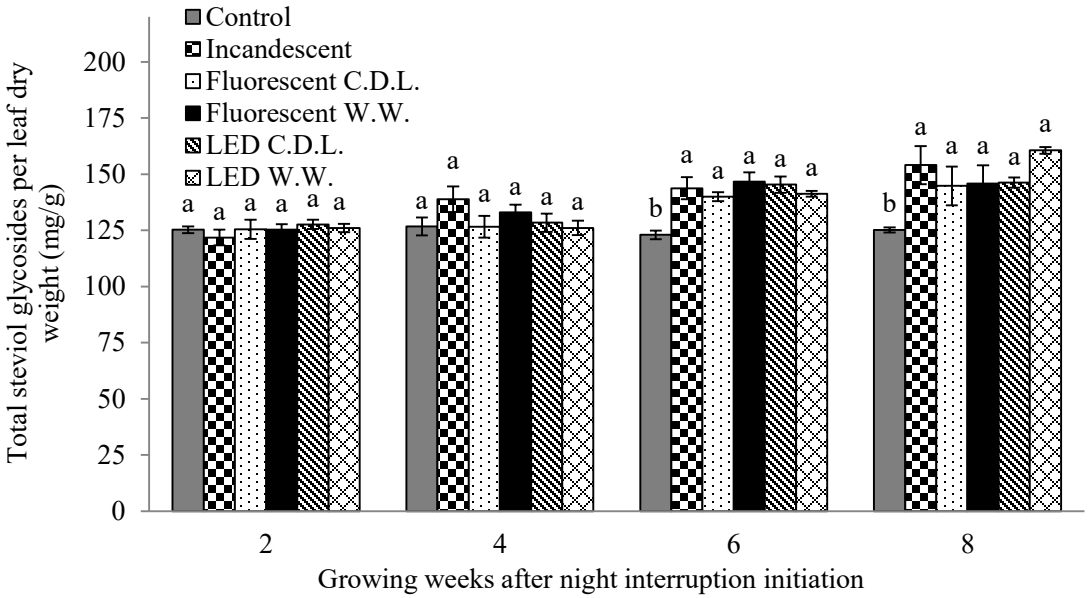

Figure 5. The effects of light sources after night interruption initiated on total steviol glycosides content per leaf dry weight of stevia. Error bars represent standard error of the means. Means followed by the same letter in the same week are not significantly different by DMRT at $p \leq 0.05$.

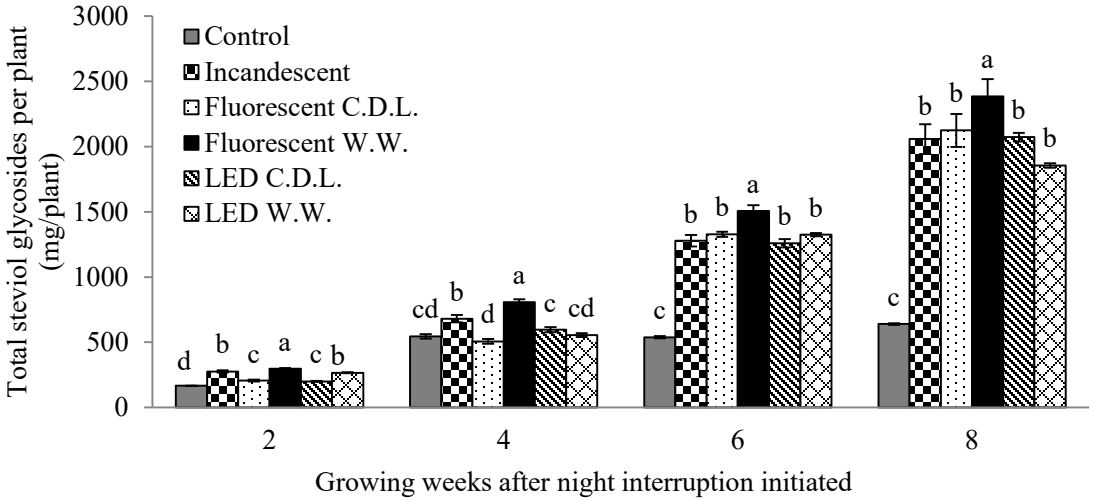

Figure 6. The effects of light sources after night interruption initiated on total steviol glycosides content per plant of stevia. Error bars represent standard error of the means. Means followed by the same letter in the same week are not significantly different by DMRT at $p \leq 0.05$.

Drying methods on steviol glycoside content

Drying duration required to achieve pre-determined moisture content of 10% was as presented in Table 4. Oven drying at 70°C took the shortest duration while freeze drying took the longest duration. Stevioside content was not significantly affected by all drying methods (Figure 7). Abou-Arab et al. (2010) also reported that sun drying of stevia under direct sunlight at temperature ranging from 25 to 30ºC for 24 to 48 h did not significantly reduce stevioside content in stevia leaves. Kinghorn and Soejarto (1985) reported that stevioside was stable at high temperature (100ºC). Periche et al. (2015) also reported the effect of high air temperature on stevioside content, however the findings were inconclusive. They found out that hot air drying at 100ºC in a convective drier reduced stevioside content by 51.4% compared to freeze drying. However, hot air drying at 180ºC in a convective drier did not show any significant difference compared to freeze drying.

Sun drying took 6 h to achieve 10% MC without any significant reduction of steviol glycoside content (Table 3 and Figure 8). Oven drying at 40°C did not cause any significant reduction in rebaudioside A content compared to freeze drying. Chranioti et al. (2016) also reported that drying methods of freeze drying and oven drying at 45ºC did not show any significant difference in rebaudioside A content. Although oven drying of stevia leaves at 40ºC did not reduce rebaudioside A content, the drying duration was longer at 9 h and increased the cost for electricity. Although oven drying at 70ºC required shorter drying period, this treatment caused significant reduction of rebaudioside A (3.38%) which indicated that thermal degradation had occurred at higher temperature. Many studies have also reported thermal degradation of phytochemicals following high temperature treatments (Lim and Murtijaya, 2007). On the contrary, Periche et al. (2015) reported that rebaudioside A content of stevia leaves under hot air drying at 100 and 180ºC did not show any significant difference compared to freeze drying.

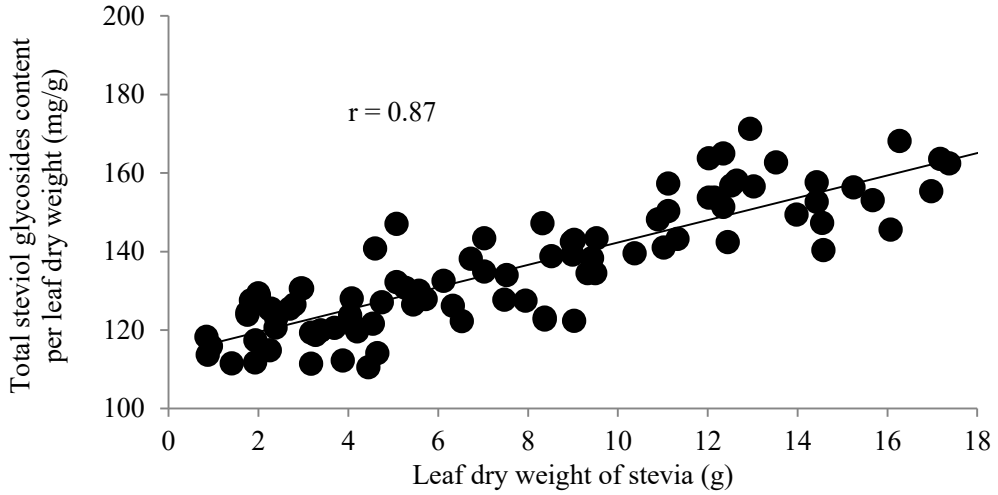

Figure 7. Positive and highly significant ($p \leq 0.001$) correlation between leaf dry weight of stevia and total steviol glycoside content per leaf dry weight of stevia. r is the Pearson's correlation coefficients.

Table 3. Drying duration required to complete freeze drying and drying duration for sun, air and oven drying to achieve pre-determined moisture content of 10%

Treatment	Drying duration (h)
Freeze drying (-79°C)	72.0
Sun drying	6.0
Air drying (25°C)	50.0
Oven drying (40°C)	9.0
Oven drying (70°C)	3.5

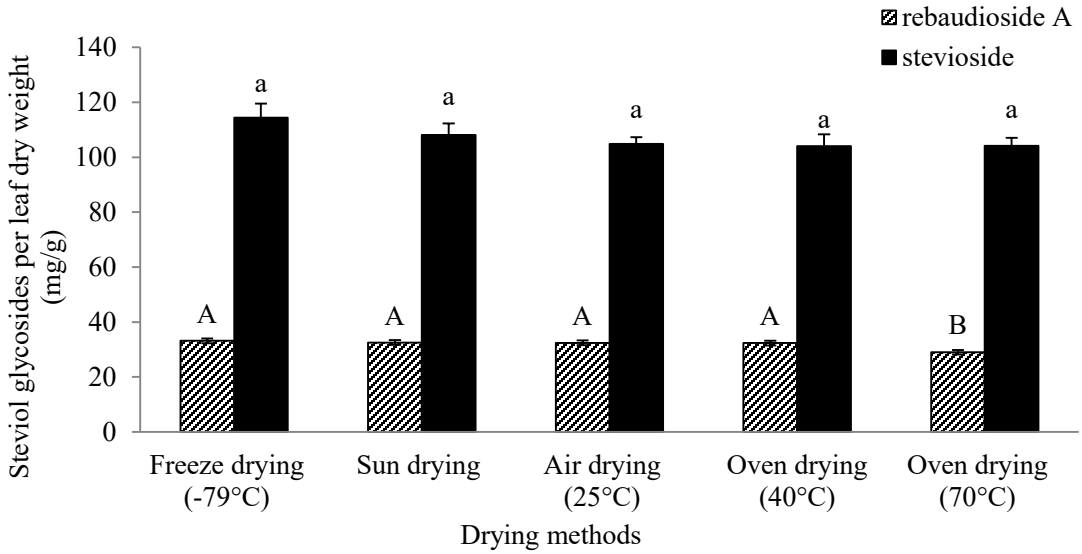

Figure 8. Effects of drying methods on rebaudioside A and stevioside content per leaf dry weight. Error bars represent standard error of the means. Means with the same capital letter and small letter are not significantly different by DMRT at $p \leq 0.05$ for rebaudioside A and stevioside, respectively.

CONCLUSIONS

Night interruption treatment using commercial light bulbs successfully extended vegetative phase for longer period and inhibited early flowering of stevia under SD conditions in Malaysia. Stevia plants under modified LD conditions created by night interruption treatment induced greater accumulation of plant biomass and steviol glycoside content in the leaves. Fluorescent and LED lights were better options to be used for night interaction due to their higher energy efficiency compared to incandescent bulbs. Fluorescent W.W. significantly induced higher plant growth performance, biomass and gave the highest total steviol glycoside content per plant probably because it contained the highest red light at 614 nm compared to other light sources. As for drying methods, steviol glycoside content could withstand temperature ranging from -79ºC (freeze drying) to 40ºC (oven drying), direct exposure of sunlight and longer drying hours at room temperature. However, oven drying at higher temperature of 70ºC was not preferable as it caused reduction of rebaudioside A content which is a more favourable steviol glycoside. Sun drying was a preferable method because it was cheaper, faster and did not cause significant reduction of steviol glycoside content compared to other drying methods.

AUTHORS CONTRIBUTION

PD and SAH conceived and designed the work. MAR and PD performed the analysis. MAR and PD wrote the paper. PD and SAH checked and approved the submission.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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