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# RESPONSE AND STEVIOSIDE LEVELS OF STEVIA (*STEVIA REBAUDIANA* BERTONI) GROWN AT DIFFERENT LIGHT INTENSITY AND WATER AVAILABILITY

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## Abstract

*Stevia rebaudiana* Bertoni has a great potency to be cultivated and developed as a raw material for natural sweetener, sugar cane substitution, and as synthetic sugar substitution because it has sweetness level of 300 times higher than sugar cane. Suitable cultivation techniques are needed to increase crop productivity. Whereas environmental factors such as light intensity and water availability play an important role on plant growth and development. The objectives of this research was to study the response and stevioside level of stevia (*Stevia rebaudiana* Bertoni) grown at different light intensities and water availability. The experiment was designed in Completely Randomized Design consisted of two factors: light intensities (100%, 50%, and 25%) and water availability (100%, 75%, 50%, and 25% of feld capacity). The variables observed were the plant height, number of nodes, number of leaves, plant biomass, shoot-root ratio, chlorophyll content, density and stomata index and stevioside level. The results showed that the light intensity and water availability significantly affected growth attibutes, but there was no effect on levels of chlorophyll as well as the density and index of stomata. Light intensity of 100% and water availability of 100% caused a greater increased of plant height, number of nodes, number of leaves, and plant biomass compared to other treatments. Stevioside level tended to increase at low light intensity, whereas water availability did not affect the stevioside level.

**Key words:** Stevia, Water, Growth, Stevioside, Natural sweetener.

## Introduction

Indonesia is facing the problem of sugar cane needs. The level of sugar demand reached 4.6 million tons and will continue to increase every year (Voboril, 2010). To overcome this problem, we need to intensify the sugar cane cultivation and also find the of sugar source alternative. Stevia (*Stevia rebaudiana* Bertoni) is a perennial herb of the family Asteraceae and its leaves contain high sweetness (250-300 times greater than sucrose) due to the presence of diterpene, a specifically steviol glycosides (Lemus *et al.*, 2012). Compared with other sweeteners, Stevia sugar is non-carcinogenic and no calorie content (Fujita & Edahiro, 1979; Lemus *et al.*, 2012). Stevia sugar can be used as an appropriate choice to replace the position of the synthetic sweeteners (cyclamate). Widodo *et al.* (2015) reported that stevia leaves is potential as sugar substitute in low calorie sweet bio-yoghurt. Stevia sugar is also very suitable for diabetics (Gregersen *et al.*, 2004). Until now, there are no reports of side effects from the use of stevia sweetener by human beings (Brusick, 2008; Brahmachari *et al.*, 2011). However stevia produced today is still of poor quality. Therefore, *Stevia rebaudiana* has a great potency to be cultivated and developed as a raw material for natural sweetener.

Stevia contains eight glycosides diterpene namely stevioside, steviolbioside, rebaudioside (A, B, C, D, E), and dulcosideA (Jeppesen *et al.*, 2006; Tavarini *et al.*, 2010). The two main glycosides of Stevia are stevioside (5% - 10% of dry leaves) and rebaudioside-A (2%-4%). Sweetener derived from the leaves of *Stevia rebaudiana* containing stevioside sugar. Due to the non-caloric and sweetening properties, stevioside has gained attention with the rise in demand for low-carbohydrate, and low-sugar food alternatives (Kalpana *et al.*, 2009).

Suitable cultivation techniques are needed to increase crop productivity. Environmental factors such as light intensity and the availability of water play an important role on plant growth and development. Light plays an important role in the life cycle of plants for the production as the energy supply are needed for photosynthesis (Cseke *et al.*, 2006). Differences in light intensity affect the growth and active compound of a plant. In addition to light, water is a major component in the process of photosynthesis and is a suitable solvent for various biochemical reactions in plants (Fitter & Hay, 2002).

Light radiation changes can alter the biochemical composition and morphology of the whole plants. Generally in the low light conditions, plants increase the efficiency of light capture, whereas in the high light conditions the plant maximizes the level of saturation of light on photosynthesis (Murchie *et al.*, 2002). Light can also affect the production of plant secondary metabolites. *Camellia sinensis* has a higher caffeine content when grown in high light intensity than under the canopy (Lambers *et al.*, 1998). Other studies showed that low light intensity affect the growth and accumulation of secondary metabolites in medicinal plant *Glycyrrhiz auralensis* Fisch. Low light intensity decreased the thickness of the leaves, photosynthesis and biomass, but increased leaf area and chlorophyll content. The low light intensity also significantly increased the accumulation of glycyrrhizic acid and liquiritin at the root *G. uralensis* (Hou *et al.*, 2010).

The low water availability may lead to an increase in plant secondary metabolites. According to Golldack *et al.* (2014) drought stress induced hormone ABA which is inhibiting hormone GA, resulting the precursor of GA is likely to be diverted towards stevioside biosynthesis. Stevioside is originated from the same precursor hormone GA, that is ent-kaurenoic acid (Brandle &Telmer, 2007). However, the mechanisms related to stevioside biosynthesis is not yet known.

Srivastava & Srivastava (2014a) reported marked alterations in physiology and biochemistry of Stevia plants grown under water stress. The objectives of this study was to evaluate the response and stevioside levels of stevia (*Stevia rebaudiana* Bertoni) grown at different light intensities and water availability. Information about the growth and stevioside levels of stevia in drought conditions as well as the different light intensities would be used as a reference in its cultivation techniques.

## Material and Methods

**Plant materials and experimental design:** The experimental soils were collected from stevia plantation. The soil predominant fraction of particle size contained clay (4.67%), silt (20.27%) and sand (75.07%). The soil were then dried and mixed with manure in the ratio 4:1. Planting medium used (3 kgs) is soil mixed with manure put into polybag (height 22 cm and diameter 15 cm). Stevia seedling was prepare as a vegetatively propagated stevia which has 3 nodes.

The experiment was conducted in the greenhouse on stevia agricultural land Tawangmangu, Central of Java from August 2014 to April 2015. The experiment was designed in *Randomized Completely Block Design* which consists of two factors: light intensity and water availability. Light intensity consists consisted of 3 levels: 100% (C1), 50% (C2) and 25% (C3). Water availability consists consisted of 4 levels, field capacity of 100% (A1), 75% (A2), 50% (A3) and 25% (A4). Each treatment combination was with five replications.

Polybags containing stevia seedlings were placed in accordance to the treatment of light intensity. Vegetatively propagated stevia plants were grown in planting medium (mixed soil and manure in the ratio of 4:1). Twenty eight days after replanting, plants were treated with different water availability using field capacity of 100%, 75%, 50% and 25%. Treatment of water availability was carried out by watering each polybag according to the volume of field capacity.

**Measurement of growth:** All the growth parameters studied were recorded at every seven days interval from day 28 up today 70 after replanting. Plant height was measured from the attachment point of root and stem up to the tips of uppermost fully opened leaf. The growth variables (plant height, number of nodes, and number of leaves) were measured every weeks. Chlorophyll content was measured one day before harvesting using spectrophotometric method (Yoshida *et al*., 1976). Plant biomass and stevioside level were measured at the time of harvesting (6 weeks after treatment).

**Density, index and size of stomata:** Density, index and size of stomata were determined from epidermal leaves prepared using leaf clearing method (Ruzin, 1999) and observed using a microscope equipped with NIS (Nicon Image System). Density of stomata was calculated based on the number of stomata /mm$^2$. Stomata sizes were determined using *Image Raster* program. Stomatal index was determined based on formula:

$$\text{IS} = \frac{\text{Number of stomata}}{\text{Number of stomata} + \text{Number of epidermis cells}} \times 100\%$$

**Sample extraction and stevioside analysis:** Plant samples (leaves) were dried at 70$^{o}$C in an oven for at least 2 days then was ground to a powder. A total of 0.5 g powder was dispersed in 100 mL of water. Aqueous extract of dried *Stevia* leaves was obtained at atmospheric pressure. Subsequently, the aqueous extract was filtered through filter paper and cooled before the analytical determination was made. Steviol glycoside extraction was carried out as described by Woelwer-Rieck (2012) with modification. Stevioside level was determined by HPLC (Shimadzu SCL 10 AVP) with detection of separation using UV/V detector at a wavelength of 210 nm and the amount stevioside per plant was calculated based on the level of stevioside multiplied by the dry weight of leaves.

**Data analysis:** The data presented are means of three replicates and were analyzed with two ways ANOVA using IBM SPPS Statistic 19. The Duncan's Multiple Range Test calculated to verify the significance of difference between the means with 5% significance level.

## Results

**Response of growth parameters to light and water availability:** Light intensity and the availability of water significantly affected the stevia plant height (Table 1). However, there is no interaction between the light intensity with the availability of water to the height of the stevia. Plant height increased by increasing the light intensity and water availability. The light intensity of 100% and water availability 100% of field capacity showed the highest plant height. Stevia plants showed greater variability in morphology under different water levels (Fig. 1). The well-watered plants which are grown in 100% of field capacity exhibited maximum growth and the highest of plant height recorded during the experimental period was found in well-watered plants and this parameter decreased with decreasing water levels. Water stress greatly suppresses cell expansion and cell growth due to low turgor pressure. Alavi-Samani *et al*. (2013) observed reduced plant height in two species of thyme in response to water deficit conditions. Water stress as a very important limiting factor for plant growth and development affects both elongation and expansion growth (Shao *et al*., 2008).

The analysis of variance showed that intensity of light and water availability treatments as well as the interaction between treatments showed significant effect on the number of nodes (Table 1). Light intensity and water availability significantly affect in increasing number of nodes.The number of leaves of the stevia increased with increasing light intensity and water availability (Table 1) but there is no interaction between the light intensity with the availability of water to the number of leaves. The light intensity of 100% treatment indicates the highest number of leaves, that was significantly different from the other light intensities treatments at all levels of water availability.

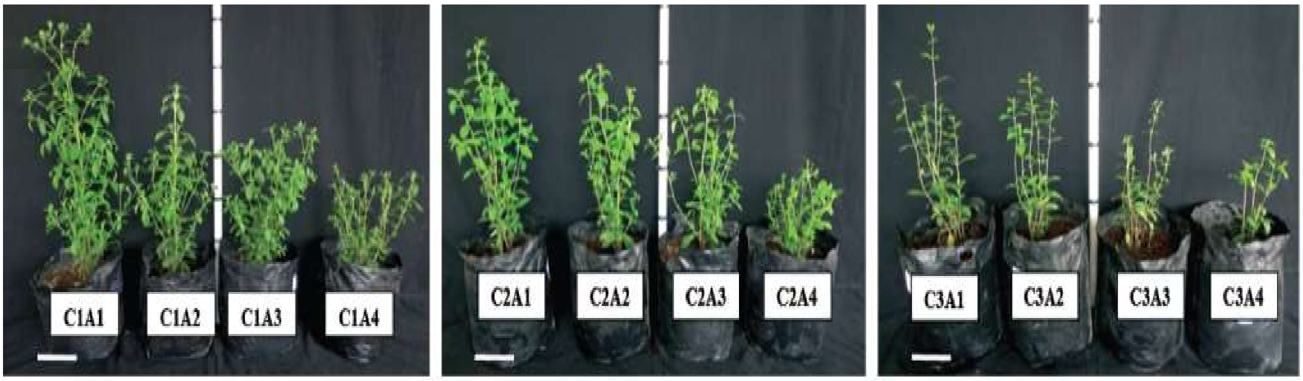

Fig. 1. Morphology of stevia (*Stevia rebaudiana* Bertoni) grown 6 weeks at different light intensity and water availability. Bar = 10 cm. A. Water availability (1: 100%, 2: 75%, 3:50%, 4:25 of field capacity) and C. Light intensity (1: 100%, 2: 50%, 3: 25%).

**Table 1. Growth attributes and Chlorophyll content of stevia (*Stevia rebaudiana* Bertoni) grown at different light intensities and water availability 6 weeks of treatments**

| Variables | Light intensity (%) | Water availability (% FC) | | | | Average |
|---|---|---|---|---|---|---|
| | | 100 | 75 | 50 | 25 | |
| Plant height (cm) | **100** | 46.00 $^{f}$ | 35.33 $^{e}$ | 31.00 $^{bcde}$ | 25.67 $^{ab}$ | **34.50** $^{x}$ |
| | **50** | 35.00 $^{de}$ | 32.33 $^{cde}$ | 29.33 $^{bcd}$ | 21.67 $^{a}$ | **29.58** $^{y}$ |
| | **25** | 32.67 $^{cde}$ | 27.67 $^{bc}$ | 22.00 $^{a}$ | 20.67 $^{a}$ | **25.75** $^{z}$ |
| | Average | **37.89** $^{p}$ | **31.78** $^{q}$ | **27.44** $^{r}$ | **22.67** $^{s}$ | **(-)** |
| Number of nodes | **100** | 199 $^{g}$ | 135 $^{f}$ | 120 $^{e}$ | 109 $^{de}$ | **141** $^{x}$ |
| | **50** | 100 $^{d}$ | 84 $^{c}$ | 71 $^{b}$ | 68 $^{b}$ | **81** $^{y}$ |
| | **25** | 68$^{b}$ | 64 $^{b}$ | 58 $^{b}$ | 41 $^{a}$ | **58** $^{z}$ |
| | Average | **122** $^{p}$ | **95** $^{q}$ | **83** $^{r}$ | **72** $^{s}$ | **(+)** |
| Number of leaves | **100** | 575 $^{g}$ | 417 $^{f}$ | 334 $^{e}$ | 267 $^{d}$ | **398** $^{x}$ |
| | **50** | 275 $^{d}$ | 187 $^{c}$ | 182 $^{c}$ | 162 $^{bc}$ | **201** $^{y}$ |
| | **25** | 183 $^{c}$ | 160 $^{bc}$ | 135 $^{b}$ | 93 $^{a}$ | **143** $^{z}$ |
| | Average | **344** $^{p}$ | **255** $^{q}$ | **217** $^{r}$ | **174** $^{s}$ | **(+)** |
| Plant biomass (g) | **100** | 5.80 $^{e}$ | 4.37 $^{d}$ | 3.87 $^{d}$ | 2.97 $^{c}$ | **4.37** $^{x}$ |
| | **50** | 2.95 $^{c}$ | 2.45 $^{bc}$ | 2.47 $^{bc}$ | 1.73 $^{ab}$ | **2.44** $^{y}$ |
| | **25** | 2.23 $^{abc}$ | 1.83 $^{ab}$ | 1.63 $^{ab}$ | 1.43 $^{a}$ | **1.82** $^{z}$ |
| | Average | **3.66** $^{p}$ | **2.84** $^{q}$ | **2.66** $^{q}$ | **2.04** $^{r}$ | (-) |
| Shoot root ratio | **100** | 2.04 $^{c}$ | 1.62 $^{bc}$ | 1.74 $^{bc}$ | 1.21 $^{ab}$ | **1.68** $^{x}$ |
| | **50** | 1.25 $^{ab}$ | 1.23 $^{ab}$ | 1.07 $^{ab}$ | 0.88 $^{a}$ | **1.12** $^{y}$ |
| | **25** | 1.08 $^{ab}$ | 1.25 $^{ab}$ | 1.18 $^{ab}$ | 1.26 $^{ab}$ | **1.18** $^{z}$ |
| | Average | **1.45** $^{p}$ | **1.35** $^{p}$ | **1.33** $^{p}$ | **1.11** $^{p}$ | (-) |
| Chlorophyll content (mg. $g^{-1}$) | **100** | 2.58 $^{abcd}$ | 2.61 $^{abcd}$ | 2.28 $^{b}$ | 2.49 $^{abcd}$ | **2.50** $^{x}$ |
| | **50** | 2.90 $^{d}$ | 2.52 $^{abcd}$ | 2.49 $^{abcd}$ | 2.37 $^{ab}$ | **2.58** $^{x}$ |
| | **25** | 2.84 $^{cd}$ | 2.55 $^{abcd}$ | 2.78 $^{bcd}$ | 2.46 $^{ab}$ | **2.63** $^{x}$ |
| | Average | **2.77** $^{p}$ | **2.56** $^{pq}$ | **2.52** $^{q}$ | **2.44** $^{q}$ | (-) |

Means within the column and row followed by different letters differ significantly at the α 5% significance level DMRT

Results of analysis of variance showed light intensity and water availability treatments as well as the interaction between treatments provide significant effect on the plant biomass. The light intensity of 100% showed the highest plant biomass, and this was significantly different from the light intensity of 50% and 25%. On water availability of 100% field capacity showed the highest plant biomass. This result was significantly different from those of water availability of 50% and 25%, but does not significantly different with water availability of 75% field capacity (Table 1).

Shoot-root ratio of stevia tend to decrease with reduced levels of water availability and light intensity (Table 1). It is possible in conditions of light intensity 100% stevia can perform photosynthesis process optimally, as well as photosynthates translocation in shoot thus increasing the shoot-root ratio. The shoot-root ratio of stevia tend to decrease with reduced levels of water availability. Shoot-root ratio is used to determine the ability of plants to maintain a functional balance in environment stress. Drought stress decreased the shoot-root ratio, more organic C being delivered to the roots and root growth is being stimulated.

Chlorophyll content decreased with decreasing water availability (Table 1). Drought stress significantly decreased chlorophyll content in cucumber (Nazi *et al*., 2016). Decreasing of chlorophyll content of leaves in the drought conditions might be caused by inhibition of the formation of chlorophyll. Chlorophyll is very sensitive to low water availability, therefore the chlorophyll level will decline due to low water availability (Pugnaire & Pardos, 1999).

**Table 2. Density, index and size of stomata of stevia (*Stevia rebaudiana* Bertoni) grownat different light intensities and water availability 6 weeks of treatments.**

| Variables | Light intensity (%) | Water availability (% FC) | | | | Average |
|---|---|---|---|---|---|---|
| | | 100 | 75 | 50 | 25 | |
| Density of stomata (/mm$^2$) | **100** | 107$^{ab}$ | 116$^{ab}$ | 120$^{ab}$ | 133$^{ab}$ | **119$^{x}$** |
| | **50** | 109$^{ab}$ | 102$^{a}$ | 112$^{ab}$ | 113$^{ab}$ | **109$^{x}$** |
| | **25** | 116$^{ab}$ | 117$^{ab}$ | 157$^{ab}$ | 186$^{b}$ | **144$^{x}$** |
| | Average | **111$^{p}$** | **112$^{p}$** | **130$^{p}$** | **144$^{p}$** | **(-)** |
| Index of stomata (%) | **100** | 10.00$^{ab}$ | 9.16$^{a}$ | 10.27$^{ab}$ | 11.41$^{ab}$ | **10.21$^{x}$** |
| | **50** | 12.17$^{ab}$ | 10.76$^{ab}$ | 10.37$^{ab}$ | 10.88$^{ab}$ | **10.98$^{x}$** |
| | **25** | 10.23$^{ab}$ | 11.21$^{ab}$ | 13.59$^{ab}$ | 13.87$^{b}$ | **12.01$^{x}$** |
| | Average | **10,74$^{p}$** | **10.46$^{p}$** | **11.21$^{p}$** | **11.94$^{p}$** | (-) |
| Size of stomata (□m) Check | **100** | 33.45$^{g}$ | 28.90$^{ef}$ | 25.56$^{bcd}$ | 25.46$^{bcd}$ | **28.34$^{x}$** |
| | **50** | 30.68$^{f}$ | 27.79$^{def}$ | 25.08$^{bcd}$ | 23.83$^{ab}$ | **26.85$^{y}$** |
| | **25** | 28.90$^{ef}$ | 27.32$^{cde}$ | 24.49$^{abc}$ | 21.89$^{a}$ | **25.65$^{y}$** |
| | Average | **31.01$^{p}$** | **28.00$^{q}$** | **25.04$^{r}$** | **23.73$^{r}$** | **(-)** |

Means within the column and row followed by different letters differ significantly at the α 5% significance level DMRT

**Table 3. Stevioside level and amount of stevioside of stevia grown at different light intensities and water availability 6 weeks of treatments.**

| Variables | Light intensity (%) | Water availability (% FC) | | | | Average |
|---|---|---|---|---|---|---|
| | | 100 | 75 | 50 | 25 | |
| Stevioside level (mg/g) | 100 | 32.38$^{a}$ | 38.32$^{a}$ | 41.54$^{ab}$ | 33.59$^{a}$ | **36.45$^{x}$** |
| | 50 | 42.06$^{ab}$ | 44.56$^{ab}$ | 35.52$^{a}$ | 43.17$^{ab}$ | **41.33$^{xy}$** |
| | 25 | 46.77$^{b}$ | 36.62$^{a}$ | 43.86$^{ab}$ | 46.85$^{b}$ | **43.52$^{xy}$** |
| | Average | **40.40$^{p}$** | **39.83$^{p}$** | **40.30$^{p}$** | **41.20$^{p}$** | **(-)** |
| Amount of stevioside (mg) | 100 | 125.62$^{c}$ | 103.46$^{c}$ | 100.93$^{c}$ | 54.76$^{ab}$ | **96.19$^{x}$** |
| | 50 | 68.56$^{ab}$ | 60.16$^{ab}$ | 45.12$^{ab}$ | 34.54$^{a}$ | **52.09$^{y}$** |
| | 25 | 53.78$^{ab}$ | 36.62$^{a}$ | 33.77$^{a}$ | 36.07$^{a}$ | **40.06$^{y}$** |
| | Average | **82.65$^{p}$** | **66.74$^{pq}$** | **59.94$^{pq}$** | **41.79$^{q}$** | **(-)** |

Means within the column and row followed by different letters differ significantly at the α 5% significance level DMRT

**Response of density, index and size of stomata to light and water availability:** The results showed the treatment of light intensity and water availability significantly affected the size of the stomata, but did not affect the density and stomatal index. Stomata size reduced with decreasing light intensity and water availability (Table 2). Small stomata could maintain the pores opening with lower guard-cell turgor pressures compared with larger stomata. Reduced stomatal size in rice responding to drought can efficiently inhibit transpirative water loss and better ensure water balance (Bosabalidis and kofidis, 2002).

**Response of stevioside levels to light and water availability:** The results showed that the levels of stevioside increased with decreasing levels of light intensity. The light intensity of 25% plants showed the highest stevioside levels, and this value was not different with the light intensity of 50% plants, whereas the light intensity of 100% indicated the lowest level of stevioside. The level of stevioside levels were identical on all levels of water availability (Table 3). The highest level of stevioside levels was observed in the water treatment 25% of field capacity.

## Discussion

Light intensity and water availability significantly affected growth of stevia. Light intensity of 100% and water availability of 100% showed the optimal growth of stevia as observed in plant height, number of nodes, number of leaves. Plant height, the number of nodes, number of leaves and plant biomass of the stevia increased with increasing light intensity and availability of water and positively correlated to plant biomass (Fig. 2). These results are consistent with research by Srivastava & Srivastava (2014b) which states that stevia growth increased in conditions of high water availability. Stevia will grow well in various soil types given adequate water supply. Stevia requires a high soil moisture and has a high tolerance of wet soils. Water stress greatly suppresses cell expansion and cell growth due to low turgor pressure. Water plays a role in maintaining turgidity necessary for cell enlargement and growth. This important role has consequences that lack of water in plants are directly or indirectly affect all metabolic processes in the plant which resulted in disruption of growth (Pugnaire & Pardos, 1999; Suryanti *et al*., 2015). Alavi-Samani *et al*. (2013) observed reduced plant height in two species of thyme in response to water deficit conditions. Water stress as a very important limiting factor for plant growth and development affects both elongation and expansion growth (Shao *et al*., 2008). Light also plays an important role in photosynthesis (Cseke *et al*., 2006). The intensity of light affect photosynthesis is correlated to the accumulation of organic matter and biomass.

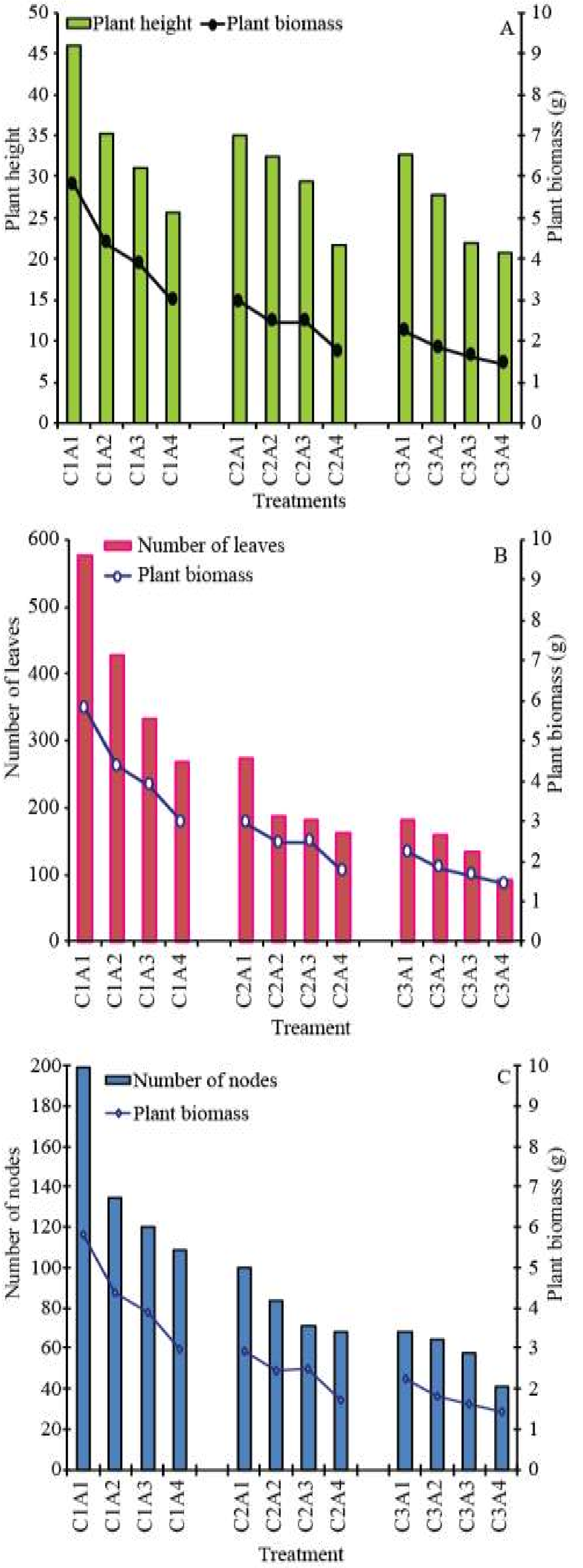

Fig. 2. Correlation between plant height, number of nodes and leaves to plant biomass of stevia (*Stevia rebaudiana* Bertoni) grown 6 weeks at different light intensity and water availability. A. Water availability (1: 100%, 2: 75%, 3:50%, 4:25 of field capacity) and C. Light intensity (1: 100%, 2: 50%, 3: 25%).

Production of plant biomass related to the ability of photosynthesis and leaf area. Drought will cause a reduction in growth and yield, this is due to decreasing photosynthesis. In the drought conditions, the process of photosynthesis is limited by the low availability of $CO_2$ due to reducing stomatal conductance. Drought stress is closely related to stomatal closure and reduction in $CO_2$ fixation (Li *et al*., 2006), which led to decreased plant biomass. The results of this research are in consistent with the findings of Srivastava & Srivastava (2014a) that biomass of stevia tends to decrease with decreasing availability of water.

The availability of water also affects the chlorophyll content of leaves of the stevia. The results showed that the levels of chlorophyll tends to decrease with decreasing availability of water. These results are in consistent with research by Srivastava & Srivastava (2014a) that the levels of chlorophyll decreased in the stevia plant on drought conditions. The decline in chlorophyll content at drought conditions was caused by inhibition of the formation of chlorophyll. Srivastava and Srivastava (2014b) mentioned a drought led to the inhibition of absorption of nutrients from the soil, thus reducing the availability of N and Mg which plays an important role in the synthesis of chlorophyll.

The availability of water significantly affected the size of the stomata of the leaves of stevia, but did not affect the density and index of stomata (Table 2). The size of stomata gets smaller with decreasing availability of water, while the density and index of stomata tends to increase with decreasing water availability. Reduced soil water content significantly stimulated stomatal generation (formation of stomata), resulting in a significant increase in stomatal density but a decrease in stomatal size. Water stress condition was significantly decrease in the parameters of length and width of stomata (Terletskaya *et al*., 2017). Small stomata could maintain the pores opening with lower guard-cell turgor pressures compared with larger stomata (Bosabalidis & Kofidis, 2002). According to Mc Cree & Davis (1994) the density, index, and size of the stomata on a plant is related to drought resistance. Increased the density of stomata and a decrease in the size of stomata can improve crop adaptation to drought (Martinez *et al*., 2007).

Plant biomass decreased with decreasing water availability and light intensity (Fig. 3A). Stevioside level tended to increase at low light intensity, whereas water availability did not significantly affect the stevioside level. High light intensity and water availability are more effective on biomass accumulation compared to the accumulation of glycosides steviol (stevioside level). The amount of stevioside was positively correlated with plant biomass (Fig. 3B). Light intensity of 100% caused an increase in the amount stevioside through increasing biomass, but not through increasing the level of stevioside. Stevioside originates from the same precursor GA hormone that is ent-kaurenoic acid (Brandle & Telmer, 2007). According to Golldack *et al.* (2014) drought induced ABA which is inhibiting GA, so that in drought conditions, the precursor hormone GA is likely to be diverted towards the biosynthesis stevioside, but the mechanisms related to stevioside biosynthesis is not yet known.

Stevioside levels increased along with reduced levels of light intensity. Twenty five percent light intensity showed highest stevioside levels. In the treatment of water availability showed the Stevioside levels similar at all levels of water availability. The highest stevioside levels was found in plant treated with-water availability of 25% field capacity. It is associated with the stevia biochemical adaptation to drought conditions.

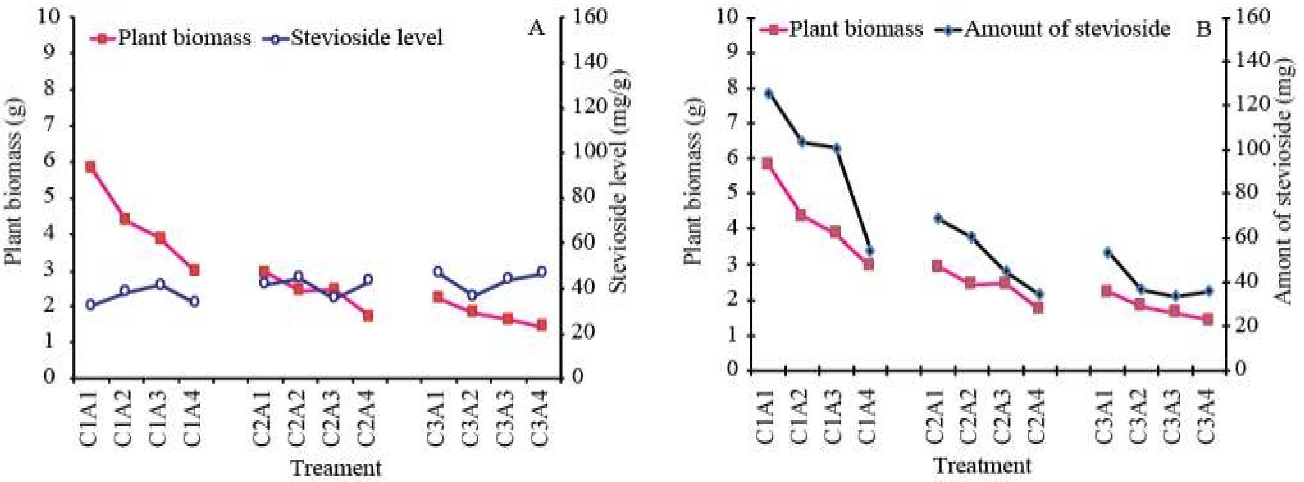

Fig. 3. Correlation between stevioside level and plant biomass (A), amount of stevioside and plant biomass (B) of stevia (*Stevia rebaudiana* Bertoni) grown 6 weeks at different light intensities and water availability. A. Water availability (1: 100%, 2: 75%, 3:50%, 4:25 of field capacity) and C. Light intensity (1: 100%, 2: 50%, 3: 25%).

## Conclusions

It can be concluded that Stevia showed the changes in physiological traits in response to drought and low light intensity. High light intensity and water availability increased plant growth (plant height, number of nodes, number of leaves and biomass), but there was no effect on leaf chlorophyll content as well as steviosida level. Light intensity of 100% and water availability of 100% showed optimal growth of stevia.

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