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Effects of kinetin on the morpho-physiological and biochemical characteristics of stevia (*Stevia rebaudiana)*

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Abstract

Khandaker, M. M., Liyana, N., Majrashi, A., Dalorima, T., Alias, N. & Mat, N. (2018). Effects of kinetin on the morpho-physiological and biochemical characteristic of stevia (*Stevia rebaudiana)*. *Bulgarian Journal of Agricultural Science*, *24*(4), 638–647

Stevia is a medicinal herb commonly known as sugar leaf under Asteraceae family. Stevia leaves are in growing demand as a natural sweetener, and the health benefits of the herb have created an interest among researchers. However, stevia plant is a slow growing herb and biomass yield is low due to slow initial plant growth rate, less number of leaves and small leaf area. In addition, less stevioside activity some times causes poor sugar content in the leaf. The aim of the study is to investigate the effects of kinetin on morpho-physiological and biochemical characteristics of *Stevia rebaudiana*. Besides that, this study was conducted to determine the effects of kinetin on antioxidant activity of *Stevia rebaudiana*. The stevia plants were sprayed with 0, 10, 20, 40 and 80 mg/L of kinetin under green house conditions. Parameters such as height of plant, leaf number, area of the leaf, leaf chlorophyll content, chlorophyll fluorescence, photosynthesis rate, stomatal conductance, total soluble solids, carotene content and antioxidant activity through DPPH assay were measured and determined. The results showed that the application of 10 mg/L kinetin increased plant physiological activities, leaf area, and photosynthetical characteristics of stevia plants. In addition, total soluble solids, carotene content and antioxidant activity were also increased significantly with 10 mg/L kinetin treatment. It can be concluded that spraying with 10 mg/L kinetin twice a week improved morpho-physiological characteristics and increased biochemical properties of stevia plants.

Keywords: stevia; kinetin; morphology; physiology; quality

Introduction

Medicinal herb *Stevia rebaudiana* belongs to Asteraceae family, native to the Amambay region of Paraguay, and stevia is also found in Argentina and Brazil (Abou-Arab et al., 2010). It is known by different names, which include sweet herb, sweet leaf, honey leaf, candy leaf and honey yerba. The Latin binomial *Stevia rebaudiana* first appeared in the literature in 1899, coined by Moises Santiago de Bertoni (Bertoni, 1899). The specific epithet *rebaudiana* was dedicated to a Paraguayan chemist, Ovidio Rebaudi, whom he admired and who later performed the first chemical study of the plant, from samples provided by Bertoni. In 1904 on his return to Asuncion, he received a live specimen of the plant from a resident of San Pedro (Kinghorn, 2003). It was this specimen that enabled him to assign the correct taxonomic placement of the plant within the genus *Stevia*

and published it as *Stevia rebaudiana* Bertoni (Bertoni, 1905). There are about 4 to 20 % of sweet deterrence in the dry leaf biomass of stevia (Ghanta et al., 2007). Stevia leaves are also the ultimate source of sweet ent-kaurene and steviol glycoside, which are responsible for sweetness. It has been reported that *rebaudiana* and *phlebophylla* species contain the steviol glycosides out of 230 species (Brandle and Telmer, 2007).

Organic chemical substances other than nutrients which are active in low concentration in promoting, inhibiting or otherwise modifying growth and development may be called growth regulators. Plant growth regulators are organic substances, active in small amount which regulate the physiological, morpho-physiological and biochemical reactions within the plants. Plant growth regulators regulate growth, development, signals transduction, stomatal conductance, photosynthesis, respiration, water uptake, phloem loading and other physiological processes. Chatsudthipong and Muanprasat (2009) reported that cytokinins are a class of plant hormones that play a crucial role from seed germination to senescence. Cytokinin regulates gene expression, promote cell division, chloroplast development, suppress apical dominance and inhibit shoot and root growth. Besides, the interaction of cytokinin with auxin and ABA has already been reported. Cytokinin can control cell division, promote growth and development, and induce lateral buds in combination with auxin.

Stevia is nutrient-rich, having considerable amount of protein, sodium, calcium, phosphorus and other macro and micro nutrient elements (Ghanta et al., 2007). Currently, stevia is using as major source of high potency sweetener in different countries. The powder of stevia leaves can be a substitute to the artificial sugar in tea, coffee and other food product without any harmful effects for diabetic patients (Anbazhagan et al., 2010). Steviol glycosides have antioxidant, antimicrobial and several pharmacological activities. However, stevia plant is a slow growing medicinal herb and biomass yield is low due to slow initial plant growth rate, less number of leaves and small leaf area. In addition, some times less stevioside activity causes poor sugar content in the leaf. It has been stated earlier that plant growth regulators can play significant role in plant growth and development. The objective of this study is to study the effect of kinetin, a cytokinin plant growth regulator, on morphological, physiological and biochemical characteristics of stevia plants.

Materials and Methods

Plant material and experimental site

Plant materials used in this study was *Stevia rebaudiana* Bertoni. Stevia plants were collected from a commercial nursery at Sungai Buloh, Kuala Lumpur. There were 25 plants that have uniform height and size that have been selected. This study was carried out in the greenhouse at the Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia.

Preparation of hormone

The mass of the kinetin were weighed by using electronic balance. Then calculated mass of kinetin was dissolved into a few drops of 1 M sodium hydroxide (NaOH) in a beaker. After that the mixture was transferred into the 1 liter Schott bottle and distilled water was added up to the 1 liter level. The solution then was stirred for 5 to 10 minutes, before being covered with aluminium foil and stored in 4°C chiller.

Treatment application and experimental design

The experiments consisted of five treatments (0, 10, 20, 40, and 80 mg/L of kinetin). Each treatment consisted of 5 replicate of stevia plant. Treatments were applied as a foliar spray twice per week, from week 1 until week 5, and a total of ten (10) sprays were carried out during the experiment. Kinetin hormone solution around 100 mL was used per treatment and applied to the leaf of stevia plants. This treatment was sprayed to all parts of stevia plant except root. Treatments have been applied at three days interval. This experiment was carried out with Completely Randomized Design (CRD) design layouts with five treatments and each treatment consisted of 5 replicates. Treatment 0 acted as a control with no kinetin application. The different treatments used were as in Table 1.

Morphological parameters: Plant height, branch and leaves number and leaf area

The measurements for the plant height were taken every week for nine weeks. These were measured by using ruler for each treatment. The average of the result was calculated and the data was recorded. The branch and leaves number were counted every week for nine weeks. The measurements for the leaf area were taken by a leaf area meter once in two week for five weeks.

Measurement of chlorophyll content and chlorophyll fluorescence

SPAD-502 meter (Minolta Japan) was used to measure the chlorophyll content in the stevia leaf. Lower (F0), variable (Fv) and higher fluorescence (Fm) were measured and recorded with a portable Handy Plant Efficiency Analyzer (PEA). Photosynthesis or quantum yield (Fv/Fm) was also calculated from the treated and control plants. The clip of this meter was clamped over the leaf for about ten minutes. The reading was observed on the meter screen and the result was recorded. The data was taken by two replicate for each treatment.

Photosynthetic rate (Pn) and stomatal conductance of stevia plants

The photosynthetic rate (*Pn*) was measured by using the portable photosynthesis system CI-340 Handheld Photosynthesis System. Net photosynthesis rate was measured at dry leaf stage after the treatment application at around 11 AM. The clip of this system was clamped over the leaf for each treatment. The reading was taken by three replicates for each treatment. The reading was observed and recorded. Stomatal conductance of stevia leaf was measured by using a leaf Porometer, model SC-1, USA. The leaf chamber was kept at a 26°C for 12 minutes before the measurement, to maintain sunlight adaptation. Then one leaf was attached with the leaf chamber and the readings were recorded.

Determination of total soluble solids (TSS) and carotene content

Hand refractometer (Atago 8469, Atago Company Limited, Tokyo, Japan) was used to evaluate the TSS content of stevia leaves and expressed as percentage Brix. The leaves samples were collected for each treatment. The samples were then cleaned and air dried. After that, veins of leaf were removed and leaves were cut into small pieces. Then it was weighted at approximately 1 g for each treatment by using the electronic balance. The weighed leaf samples were later crushed in pestle and mortar and few drops of distilled water added to make juice of stevia leaf. Two drops of leaf juice were placed to the refractometer sensor. The readings showed in percentage and data was recorded.

The carotenoid content was measured by using spectrophotometer. This measurement was taken to measure the photosynthetic pigments of the leaf. This method has followed the Lichtenthaler method (Lichtenthaler and Wellburn, 1983) with slight modification. The leaves samples were collected for each treatment. Then, samples were cleaned and air dried. The veins of the leaf were removed and the leaf was cut into small pieces. Then, samples were weighed about one g by using electronic balance for each treatment. The weighed samples were crushed by using pestle and mortar and were homogenized in 10 mL of 80% acetone for each 0.25 g samples. Then, the homogenate was filtered by using filter paper Whatman no 1 in a glass funnel. The filtrate was poured in 3 mL cuvette and its absorbance was measured in wavelengths of 663 nm, 645 nm and 480 nm, for measurements of chlorophyll *a*, chlorophyll *b* and carotenoid, respectively. These readings were taken by spectrophotometer devices.

Antioxidant activity (DPPH assay)

In a 96 wells microplate, 20 µL of 100% DMSO was added to the wells B until H, followed by 20 µL of sample solution added with different concentration in wells A and B only. Then, serial dilution was performed from well B to G. Exactly 200 µL of DPPH solution was added next and the solution was vortexed. Then, the diluted sample was incubated in darkness at 30°C since the test is light sensitive. By using the microplate reader the absorbance A was read at 517 nm. Absorbance of reading samples for all the treatments was carried out in triplicate. The following equation was used to calculate the DPPH inhibitory effect.

Percentage Inhibition = [(A control – A sample)]/ [A control] x100

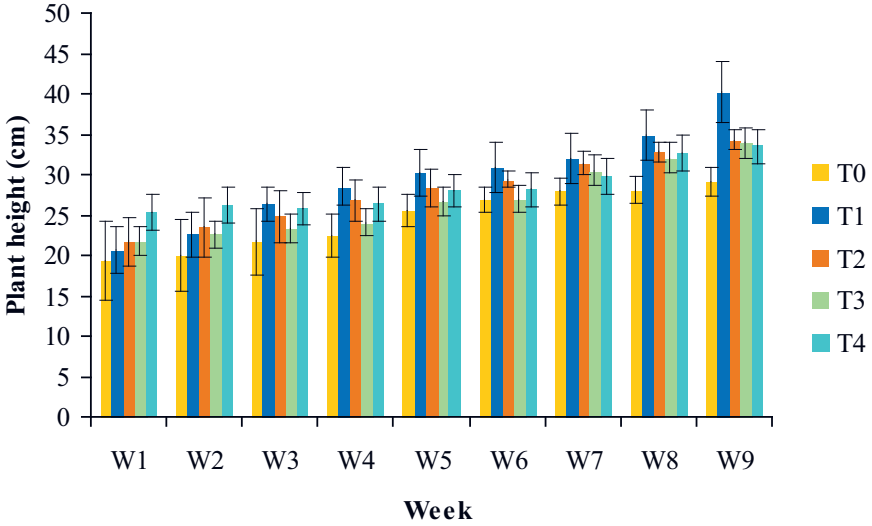

Fig. 1. Effects of kinetin on plant height of stevia plant
Bars indicates ± S.E.T0 (Control), T1 (10 mg/L), T2 (20 mg/L), T3 (40 mg/L) and T4 (80 mg/L)

Statistical analysis

A Completely Randomized Design (CRD) with five replications was used to arrange all the experimental treatments. For evaluation of the significance level among the studied parameters of this current experiment, a one way repeated ANOVA was used. The Fishers protected LSD (Least significant difference) was calculated following the F test at $p > 0.05$).

Results and Discussion

Plant height and number of branches

Kinetin is known to be a promoter of cell division, seed germination and the growth of new buds and tillers (Moore, 1989). Figure 1 shows the plant height for stevia plants as affected by different concentrations of kinetin. All treatments produced significant effects on plant height. Based on Figure 1, it clearly shows that plant treated with kinetin treatment has higher plant height as compared to the untreated plant (T0).

T1 stated the highest plant height in week 3 until week 9 while T0 showed the lowest plant height from week 1 until week 9. T1 stated the highest means of increment from week 1 to week 9 with 19.5 cm followed by T2 and T3 with 12.6 cm and 12.1 cm, respectively, while the lowest reading came from T4 with 8.2 mm. It shows that T1 (10 mg/L) was the best kinetin concentration for the plant height of stevia plant. Number of branch of stevia was also affected significantly by kinetin application (Figure 2). Figure 2 shows the means of the number of branch for treated and untreated plants from week 1 to week 9. All treatments were significantly different than the control at 5% level of significance. The result shows that T1 produced the highest number of branch in week 5 to week 9 while T3 showed the lowest number of branch from week 2 to week 9. Similar results were reported by Shah (2007) who stated that kinetin enhances the meristematic activity of plant and increases the growth of plant.

T1 stated the highest means of increment from W1 to W9 at 17 followed by T0 and T2 at 13 and 9, respectively, while the lowest reading came from T3 at 1. It shows that T1 (10 mg/L) was the best kinetin concentration for the number of branch of stevia plant. El-Badawy and Abd El-Aal (2013) also reported similar positive effects of kinetin on morpho-physiological properties of plants.

Number of leaves

As can be seen from Figure 3, all the treatments produced significant effect on leaf number of stevia plant. T1 stated the highest number of leaf in week 1 to week 9 and the highest number of leaves was recorded in week 9 with a value of 279. T1 produced the highest leaf number from week 1 to week 9 at 167, followed by T4 and T2 at 117 and 98, respectively, while the lowest reading came from T3 with 88. It shows that T1 (10 mg/L) was the best kinetin concentration for the number of leaves of stevia plant. Khandaker et al. (2017) also reported that plant growth regulators application increased the leaf number of orchid but the effect was not statistically significant.

Leaf area

Leaf area is important parameters that regulates the light interception, photosynthetic capacity, chlorophyll content,

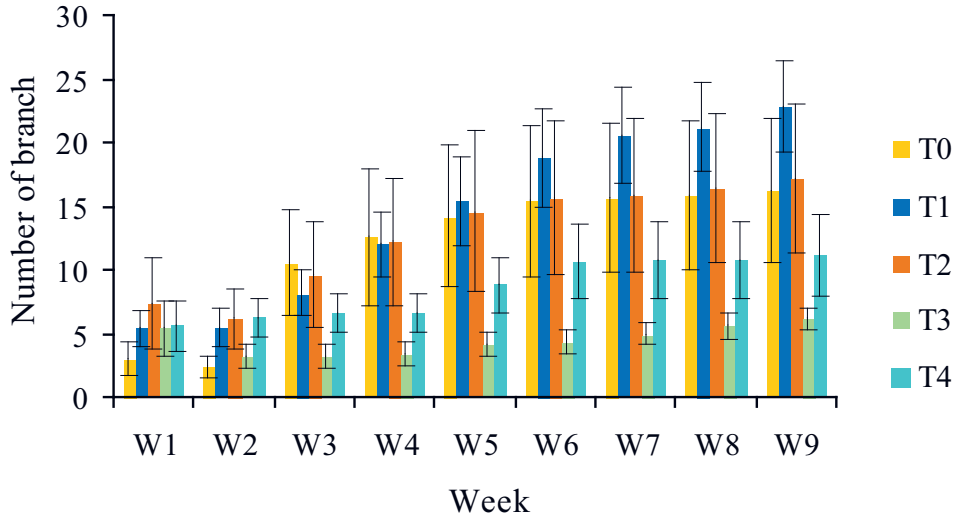

Fig. 2. Effects of kinetin on the number of branchs of stevia plant
Bars indicates ± S.E. T0 (Control), T1 (10 mg/L), T2 (20 mg/L), T3 (40 mg/L) and T4 (80 mg/L)

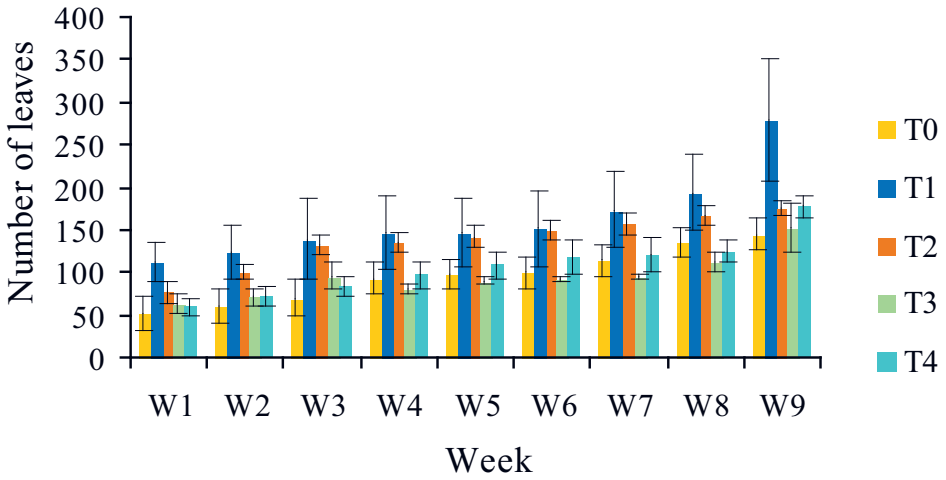

Fig. 3. Effects of kinetin treatment on the number of leaves of stevia plant
Bars indicates ± S. E. T0 (Control), T1 (10 mg/L), T2 (20 mg/L), T3 (40 mg/L) and T4 (80 mg/L)

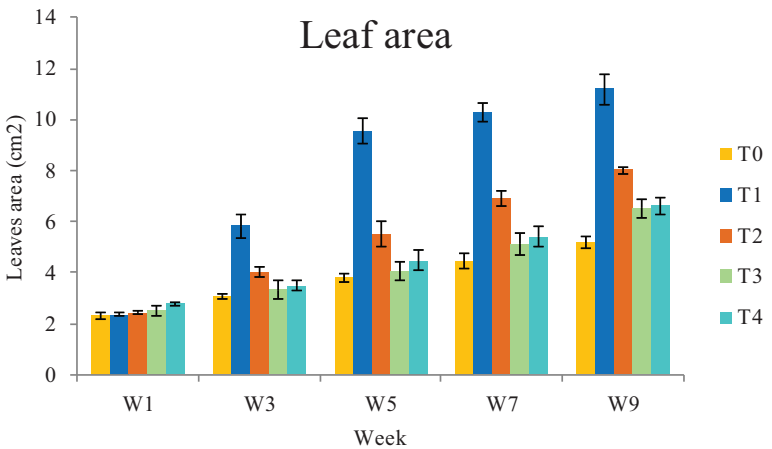

Fig. 4. Effect of kinetin treatment on the leaf area of stevia plant
Bars indicates ± S.E. T0 (Control), T1 (10 mg/L), T2 (20 mg/L), T3 (40 mg/L) and T4 (80 mg/L)

transpiration, fertilizer and irrigation use efficiency. Figure 4 shows the leaf area for T0, T1, T2, T3 and T4. All the treatments were significantly different than the control at the 5 % significance level.

According to Figure 4, T1 showed greater number of leaf area in week 3, week 5, week 7 and week 9, respectively. The leaf area for T1 were 5.82, 9.54, 10.28 and 11.19 cm^2 in W3, W5, W7 and W9 respectively. It was followed by T2 and T4. Pospíšilová et al. (2000) stated that exogenous application of kinetin increases the leaf area of stressed plants. In our study all the kinetin treated plants increased the plant height, number of leaves, branch number and leaf area of stevia plants. This increased growth may be the results of stimulated activity of apical and lateral meristems. It has been reported earlier that kinetin may stimulate cell division, suppress senescence promoting enzyme and minimize the contents of abscisic acid, ethylene or other plant growth retardants. Mukherjee and Kumar (2007) confirmed that exogenous application of kinetin stimulates cell division, suppresses proteolysis and declines protease activity.

Chlorophyll content (SPAD value)

The amount of chlorophyll content (SPAD) for treated and untreated plants was as shown in Table 1. In week 9, T3 treatment produced the highest amount of chlorophyll content at 39, while the control shows the lowest reading at 36.

Table 1
Leaf chlorophyll content (SPAD) of stevia plants as affected by kinetin application

Treatment	Week 1	Week 3	Week 5	Week 7	Week 9
T0	29.10[a]	34.56[a]	33.4[a]	35.94[a]	36.06[a]
T1	33.84[a]	28.58[a]	32.28[a]	38.10[a]	36.32[a]
T2	36.80[a]	29.66[a]	30.14[a]	33.94[a]	36.84[a]
T3	36.80[a]	33.74[a]	32.92[a]	35.82[a]	39.74[a]
T4	38.16[a]	36.72[a]	35.32[a]	36.92[a]	36.80[a]

Different letter in same column are significantly different at 5% level of significance. Treatment (0, 10, 20, 40 and 80 mg/L Kinetin)

In this study all the treatments increased the leaf chlorophyll content of stevia but their differences were not statistically significant than the control at the 5% level of significance (Table 1). Our results were in agreement with the findings of Kaya et al. (2010), who reported that kinetin increases photosynthetic pigments and leaf chlorophyll contents. Growth regulators application increases the chlorophyll content of plant parts at early growth stage, and later on this chlorophyll was converted into other pigments (Khandaker et al., 2013a).

Chlorophyll fluorescence

The chlorophyll fluorescence is a signal of the fate of excitation energy in the chloroplast that is used to detect the physiological injury in the plant leaves under different environmental conditions. Chlorophyll fluorescence also gives the information of photosystem II and stomatal density in the leaves. Based on Table 2, the result for the kinetin treatment shows the highest value of the higher fluorescence (Fm) in T4 at 2400, followed by T1 at 2293.

While, the lowest reading was as shown in T3 with the value 1957. All treatments were not significantly different at $p > 0.05$. For lower fluorescence (Fo), T2 produced the highest value at 639 followed by T1 and T0 at 624 and 606, respectively. The lowest reading was shown in T4 at 574. The results for relative variable fluorescence (Fv) showed the highest reading in T4 at 1826, followed by T1 and T0 at 1668 and 1590, respectively, and the lowest relative variable fluorescence was shown in T3 at 1378. The positive effect of kinetin on chlorophyll fluorescence was also recorded by Moneruzzaman et al. (2010a).

Photosynthetic yield is positively correlated with photosystem II and chlorophyll fluorescence is an indirect measurement of plant health status for which values of 8 indicates the plant is highly efficient in photochemical processes. It has been reported that photosynthetic yield of below 0.65 as a sign of severe stress, and normal growth and development of plant hampered (Maki and Colombo, 2001). The results for photosynthetic yield shown the highest value in T4 at 0.756, followed by T1 and T0 with values of 0.726 and 0.71, respectively (Table 2). The means difference of T4 from other treatments and control were significantly different at p value greater than 0.05. Our results were supported by the findings of Saifuddin et al. (2009), who reported that GA_3 treatment plus phloemic stress increase the quantum yield (Fv/Fm) in potted Bougainvillea plant. On the other hand, another research reported that cytokinin treatment did not produce any effect on chlorophyll fluorescence (Xiaotao et al., 2013).

Photosynthetic rate

Figure 5 shows the net photosynthetic rate for stevia plants as affected by different concentrations of kinetin. All the treatments produced significant effects on photosynthetic rate. Based on the results it clearly shows that plant treated with kinetin has higher photosynthetic rate as compared to the untreated plants. The highest photosynthetic rate was recorded in T1 treatment for both months were followed by T2. While T0

Table 2
Effect of kinetin treatment on the leaf chlorophyll fluorescence of stevia plant

Treatment	Fm	Fo	Fv	Fv/Fm
T0	2169.55[a]	606.25[a]	1590.25[a]	0.710[a]
T1	2293.30[a]	624.75[a]	1668.25[a]	0.726[a]
T2	2060.25[a]	639.25[a]	1421.90[a]	0.684[a]
T3	1957.75[a]	579.20[a]	1378.75[a]	0.702[a]
T4	2400.75[a]	574.25[a]	1826.50[a]	0.756[b]

Different letters in same column are significantly different at 5% level of significance
Treatments: 0, 10, 20, 40 and 80 mg/L kinetin

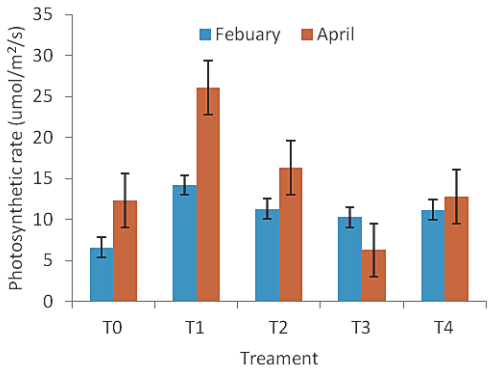

Fig. 5. Effect of kinetin treatment on the photosynthetic rate of stevia plant
Bars indicates ± S. E. T0 (Control), T1 (10 mg/L), T2 (20 mg/L), T3 (40 mg/L) and T4 (80)

shows the lowest reading in February, T3 gave higher reading at in April (Figure 5). Shah (2011) also reported that kinetin treatment increases the net photosynthesis rate and yield of black cumin. Cytokinin may stimulate the stomatal opening and affect photosystems I and II at the mesophyll and chloroplast levels. It has been reported that exogenous application cytokinin increases the level of endogenous cytokinin, thus elevated the level of cytokinin, increases the CO_2 assimilation, net photosynthetic rate and lengthens the active photosynthetic period (Chen et al., 2010).

Stomatal conductance

Stoma plays a significant role in diffusion of water vapour, maintaining of leaf temperature, and exchange of carbon-dioxide and oxygen levels in plant. An enhancement of the stomatal conductance after treatment occurs because cytokinins can increase stomatal opening and counteract the closing effects (Wang et al., 1994). Based on the results of the current study, the mean differences of stomatal conductance were not significantly different from week 1 to week 5 (Table 3).

On the other hand, from the week 7 and onwards, the different kinetin treatments produced significant effects on stomatal conductance of stevia plants, where p value was greater than 0.05 with the highest reading in week 9 came from T1 with 500 mmol/m²/s, followed by T2 and T3 with the values of 378 and 329 mmol/m²/s, respectively, while T4 stated the lowest reading with 289 mmol/m²/s (Table 3). These results were in agreement with the findings of Shah (2011), who stated that foliar application of kinetin increases the leaf chlorophyll content and stomatal conductance of black cumin plants. The results of this study showed that kinetin treatment increased the stomatal conductance of stevia plants at the later stage. Merewitz et al. (2011) reported that stomatal opening may be associated with the reduction in the production or degradation of ABA or a lower ratio of ABA/cytokinin that decrease resistance of stomata. Cytokinin regulate stomatal opening by enhancement of ethylene production, stimulation of osmotic adjustment and or by decreasing hydrogen peroxide and nitric oxide levels in the guard cells (Song et al., 2006). Cytokinin may also stimulates electrogenic H^+-pump, adenylate cyclase and guanylate cyclase in the guard cell, thus leading to stomatal opening (Pharmawati et al., 1998).

Total soluble solids

In this study, the results showed that TSS content in the leaf juice of stevia was significantly increased after kinetin application. Figure 6 shows the means of the total soluble solids for all treatments from week 1 to 9. Based on the results, T1 has the highest TSS content in week 5 to week 9, while T3 showed the lowest TSS content in the same weeks.

It was shown that T1 (10 mg/L) was the best kinetin concentration for the total soluble solids of stevia plants. Khandaker et al. (2011) reported that exogenous plant growth regulators increase total soluble solids in plant parts. May be the growth regulators increase the accumulation of carbohydrate and starch content in plants. This might be due to increases of pedicel vascularization, sink strength, and reduced senescence and respiration by the exogenous treat-

Table 3
Effect of kinetin treatment on the leaf stomatal conductance of stevia plants

Treatment	Week 1	Week 3	Week 5	Week 7	Week 9
T0	315.80^a	376.26^a	350.20^a	236.04^c	321.15^b
T1	285.54^a	372.98^a	291.12^a	438.58^a	500.64^a
T2	240.64^a	319.34^a	345.32^a	354.04^b	378.58^b
T3	305.88^a	359.66^a	347.86^a	368.88^b	329.54^b
T4	288.68^a	284.50^a	216.66^a	353.20^b	289.38^b

Different letters in same column are significantly different at 5% level of significance;
Treatments: 0, 10, 20, 40 and 80 mg/L kinetin

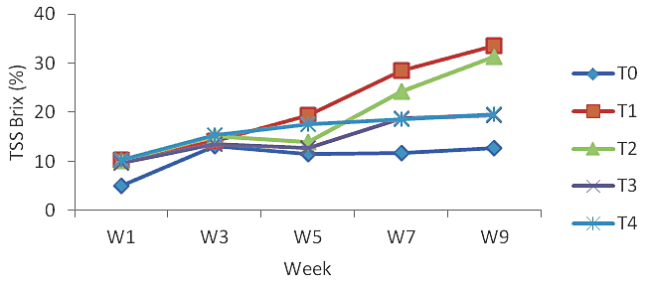

Fig. 6. Effect of kinetin treatment on the total soluble solids of stevia plant
Bars indicates ± S.E. T0 (Control), T1 (10 mg/L), T2 (20 mg/L), T3 (40 mg/L) and T4 (80 mg/L)

ment of kinetin (Dhillion et al., 1985). Kinetin treatment also affect the physiological ageing and change in metabolism, which eventually resulted in more TSS content as compared to control (Bhardwaj et al., 2010). Contradictory result, like kinetin decreasing the leaf soluble sugar was reported by Niakan and Ahmadi (2014).

Pigments contents

Pigments concentrations in a plant parts is an important quality characteristic which is important not only for consumer acceptability but also in association with aroma, flavour and health benefits (Al-Saif et al., 2011). Table 4 represents the means of chlorophyll and carotene contents of treated and untreated stevia leaves that were determined by using spectrophotometer. The results showed that all the treatments increased the chlorophyll content compared to control but it was not statistically significant (Table 4).

Based on the results on chlorophyll *a*, T3 showed the highest reading at 20 mg/L, followed by T4 then T2 with the readings of 19 and 18 mg/L, respectively. The lowest reading of chlorophyll *a*, was observed in T1 with the values 16 mg/L. For chlorophyll *b*, T3 shows the highest reading with 10 mg/L, followed by T4 and T2 with readings of 10 and 9 mg/L, respectively. The lowest reading of chlorophyll *b*, was from T1, with reading 8 mg/L. Our results were supported by the findings of Khandaker et al. (2013b), who reported that application of growth regulators increases the accumulation of pigments contents in the plant parts. Kinetin may increase the membrane osmosis and chlorophyll content in chloroplast. It has also been reported that kinetin control the chlorophyll breakdown (Thimann, 1985). Previous study reported that chlorophyll content increases with carotenoids content because carotenoids protect chlorophyll from photooxidative destruction. Cytokinin may eventually increases the number of leaf chloroplast by increasing cell growth and cytoplasm activity, and consequently enhances the level of chlorophyll synthesis.

In the light harvesting complex and photo protection carotenoids play a significant role. It has been reported that pigments are very important in protecting the photosystem apparatus against photodamage. Kinetin treatment also improves the quality of plant parts by reducing the loss of colour during storage (Moneruzzaman et al., 2010b). Based on results, the mean differences of carotenoid was not significantly different at p value greater than 0.05. From Table 4, the highest reading for carotenoid from T4 and T1 at 0.0109 and 0.019 ug/g values, respectively, while the lowest reading was from T0 at 0.009 ug/g. But, another study reported that growth regulators had positive effects on anthocyanin and carotenoids contents in wax apple fruits (Moneruzzaman et al., 2012).

Antioxidant activity

In this experiment, the samples were compared on the basis of their half maximal inhibitory concentration (IC_{50}) which is a measure of the effectiveness of a substance in inhibiting a specific biochemical function. Ghanta et al. (2007) reported that IC_{50} represents the potential antioxidant activity of a plant extract. Antioxidant activities of plant extracts positively correlated with the total phenolic contents (Haq et al., 2011).

Table 4
The effects of kinetin treatment on the leaf chlorophyll and carotene content of stevia plant

Treatment (mg/L)	Chlorophyll *a* (mg/L)	Chlorophyll *b* (mg/L)	Chlorophyll *a* +*b* (mg/L)	Carotenoid (ug/g)
T0	16.23[a]	8.70[a]	24.94[a]	0.0091[a]
T1	16.22[a]	8.09[a]	24.32[a]	0.0109[a]
T2	16.46[a]	9.25[a]	27.72[a]	0.0098[a]
T3	20.01[a]	10.44[a]	30.45[a]	0.0107[a]
T4	19.50[a]	9.98[a]	29.49[a]	0.0109[a]

Different letters in same column are significantly different at 5% level of significance;
Treatments: 0, 10, 20, 40 and 80 mg/L kinetin

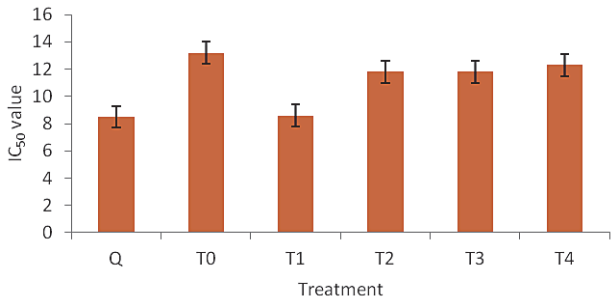

Fig. 7. Effect of kinetin treatment on the antioxidant activity of stevia plant
Bars indicates ± S.E. T0 (Control), T1 (10 mg/L), T2 (20 mg/L), T3 (40 mg/L) and T4 (80 mg/L)

From Figure 7, T2 showed the highest percentage inhibition of DPPH free radical activity. In other words, T2 showed high DPPH activity compare to other concentrations. In the presence of antioxidant compounds, the DPPH which were the stable free radicals will donate hydrogen and be reduced to diphenylpicryhydrazine. The colour change cause by the loss of DPPH can be measured. Based on the results on the IC_{50} value, T0 showed the highest value followed by T4 while T1 produced the lowest IC_{50} value. In our study, the antioxidant content of stevia plant was increased with cytokinin application. This is in agreement with results of Karalija et al. (2017), who reported that application of cytokinin increase the antioxidant potential of plant parts. They also reported that cytokinin treatment increases the accumulation of phenolic compounds and this elevated level of phenolic compounds may be correlated with antioxidant activities.

Conclusion

From the above results, we concluded that the morpho-physiological and biochemical characteristics of stevia can be improved by the exogenous application of kinetin. The results showed significant effects of kinetin on plant height of stevia, lateral branch, leaf number and area, net photosynthetic rate, total soluble solids and pigments contents in the leaves. From this current study, it can be concluded that 10 mg/L kinetin concentration gave the best results to improve the morpho-physiological and biochemical characteristics and antioxidant activity of stevia plant.

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