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Influence of ethephon spraying on the growth and yield of Stevia (*Stevia rebaudiana* Bertoni.)

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Abstract The effects of ethephon spraying on the growth and yield of stevia (*Stevia rebaudiana* Bertoni) was investigated. The results indicated that spraying ethephon for three times leaded to the highest growth parameters in term of stem, leaf, root and total dried weight and yield, followed by spraying two and one times. The ethephon spraying to stevia at concentration of 200 ppm gave the highest total dried weight and yield. It is concluded that application of ethephon at concentration of 200 ppm to stevia for three times at 30, 60 and 90 DAT gave the highest vegetative growth and yield.

Keywords: Ethephon, Growth, Yield, Stevia

Introduction

Stevia (*Stevia rebaudiana* Bertoni.) is an abiding herb belonging to the Asteracceae, it is originated in South America, Misiones, Paraguay (Shaafi *et al.*, 2021; Ghaheri *et al.*, 2019; Akbari *et al.*, 2018). The leaves have a great potential for natural non-caloric sweeteners known as steviol glycosides (Wölwer - Rieck, 2012). Among steviol glycosides, the most substantial glycoside in stevia leaf is stevioside, which is about 300 times sweeter than sucrose, that does not generate any energy, and has a glycemic index of zero (Puri *et al*., 2011). Generally, the content of steviol glycosides may vary from 4-20 % of leaf dry matter (Starrat *et al*., 2002). Stevioside is the main steviol glycoside. It has been reported that the compound can lower the postcibal blood glucose level in Type II diabetic patients and the blood pressure in mildly hypertensive patients (Gregersen *et al*., 2004). The stems and leaves of the plant often stop growing when flowering occurs, resulting to reduce leaf yield. The solution to this problem is to remove the flowers which will delay their senescence as well as boost their leaf productivity. The stevia plant is deflowered leading to be higher number of branches and more vegetative growth. Flower removal was reported to increase leaf yield from 21% to 62% (Chumthong and Detpiratmongkol, 2018). The study also reported that removing flowers by hand for four times

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at 30, 60, 75 and 85 days after transplanting gave the highest number of leaves, total biomass dry weight, and leaf dry weight yield compared to the control (no flower removal). However, the manual deflowering or cutting flowers by hands required a lot of time and costs. Therefore, spraying ethephon hormone on stevia to deflower instead of manual deflowering. Ethephon is a gaseous phytohormone that controls various biological processes including growth, flowering, fruit ripening, abscission, and senescence (Seesangboon *et al.*, 2018). Knowledge, there has not been reported on how much the dose should be treated and how often ethephon should be sprayed to achieve the desired deflowering without harmful side effects.

Therefore, the objective was to investigate the effects on the growth and yield of stevia to various ethephon doses, and the number of application to spray on stevia.

Materials and methods

Experimental design and plant materials

A split-plot in randomized complete block design with three replications was used for the experiment. The main plots were assigned to three application times of ethephon as follows: 1) spraying for first time at 30 days after transplanting (DAT), 2) spraying for second times at 30 and 60 DAT, and 3) spraying for third times at 30, 60 and 90 DAT. Subplots were assigned seven doses of ethephon of 0, 50, 100, 150, 200, 250 and 300 ppm.

Stevia rebaudiana Bertoni were used in this study. The experiments were conducted under glass-house conditions at the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The stevia cuttings from the mother plant were raised in a seedling tray and regularly watered. One-month-old healthy stevia seedlings of the same height were transplanted into pots. Further, each pot was irrigated to keep soil moisture at 65–75 % of field capacity throughout the experimental period. Thirty days after transplanting, the plants were foliar sprayed following the treatments.

Data collection

Plants were harvested at 120 days of transplanting by cutting the plant at 5 cm above the ground. Plant height, number of branches, stem, leaf, flower and pod, and root dried weights were recorded. Plant height was measured from the base of stem at ground level to the upper most leaf (cm). The branch number of each plant was counted. The stems, leaves, flowers, and roots were dried at 50 ℃ in a hot air oven for 48 hours. The leaf area

was measured by using a LI-3100 leaf area meter (Licor Inc., Lsincoln, USA.), and then leaf area index (LAI) was calculated by Chen and Black (1991) in the following equation.

$$LAI = \frac{\text{Leaf area}}{\text{Ground area}}$$

Determination of chlorophyll

Chlorophyll contents from fully developed leaves were determined using a mobile chlorophyll meter (SPAD-502 Konica Minolta, Inc., Tokyo, Japan). Chlorophylls (Chlorophyll a, b and total) were extracted in a solution of 80% acetone (v/v) following a method of Arnon (1967). The absorbance of the extracts was recorded at 645 and 663 nm. Chlorophyll a, b and total were calculated to mg g^{-1} leaf from the absorption values as standard equations Arnon (1967).

Statistical analysis

For statistical analysis of the data, Statistix (8.0) software was used. All the collected data for various variables were statistically analyzed using split-plot arrangement in randomized complete block design. The difference between the treatments were further analyzed through the least significant difference (LSD) test.

Results

Growth parameters

Plant heights were significantly different ($P \leq 0.05$) in each treatment at harvesting time (Table 1). The maximum plant height (68.79 cm) was observed in the treatment that sprayed ethephon on the plant for one time at 30 DAT, and the minimum plant height (42.77 cm) was observed in the treatment that sprayed ethephon for three times at 30, 60, and 90 DAT. Plant height was decreased in relation to the increase in the concentration of ethephon. The tallest plant (73.13 cm) was recorded from the untreated plant (0 ppm). The treated plants had different plant height due to the concentration of ethephon. The treatment of ethephon spray with the lowest concentration of 50 ppm gave the highest average of 65.65 cm compared to the comparison treatment and the highest concentration of 300 ppm, which gave the lowest mean of 37.38 cm.

The number of branches were significantly affected by application times of ethephon spraying (Table 1). The highest number of branches (13.85 branches $plant^{-1}$) was obtained at three times of ethephon spraying,

and the lowest (8.38 branches plant^{-1}) was obtained at one time of ethephon spraying. Application of ethephon at 200 ppm dose gave the highest number of branches (13.66 branches plant^{-1}). The lowest number of branches (8.44 branches plant^{-1}) was obtained from application of ethephon at 0 ppm dose (control).

The stem and leaf dry weight significantly differed in application time of ethephon (Table 1). The highest stem and leaf dry weight (16.59 g plant^{-1} and 11.21 g plant^{-1}) were obtained after three times of ethephon foliar application. The lowest stem and leaf dry weight (10.93 g plant^{-1} and 6.34 g plant^{-1}) were obtained after one time of ethephon foliar application. There were significant ($P \leq 0.05$) differed in stem and leaf dry weight of stevia plant grown under different ethephon foliar spraying. Stevia plant grew after ethephon foliar spraying dose at 200 ppm had the highest stem dry weight (18.02 g plant^{-1}) and leaf dry weight (11.79 g plant^{-1}), followed by 250, 300, 150, 100, and 50 ppm, whereas the lowest (9.35 g plant^{-1} and 6.22 g plant^{-1}) was observed in the non-treated control (0 ppm).

Table 1. Effects of ethephon spraying on plant height, number of branches, stem, leaf and flower and pod dry weights of *Stevia rebaudiana* Bertoni. at 120 days after transplanting

Treatments	Plant height (cm)	No. of branches (branch plant^{-1})	Stem DW. (g plant^{-1})	Leaf DW. (g plant^{-1})	Flower and pod DW. (g plant^{-1})
Number of times of spraying (A)					
first time	68.79[1]	8.38	10.93	6.34	1.95
second times	55.05	10.90	13.67	9.30	1.61
third times	42.77	13.85	16.59	11.21	1.13
Ethephon doses (B)					
0 ppm	73.13	8.44	9.35	6.22	1.95
50 ppm	65.65	9.33	10.28	7.28	1.81
100 ppm	60.75	10.66	12.17	7.94	1.71
150 ppm	57.54	11.55	14.44	9.29	1.63
200 ppm	48.32	13.66	18.02	11.79	1.42
250 ppm	45.98	12.44	16.72	10.57	1.31
300 ppm	37.38	12.22	15.11	9.54	1.12
Mean	55.54	11.19	13.73	8.95	1.56
LSD (0.05)	9.41	1.38	2.38	1.38	0.14
C.V. (%)	**19.79**	**14.46**	**20.29**	**18.07**	**10.43**

[1]Mean of three replications, and treatment means were compared with Least Significant Different (LSD) at P= 0.05.
DW = dry weight

Flower and pod dry weight was significantly affected by application time of ethephon spraying (Table 1). The highest flower and pod dry weight were observed after one time of ethephon spraying. Flowers and pod dry weight decreased with increasing ethephon dose. The highest flower and

pod dry weight (1.95 g plant^{-1}) was observed in the non-treated (0 ppm ethephon), whereas the lowest (1.12 g plant^{-1}) was obtained after applying at the concentration of 300 ppm.

Root dry weight of stevia significantly differed in different application times of ethephon (Table 2). The highest (0.77 g plant^{-1}) was obtained after three time applications of ethephon, and the lowest (0.52 g plant^{-1}) was observed for one time of ethephon application. The highest root dry weight (0.81 g plant^{-1}) was observed at concentration of 200 ppm ethephon and followed by 250, 300, 150, 100, and 50 ppm. The lowest root dry weight (0.45 g plant^{-1}) was observed in the control treatment (0 ppm).

Leaf area and leaf area index were significantly affected by different numbers of times of ethephon spraying (Table 2). The highest leaf area (1,030 cm^2) and leaf area index (1.41) were observed under three times of ethephon spraying, and the lowest (585 cm^2 and 0.80) were obtained at one time of ethephon spraying. Application of different ethephon doses, leaf area and leaf area index were increased in relation to the increase in the concentration of ethephon. The highest leaf area (1,084 cm^2) and leaf area index (1.48) were found in stevia grown under 200 ppm ethephon dose, and then increased in ethephon doses leaf area and leaf area index were decreased. However, the leaf area (864 cm^2) and leaf area index (1.18) were recorded in stevia grown under the highest ethephon dose (300 ppm).

Table 2. Effects of ethephon treatments on root dry weight, leaf area, leaf area index (LAI), total dry weight and leaf dry weight yield of *Stevia rebaudiana* Bertoni. at 120 days after transplanting

Treatments	Root DW. (g plant^{-1})	Leaf area (cm^2)	LAI	Total DW. (g plant^{-1})	LDW Yield (g m^{-2})
Numbers of times of spraying (A)					
first time	0.52	585	0.80	19.75	86.96
second times	0.64	846	1.16	25.23	127.52
third times	0.77	1,030	1.41	29.71	153.82
Ethephon doses (B)					
0 ppm	0.45	574	0.78	17.99	85.40
50 ppm	0.50	676	0.92	19.88	99.92
100 ppm	0.59	732	1.00	22.42	109.00
150 ppm	0.68	852	1.16	26.05	127.45
200 ppm	0.81	1,084	1.48	32.05	161.73
250 ppm	0.78	961	1.31	29.39	145.04
300 ppm	0.70	864	1.18	26.48	130.84
Mean	0.64	820	1.12	24.90	122.77
LSD (0.05)	0.08	142	0.19	2.82	19.00
C.V. (%)	**15.50**	**20.31**	**20.29**	**13.22**	**18.07**

[1]Mean of three replications, and treatment means were compared with Least Significant Different (LSD) at P= 0.05.
DW = dry weight; LDW = leaf dry weight yield

Total dry weight and leaf dry weight yield

The results showed that the total dry weight and leaf dry weight yield were significantly affected by different application time of ethephon (Table 2). The highest total dry weight (29.71 g plant^{-1}) and leaf dry weight yield (153.82 g m^{-2}) were observed after three times spraying of ethephon, followed by two and one application time. The ethephon at 200 ppm was significantly highest in total dry weight (32.05 g plant^{-1}) and leaf dry weight yield (161.73 g m^{-2}).

Chlorophyll contents, chlorophyll a, chlorophyll b, and total chlorophyll

The result showed that the chlorophyll contents, chlorophyll a, chlorophyll b, and total chlorophyll were non-significantly affected by number of times spraying and different application doses of ethephon (Table 3).

Table 3. Effects of ethephon spraying on chlorophyll contents, chlorophyll a, chlorophyll b, and total chlorophyll of *Stevia rebaudiana* Bertoni. at 120 days after transplanting

Treatments	Chlorophyll content (SPAD)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total Chlorophyll (mg g^{-1})
Number of times of spraying (A)	35.29	6.42	0.80	7.23
first time	35.08	6.22	0.80	7.02
second times	34.96	6.17	0.77	6.94
third times				
Ethephon doses (B)	35.96	6.50	0.81	7.32
0 ppm	35.56	6.47	0.81	7.28
50 ppm	35.37	6.38	0.79	7.17
100 ppm	35.14	6.31	0.79	7.11
150 ppm	35.08	6.16	0.78	6.95
200 ppm	34.93	6.05	0.78	6.83
250 ppm	33.71	6.01	0.77	6.79
300 ppm				
Mean	35.11	6.27	0.79	7.06
C.V. (%)	**11.46**	**10.78**	**14.12**	**9.61**

[1]Mean of three replications, and treatment means were compared with Least Significant Different (LSD) at P= 0.05.

Discussion

One of the previous studies, Chumthong and Detpiratmongkol (2018) reported that flower removal significantly affected the growth and yield of stevia. Flower removal treatment resulted in increased stem, leaf and root fresh weight, total biomass dry weight and leaf dry weight yield compared to control. Furthermore, Masinde and Agong (2011) indicated

that removal of flowers encouraged the vegetative growth of *Cleome gynandra*.

In this study, the biggest problem of stevia plant cultivation was early flowering which reduced vegetative growth and leaf dry weight yield. However, a lot of time and labor are needed to manually remove stevia flowers, therefore, a plant growth regulator (PGR) was used instead. PGR was applied to alleviate the issue of early flowering that had been successful in many plants. It is suggested that application of PGR improved plant growth under stress, depending on application method and dose used (Joshi *et al*., 2011). Ethephon is not only inhibiting flowering but also plays other important roles in the growth and development of plants e.g. ethylene promotes growth and yield of *Jatropha curcas* (Joshi *et al*., 2011).

The results showed that ethephon application decreased flowers and pod dry weight, shorter stems, and increased stem internodes. It explained the effect of ethylene degradation, blocking the growth of stem tissue and suppressing the elongation of the meristem (Thomas, 1980). The resulting data on flowers and pod dry weight agreed with the finding by Hashim (2014) and Shekoofa and Emam (2008). Therefore, a PGR was applied to alleviate the issue of early flowering that had been successful in many plants. Ethylene is one of the effective stimulators of flower abortion and can be released by the breakdown of the applied ethephon (Wilmowicz *et al*., 2016). In addition, promotion in the number of branches from various ethephon treatments more than the untreated plants can be mainly attributed to the inhibitory effect of these PGR on the cell division in the apical bud, which after that might have stopped the growth of the main axis and resulted in more laterals production. Furthermore, PGR activates lateral buds to grow well and produce more branches (Benjawan *et al*., 2007). The increased number of branches could be inhibited auxin activity in the apical buds because PGR acts as an anti-auxin. A PGR treatment suppresses the apical dominance by diverting the polar transport of auxins towards the basal nodes leading to increase branching rate (Dole and Wilkins, 1999; Reddy, 2005). The promoting effect of ethephon on the number of branches of *Rosa damascena* had been reported by Abbas *et al*. (2007). Moreover, after reducing stevia flower, at 200 ppm ethephon for 30, 60, and 90 DAT was found to be more efficient in the increased stem, leaf, and root dry weight total dry weight, and leaf dry weight yield. Flower abscission is due to the activation of the abscission zones, which are strongly stimulated by ethylene (Frankowski *et al*., 2015; Kućko *et al*., 2019).

In comparison to the control and other treatments, 200 ppm ethephon application at 30, 60, and 90 DAT resulted in greater leaf dry weight, leaf area, and leaf area index. Even though the plant height was lower than the control because there were more leaves than there were under the control and other treatments. These results were in agreement with Deepa *et al*. (2016) and Zeboon *et al*. (2017). Application of ethephon at a

higher dose of 200 ppm decreased leaf dry weight and leaf area because it caused necrosis on leaves. The effect was clearly seen at the edge of the leaves. Most of the treated leaves were shrank and bent upward after treatment. Approximately 20-30% of leaves were abscised after 72 hours of ethephon treatments which is consistent with the results reported by Rosli *et al*. (2012).

Total dry weight and leaf dry weight yield were significantly affected by the dose of ethephon and the number of application times. It was clearly observed that spraying 200 ppm ethephon for three times at 30, 60, and 90 DAT that significantly higher total dry weight and leaf dry weight yield than untreated plants. It produced the highest number of leaves, leaf dry weight and number of branches per plant, and provided the highest total dry weight and leaf dry weight yield at harvest. In contrast, plants treated with ethephon at a higher dose of 250 to 300 ppm or a lower dose of 50 to 150 ppm tended to reduce the number of leaves and branches (Deepa *et al*., 2016). It concluded that treating stevia plants with a moderate dose of ethephon (200 ppm) for three times DAT leads to produce the maximum total dry weight and leaf dry weight yield.

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