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### Influence of different plant growth regulators on flower suppression, herb yield and stevioside content of Stevia (*Stevia rebaudiana* Bertoni.)

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#### Abstract

*Stevia rebaudiana* Bertoni, an emerging perennial herb contains stevioside as a therapeutic principle and thus, is having diversified uses in diabetes, hypertension, tooth ache and variety of beverages. A field experiment was conducted during year 2016-18 to find out the influence of plant growth regulators on flower suppression, herb yield and stevioside content of stevia. In order to determine the effect of different plant growth regulators on flower suppression four different growth regulators each at two levels along with manual deflowering at 15 days interval and control were assessed. The design followed was Complete Randomized Block design with 10 treatments and 3 replications. T<sub>9</sub> (GA<sub>3</sub>-500 ppm) significantly affected and found superior on plant height (53.68 cm), number of branches (38.78), dry herb yield/plant (31.96 g) and dry herb yield/ha (2.37t) whereas it was observed lowest 17.48 cm, 21.04, 17.46 g, and 1.29 t, respectively. The significantly minimum number of inflorescence (6.44) was recorded in T<sub>9</sub> as compared to maximum (11.06) in control (T<sub>1</sub>). T<sub>8</sub> (GA<sub>3</sub>-250 ppm) was found on par with T<sub>9</sub> with respect to all above parameters. The highest stevioside content (0.99%) was recorded in T<sub>9</sub> and lowest content (0.32%) was in T<sub>4</sub> (ABA -250ppm).

**Keywords:** stevia, growth regulators, GA<sub>3</sub>, stevioside

#### Introduction

From the most distant to most recent times, sugar has formed an indispensable constituent in the food of mankind. The major source of sugar has long been sugarcane (60%), and sugar beet. Though the prescribed sugars have sweetening properties, they are not advised for diabetics, for such people, sugar obtained from stevia considered to be the best alternative. This sweetener imparts 250 times more sweetness than table sugar and 300 times more than sucrose. Stevia has become a potential alternative source replacing artificial sweeteners like Saccharin, Aspartame, Acesulfame, Saccharin etc. So far there have been no reports of adverse effects from the use of stevia products by humans. Hence stevia has been named as calorie free biosweetener of high quality.

*Stevia rebaudiana* Bertoni. native to Paraguay (South America), is an herbaceous perennial (2n = 22) shrub of the asteraceae family. The plant contains stevioside and rebaudiside as a therapeutic and sweetening principle and thus, is having tremendous application to sweeten soft drinks, soy sauce, yoghurt and other foods in Japan, Korea and Brazil (Taylor, 2005; Tadhani *et al.*, 2007) <sup>[1, 2]</sup>. It is recommended for diabetes and has been used by humans with no side effects (Megeji *et al.*, 2005) <sup>[3]</sup>. Due to its sweetener components, the plant would get the place in natural food market in the future (Starratt and Gijzen, 2004) <sup>[4]</sup>.

Plant growth, their primary and secondary metabolite production is controlled by the plant growth regulators. It has been demonstrated that the herb yield in basil is enhanced by plant growth regulator, similarly in coriander and fenugreek herb yield is enhanced (Verma and Sen., 2008) <sup>[5]</sup>. Plant growth regulators results the effects on plant like plant growth, number of essential oil storage structures and biosynthesis of essential oil which can alter the yield of essential oil. Since 1940, in agriculture areas to control the developmental processes like reproduction, maturation, vegetative growth, post harvest senescence and germination growth regulators have been used. The effect of growth regulator on secondary metabolite

production in agriculture has been little known although it is used from many years in agriculture.

The emerging stevia gaining momentum for its large scale cultivation among farming community as it is considered to fetch better returns as compared to other traditional crops. However, this wonder crop is of apical dominance in nature that leads to frequent flowering and in turn lower herbage yield.

Hence, considering the importance of growth regulators for flower reduction and flower suppression, an attempt was made to evaluate the "Influence of plant growth regulators on flower suppression, herb yield and stevioside content of stevia (*Stevia rebaudiana* Bertoni.)" at College of Horticulture, Bagalkot which comes under northern dry zone of Karnataka.

### Material and Methods

The field experiment was conducted at Haveli farm, College of Horticulture, Bagalkot in a complete randomized block design (CRBD) with ten treatments and three replications. Forty five days old healthy seedlings were transplanted by flat bed method at the spacing of 45×30 cm. Recommended dose of N, P, K (60: 30: 45 Kg/ha and FYM 10t/ha) were applied to all the treatments. The foliar spray of IAA, ABA, MH and GA<sub>3</sub> each at 250 and 500 mg L<sup>-1</sup> (ppm) concentration were made on planted field at time of transplanting, 30 and 60 days after transplanting. The treatments were as T<sub>1</sub>= Control, T<sub>2</sub>= Indole acetic acid (250ppm), T<sub>3</sub>= Indole acetic acid (500ppm), T<sub>4</sub>= Abscisic acid (250ppm), T<sub>5</sub>= Abscisic acid (500ppm), T<sub>6</sub>= Maleic hydrazide (250ppm), T<sub>7</sub>= Maleic hydrazide (500ppm), T<sub>8</sub>= Gibberellic acid (250ppm), T<sub>9</sub>= Gibberellic acid (500ppm), T<sub>10</sub>= Manual deflowering at 15 days interval. Spacing followed was 45cm x 30 cm. the standardized inter cultivation practices like irrigation, weeding, common manuring were followed during the entire crop period.

Plant height was measured from the ground level to the growing tip of the plant at monthly interval starting from 30 days after transplanting (DAT) and it was expressed in centimeter. The number of inflorescence per plant was counted from five randomly selected plants at 30, 60 and 90 DAT and mean was expressed as number of inflorescence per plant. The number of branches per plant was counted from five randomly selected plants at 30, 60 and 90 DAT and mean was expressed as number of branches per plant. The fresh leaves of individual labeled plants were harvested and weighed by using the electronic balance and the mean was worked out. The two years observations data (2016-18) were recorded and pooled data were analyzed and presented in this study.

### Statistical analysis and interpretation of data

The data on growth and yield parameters were subjected to Fisher's method of analysis of variance as outlined by Sundararaj *et.al.*, [1972] [6]. Wherever the 'F' test was significant for comparison of treatment means, critical difference [C.D] values were calculated at 5 per cent probability level. The critical variance was calculated on percent basis.

### Results and Discussions

All the growth attributes like plant height, number of branches and number of inflorescence were significantly enhanced by exogenous application of plant growth regulators as compared to unsprayed plants.

The plant height was significantly higher (53.68 cm) with

GA<sub>3</sub>-500 ppm (T<sub>9</sub>) at 90 DAT, which was found on par (50.02cm) with GA<sub>3</sub>-250 ppm (T<sub>8</sub>) and lowest plant height (17.48 cm) was observed in T<sub>1</sub>-control (Table No.1). Increased plant height by GA<sub>3</sub> application could be due to the stimulation of cell division and cell elongation while increasing plasticity of cell wall. Auxin content was reported due to the application of GA<sub>3</sub> and resulting in apical dominance, which might also have contributed to the increased plant height (Scott *et al.* 1967) [7].

Though the plant height is a genetically controlled character, it is evident from our results that, GA<sub>3</sub> has played a significant role in increasing the plant height which is in conformity with the findings of Bhat *et al.* (1990) [8] in davana and Lokesh *et al.* (2018) [8, 9] in stevia who showed increase in plant height, due to application of GA<sub>3</sub>.

The application of growth regulators at different concentration affected significantly the number of branches. The application of GA<sub>3</sub>-500 ppm (T<sub>9</sub>) in increased number of branches per plant (38.78) and it was noted on par with GA<sub>3</sub>-250 ppm (T<sub>8</sub>) *ie.*, 36.55 and found minimum in T<sub>1</sub>-control (21.04) at 90 DAT. This might be due to suppression of apical dominance which has brought functionality of several meristems on the nodal regions at a time leading to maximum number of branches (Table No.2). Similar findings were observed by Salama, 2008 [10] in stevia and Lokesh *et al.* (2018) [9] in stevia.

Analysis of variance showed that the application of plant growth regulators had significant effect on number of inflorescence. The minimum number of inflorescence (6.44) was observed in GA<sub>3</sub>-500 ppm (T<sub>9</sub>) and was on par with all other treatments except T<sub>1</sub>-control where as maximum number of inflorescence (11.06) were noticed in T<sub>1</sub>-control (Table. 3). It might be due to established fact that GA<sub>3</sub> promotes vegetative cell enlargement and activated functions were responsible inflorescence in stevia. It could be emphasized that GA<sub>3</sub> could act as a factor affecting on assimilate convey towards vegetative parts instead of flowers resulting in decreased number of inflorescence.

Result indicated that among all the treatments GA<sub>3</sub>-500 ppm (T<sub>9</sub>) recorded significantly highest fresh herbage yield per plant (164.25 g) which was on par with GA<sub>3</sub>-250 ppm (T<sub>8</sub>) *ie.* 155.76 g. Similarly, GA<sub>3</sub>-500 ppm (T<sub>9</sub>) recorded highest fresh herb yield per hectare (12.16 t) which is on par with GA<sub>3</sub>-250 ppm (T<sub>8</sub>) *ie.* 11.54 t. The lowest fresh herb yield per plant and per hectare was observed in T<sub>1</sub>-control as 102.43 g and 7.64 t, respectively (Table No.4). This might be the increased auxin content due to the application of GA<sub>3</sub> resulting in maximum cell growth and other aerial parts in turn contributed to the increased herb yield (Scott *et al.*, 1967) [7].

Results indicated that application of growth regulator had significant effects over dry herb yield. Among the treatments, GA<sub>3</sub>-500 ppm (T<sub>9</sub>) recorded highest dry herbage yield per plant (31.96 g) which was on par with GA<sub>3</sub>-250 ppm (T<sub>8</sub>) *ie.*, 30.29 g and lowest (17.46 g) was recorded in T<sub>1</sub>-control. Similarly, dry herb yield per hectare was recorded highest (2.37 t) in GA<sub>3</sub>-500 ppm (T<sub>9</sub>) and is on par with GA<sub>3</sub>-250 ppm (T<sub>8</sub>) *ie.*, 2.25 t and lowest (1.29 t) T<sub>1</sub>-control (Table No.5).

Application of growth regulators also had an effect over stevioside content in stevia (Table No.5). The maximum stevioside content (0.99%) was recorded with the application at GA<sub>3</sub> 250 ppm where as minimum (0.32%) was recorded in ABA 250 ppm (T<sub>4</sub>). It was tempting to suppose that, the balance level of application of GA<sub>3</sub> might contribute to some mechanism for stimulating stevioside biosynthetic pathway.

**Table 1:** Effect of growth regulators on plant height (cm) in stevia at different growth stages

Treatment	30 DAT			60 DAT			90 DAT (at harvest)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T1	16.30	11.60	13.95	17.20	17.80	17.50	17.50	17.46	17.48
T2	18.60	12.67	15.63	22.60	25.47	24.03	41.20	33.13	37.16
T3	19.40	15.40	17.40	27.50	25.60	26.55	40.60	33.40	37.00
T4	15.00	14.47	14.73	33.50	26.00	29.75	40.75	34.34	37.54
T5	16.90	15.27	16.08	30.50	26.33	28.42	42.36	34.34	38.35
T6	18.20	12.87	15.53	31.00	25.26	28.13	38.50	35.13	36.82
T7	20.80	15.47	18.13	36.30	28.86	32.58	43.00	36.06	39.53
T8	26.40	17.60	22.00	45.08	37.60	41.34	57.50	42.53	50.02
T9	29.10	23.07	26.08	45.70	40.46	43.08	60.17	47.20	53.68
T10	20.74	22.27	21.50	33.40	37.46	35.43	52.00	39.40	45.70
SEm ±	0.74	1.79	1.04	1.09	2.45	1.53	1.59	3.27	2.13
CD at 5%	2.11	5.14	2.98	3.11	7.01	4.37	4.54	9.36	6.08
CV (%)	4.48	13.71	7.07	5.13	10.33	6.11	5.48	11.35	6.62

DAT: Days after Transplanting

**Table 2:** Effect of growth regulators on number of branches in stevia at different growth stages.

Treatment	30 DAT			60 DAT			90 DAT (at harvest)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T1	17.50	13.87	15.68	21.30	18.20	19.75	21.75	20.33	21.04
T2	17.60	15.20	16.40	20.40	19.60	20.00	37.50	22.67	30.08
T3	19.45	17.87	18.65	34.30	21.13	27.72	38.90	24.33	31.62
T4	16.40	13.80	15.10	39.50	16.93	28.22	39.60	19.67	29.63
T5	17.10	14.93	16.02	39.65	18.40	29.03	40.90	21.40	31.15
T6	19.75	17.53	18.64	26.00	21.00	23.50	35.70	23.00	29.35
T7	20.10	18.53	19.32	28.30	22.53	25.41	35.90	23.87	29.88
T8	27.40	22.00	24.70	43.00	23.80	33.40	47.50	25.60	36.55
T9	29.15	23.00	26.08	48.50	24.47	36.48	49.10	28.47	38.78
T10	17.78	14.93	16.36	22.80	18.33	20.57	22.85	20.93	21.89
SEm±	1.08	1.27	0.68	1.72	1.64	1.01	1.51	1.19	1.55
CD at 5%	3.07	3.64	1.94	4.90	4.69	2.88	4.32	3.40	4.44
CV (%)	6.52	9.08	5.44	6.49	9.85	4.66	5.01	6.33	6.35

DAT: Days after Transplanting

**Table 3:** Effect of growth regulators on number of inflorescence in stevia at different growth stages.

Treatment	30 DAT			60 DAT			90 DAT (at harvest)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T1	7.76	6.60	7.18	10.88	8.27	9.57	13.60	8.53	11.06
T2	8.14	6.27	7.20	8.86	7.87	8.36	8.88	8.07	8.47
T3	7.96	6.07	7.01	8.36	7.40	7.88	8.32	7.60	7.96
T4	8.12	7.27	7.69	8.60	7.80	8.20	8.30	8.20	8.25
T5	8.87	7.80	8.34	8.96	8.27	8.61	9.10	8.47	8.78
T6	8.42	7.67	8.04	8.36	7.53	7.94	8.36	7.73	8.05
T7	8.40	7.27	7.83	8.06	7.00	7.53	8.20	7.27	7.73
T8	7.10	5.87	6.48	6.90	6.20	6.55	6.86	6.53	6.70
T9	6.15	5.73	5.94	6.84	5.73	6.28	6.82	6.07	6.44
T10	6.96	6.27	6.61	7.78	7.13	7.45	7.78	7.67	7.72
SEm ±	0.51	0.49	0.29	0.51	0.33	0.39	0.45	0.50	0.55
CD at 5%	1.45	1.42	0.83	1.46	0.96	1.13	1.29	1.43	1.58
CV (%)	7.97	9.06	4.93	7.42	5.59	6.17	6.43	8.14	8.35

DAT: Days after Transplanting

**Table 4:** Effect of growth regulators on fresh herb yield in stevia at 90 DAT (at harvest)

Treatment	Fresh herb yield per plant (g)			Fresh herb yield per hectare (t)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
T1	95.40	109.46	102.43	7.16	8.11	7.64
T2	96.10	112.20	104.15	7.12	8.31	7.72
T3	105.20	114.40	109.80	7.79	8.47	8.13
T4	95.22	116.73	105.97	7.13	8.65	7.89
T5	106.70	118.86	112.78	7.90	8.80	8.35
T6	106.85	122.26	114.55	7.92	9.06	8.48
T7	109.50	125.73	117.62	8.11	9.31	8.71
T8	182.76	128.73	155.76	13.54	9.54	11.54
T9	194.96	133.53	164.25	14.44	9.88	12.16

T10	95.30	110.46	102.88	7.06	8.17	7.61
SEm±	4.11	4.24	4.92	0.52	0.37	0.43
CD at 5%	11.76	12.13	14.06	1.49	1.07	1.24
CV (%)	5.24	5.35	5.07	6.66	5.17	6.01

DAT: Days after Transplanting

**Table 5:** Effect of growth regulators on dry herb yield and stevioside content at 90 DAT (at harvest)

Treatment	Dry herb yield per plant (g)			Dry herb yield per hectare (t)			Stevioside (%)
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled	
T1	18.13	16.79	17.46	1.34	1.24	1.29	0.39
T2	18.74	17.96	18.35	1.39	1.33	1.36	0.78
T3	20.51	18.30	19.41	1.52	1.37	1.44	0.36
T4	18.00	18.67	18.33	1.33	1.38	1.36	0.32
T5	20.56	19.02	19.79	1.52	1.41	1.46	0.48
T6	20.60	19.55	20.07	1.53	1.45	1.49	0.57
T7	20.81	20.12	20.46	1.54	1.49	1.52	0.55
T8	36.55	24.05	30.29	2.71	1.78	2.25	0.99
T9	38.99	24.94	31.96	2.89	1.84	2.37	0.58
T10	18.10	17.66	17.87	1.34	1.31	1.32	0.59
SEm ±	1.17	0.80	0.99	0.18	0.06	0.08	0.051
CD at 5%	3.36	2.29	2.85	0.51	0.17	0.24	0.15
CV (%)	6.23	4.99	5.69	12.74	4.94	6.44	11.22

DAT: Days after Transplanting

### Conclusion

Stevia growth yield and stevioside content were affected with exogenously applied plant growth regulators. GA<sub>3</sub> 500 ppm performed well with maximum dry herb yield (2.37 t/ha) which was on par with GA<sub>3</sub> 250 ppm (2.25 t/ha) where as highest stevioside content (0.999 %) was also recorded in GA<sub>3</sub> 250 ppm. Therefore, it may be suggested that the application of GA<sub>3</sub> 250 ppm was highly effective for the production dry herb yield and stevioside content and improved the overall performance of the crop. Thus, GA<sub>3</sub> 250 ppm concentration might presumably be recommended for maximizing productivity and quality of stevia through the flower suppression.

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