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Influence of pinching and growth regulators on yield and stevioside content in stevia (*Stevia rebaudiana* Bertoni)

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Abstract
A field trail was conducted to investigate the effect of pinching and growth regulators on yield and stevioside content instevia (*Stevia rebaudiana* Bertoni) in the Department of Horticulture, GKVK, Bangalore during the year 2019-2020. The two main plot treatments (with and without pinching) and three growth regulators (Benzyl adenine, Maleic hydrazide and Mepiquat chloride) each at 250 and 500 ppm were replicated thrice under split plot design. Pinching followed by spraying of Malic hydrazide (MH) at 500 ppm recorded maximum fresh (85.60 g) and dry (42.56 g) herbage yield per plant. Maleic hydrazide at 500 ppm recoded maximum stevioside content (5.35%).The study concluded that combined treatment of pinching and growth regulators significantly improved the yield and quality in stevia as compared to its alone applications.

Keywords: Pinching, Growth regulators, Yield. Stevioside content

Introduction
Stevia (Stevia rebaudiana (Bertoni) belongs to the family Asteraceae and is native to Paraguay and South-West Brazil. Now stevia is grown in many parts of India and the leaves of stevia contain sweetening compounds called steviol glycosides, viz., stevioside, rebaudioside –A, B, C, D, E and dulcoside-A which are more than 200 times sweeter than sugar. The sweetening property of stevia is well documented in literature and its extracts (stevioside and rebaudioside–A) are considered safe for human consumption without any side effects (Kumar *et al*., 2014) [7]. In this modern era, with people becoming more and more health and diet conscious, sugar is being replaced by lowcalorie sweeteners. Stevia plant has garnered attention with the rise in demand for low-carbohydrate, low-sugar food alternatives. The glycosides extracted from the stevia leaf, as all-natural low-calorie sweeteners, are nontoxic and nonmutagenic compounds.
In stevia, leaf is the main plant part for extracting sweeteners. So increasing the number of branches and leaves is the main goal of plant scientists. Among several approaches of enhancing leaf biomass, pinching is practiced to enhance branches by curbing vegetative growth. Pinching and removal of the apical bud kept the plant in their proper shape and size especially due to utilization of photosynthates towards increased yield even though the total growth of the plant was reduced and restructured plant. It is a well established fact that arresting vegetative growth by pinching of apical bud in several flower and vegetable crops results in increased number of productive branches, which in turn influence yield and yield parameters in stevia (Kumar *et al*., 2014) [7].
Steviol glycosides are diterpenoids whose biosynthetic pathways share four steps in common with gibberellic acid formation. Steviol glycosides are produced by the action of many glucosyl transferases that transfer glucose units to steviol (Shibata *et al.* 1991) [10]. In this way, several steviol glycosides are produced, depending on bond type and number of glucose units. Therefore, gibberellin and steviol glycosides biosynthetic pathways have many common stages and intermediate metabolites, although it has been reported that they are present in Stevia leaf in different amounts (Alves and Ruddat, 1979) [1]. The plant growth retardants are chemical compounds that are frequently used in agriculture to control the plant growth through gibberellins biosynthesis inhibition. Therefore, the present investigation was undertaken to determine the effect of growth regulators mainly retardants along with pinching of apical portion on herbage yield and stevioside content

Material methods
A field trail was conducted in the Department of Horticulture, College of Agriculture,

University of Agricultural sciences, GKVK, Bangalore during the year 2019-2020. The experiment consist of three growth regulators namely, Benzyl adenine, Maleic hydrazide and Mepiquat chloride each at 250 and 500 ppm as subplot and 2 levels of pinching as main plot under split plot deign replicated thrice. Thirty days old seedlings were transplanted by ridge and furrow method at the spacing of 60 x 45cm. Pinching was done on 30th day after transplanting. The growth regulators were sprayed at 30th and 60th day after transplanting.

Table 1: Total of 14 treatment combination was followed in the experiment as follows

Treatment	Components / materials
(P_0G_0)	Control (No pinching + No growth regulator spray)
(P_0G_1)	No pinching + Benzyl adenine @ 250 ppm
(P_0G_2)	No pinching + Benzyl adenine @ 500 ppm
(P_0G_3)	No pinching + Maleic hydrazide @ 250 ppm
(P_0G_4)	No pinching + Maleic hydrazide @ 500 ppm
(P_0G_5)	No pinching + Mepiquat chloride @ 250 ppm
(P_0G_6)	No pinching + Mepiquat chloride @ 500 ppm
(P_1G_0)	Only pinching at 30th day after transplanting
(P_1G_1)	Pinching at 30th day after transplanting Benzyl adenine @ 250 ppm
(P_1G_2)	Pinching at 30th day after transplanting Benzyl adenine @ 500 ppm
(P_1G_3)	Pinching at 30th day after transplanting Maleic hydrazide @ 250 ppm
(P_1G_4)	Pinching at 30th day after transplanting Maleic hydrazide @ 500 ppm
(P_1G_5)	Pinching at 30th day after transplanting Mepiquat chloride @ 250 ppm
(P_1G_6)	Pinching at 30th day after transplanting Mepiquat chloride @ 500 ppm

Fresh leaf yield per plant (g) was recorded after harvest and it was calculated per hectare (kg) by multiplying with the plant population per hectare and was expressed in tonnes. The harvested fresh leaves were dried and dry weight was recorded and expressed in grams per plant. Dry leaf yield per hectare was calculated by multiplying with the plant population per hectare and was expressed in tonnes.

Stevioside content was determined through high performance liquid chromatography (HPLC) using standards of stevioside as described by Hashimoto *et al*. (1978) [3]. The procedure involves two steps *i.e*., solvent extraction followed by isocratic HPLC analysis. Extracts were obtained from 50mg sample of air-dried, powdered leaves of stevia. Each of three extractions was prepared with 10 ml methanol for 6 to 8 h. All the extracts were concentrated to dryness under reduced pressure. Dried extracts were redissolved one by one in 5 ml methanol. Dried extracts were filtered for one minute through 0.45 lm filter and degased by sonicator to remove air. The filtrate was used for HPLC analysis using NH_2 column and a mixture of acetonotriel / water (80:20 v/v) as mobile was done in UV range at 210 nm. Quantization was performed by means of an external standard calibration curve for each analyte, which has been obtained from standard solution.

The data on yield parameters were subjected to Fisher's method of analysis of variance and 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.

Result and discussion

The yield attributing characters includes fresh and dry leaf yield per plant and stevioside content varied significantly among the treatments in the present experiment. The results obtained are discussed in details hereunder.

Fresh and dry leaf yield per plant (g)

The mean data on fresh and dry leaf yield per plant recorded on 90th day after transplanting (Table 2). Pinching treatment had significant effect on both fresh and dry herbage yield per plant. Maximum fresh and dry leaf yield per plant (77.33 g and 36.27 g, respectively) was recorded in P_1 treatment (pinching), while minimum (70.96 g and 28.14 g respectively) was recorded in P_0 treatment (no pinching) on 90th day after transplanting.

Growth regulators treatment had significant effect on the fresh and dry leaf yield per plant. Maximum fresh and dry leaf yield per plant (82.10 g and 38.43 g, respectively) was recorded in G_4 treatment (MH 500 ppm), while minimum fresh and dry leaf yield per plant (66.55 and 27.39 g, respectively) was recorded in G_0 treatment (no growth regulator spray) on 90th day after transplanting.

Interaction effects of pinching and growth regulators had significant effect on fresh and dry leaf yield per plant. On 90th day after transplanting maximum fresh and dry leaf yield per plant (85.60 and 42.56 g, respectively) was recorded in P_1G_4 treatment (pinching + MH @

Table 2: Influence of pinching and plant growth regulators on fresh and dry leaf yield of stevia

Treatment	Fresh leaf yield / plant	Fresh leaf yield / ha	Dry leaf yield / plant	Dry leaf yield / ha
Levels of pinching (P)				
P_0 : No pinching	70.96	2.63	28.14	1.04
P_1 : Pinching at 30th DAT	77.33	2.86	36.27	1.33
'F' test	*	*	*	*
S Em±	**0.35**	**0.02**	**0.45**	**0.05**
CD (P=0.05)	**2.13**	**0.09**	**2.79**	**0.19**
Levels of growth regulators (G)				
G_0 : No growth regulator spray	66.55	2.46	27.39	1.01
G_1 : Benzayl adenine @ 250 ppm	71.70	2.65	27.48	1.01
G_2 : Benzayl adenine @ 500 ppm	73.65	2.73	29.69	1.09
G_3 : Maleic hydrazide @ 250 ppm	77.80	2.88	35.47	1.31

G_4 : Maleic hydrazide @ 500 ppm	82.10	3.04	38.43	1.42
G_5 : Mepiquat chloride @ 250 ppm	75.10	2.77	34.27	1.26
G_6 : Mepiquat chloride @ 500 ppm	72.10	2.67	32.73	1.20
‘F’ test	*	*	*	*
S Em±	**0.25**	**0.01**	**0.22**	**0.01**
CD (P=0.05)	**0.79**	**0.01**	**0.74**	**0.07**
Interaction (P x G)				
P_0G_0	62.50	2.31	20.98	0.77
P_0G_1	70.80	2.62	25.12	0.93
P_0G_2	67.90	2.51	21.88	0.81
P_0G_3	73.80	2.73	30.80	1.14
P_0G_4	78.60	2.91	34.30	1.27
P_0G_5	72.30	2.67	31.15	1.15
P_0G_6	70.80	2.62	32.80	1.21
P_1G_0	70.60	2.61	33.81	1.25
P_1G_1	72.60	2.68	29.84	1.10
P_1G_2	79.40	2.94	37.50	1.37
P_1G_3	81.80	3.02	40.14	1.48
P_1G_4	85.60	3.17	42.56	1.57
P_1G_5	77.90	2.88	37.40	1.37
P_1G_6	73.40	2.71	32.66	1.20
‘F’ test	*	*	*	*
S Em±	**0.86**	**0.032**	**1.08**	**0.01**
CD (P=0.05)	**2.69**	**0.099**	**3.91**	**0.15**

500ppm), while the minimum fresh leaf yield per plant (62.50 and 20.98 g, respectively) was recorded in P_0G_0 treatment (No pinching + no growth regulator spray).

Improvement in yield, according to Humphrieg (1979) could happen in two ways i.e., by adopting the varieties to grow better in their environment or by altering the relative production of different plant parts so as to increase the yield of economically important parts. Pinching and plant growth regulator interactions are capable of redistributing energy and improving the yield. Increased fresh and dry leaf biomass caused by apical pinching of plants can be attributed to the proportionate increase in yield-contributing characters, i.e., number of branches per plant, number of leaves per plant and spread of plant as compared no pinching. This is in confirmity with the results of Kumar *et al.* (2014) [7] in stevia, Vasudevan *et al.* (2008) [9] in fenugreek and Joshi *et al.* (2002) [5] in marigold.

Among growth regulators, Maleic hydrazide at 500 ppm concentration recorded significantly higher leaf yield compared to other treatments. Higher leaf yield might be due to enhancement in yield contributing factors like greater number of leaves, number of branches per plant, spread of plant and leaf area. These results are in accordance with Kumuda (2006) [6] in stevia.

Stevioside content (%)

Observations on stevioside content was recorded on 90th day after transplanting are presented in Table 3. Pinching and interactions of pinching and growth regulators treatment had non-significant effect on stevioside content per plant.

Growth regulators treatment had significant effect on stevioside content. Higher stevioside content per plant (5.35%) was recorded in G_4 treatment (MH 500 ppm) on 90thday after transplanting. Lower stevioside content (4.40) was recorded in G_0 treatment (No growth regulator spray) on 90th day after transplanting.

Maleic hydrazide at 500 ppm concentration recorded higher stevioside content. Delayed flowering due to the application of Maleic hydrazide at the same concentration might have increased the stevioside content in the leaves because of the fact that once flowering is initiated glycoside concentrations in the leaves start declining (Singh and Rao, 2005) [8].

Table 3: Influence of pinching and plant growth regulators on stevioside content in stevia

Treatment	Stevioside content (%)
Levels of pinching (P)	
P_0 : No pinching	4.91
P_1 : Pinching at 30th DAT	4.58
‘F’ test	**NS**
S Em±	**0.018**
CD (P=0.05)	-
Levels of growth regulators (G)	
G_0 : No growth regulator spray	4.40
G_1 : Benzayl adenine @ 250 ppm	4.59
G_2 : Benzayl adenine @ 500 ppm	4.71
G_3 : Maleic hydrazide @ 250 ppm	4.56
G_4 : Maleic hydrazide @ 500 ppm	5.35
G_5 : Mepiquat chloride @ 250 ppm	4.86
G_6 : Mepiquat chloride @ 500 ppm	4.75
‘F’ test	*
S Em±	**0.028**
CD (P=0.05)	**0.123**
Interaction (PxG)	
P_0G_0	4.90
P_0G_1	4.98
P_0G_2	4.82
P_0G_3	4.79
P_0G_4	5.10
P_0G_5	4.60
P_0G_6	4.70
P_1G_0	4.81
P_1G_1	4.20
P_1G_2	4.60
P_1G_3	4.32
P_1G_4	5.60
P_1G_5	4.20
P_1G_6	4.80
‘F’ test	**NS**
S Em±	**0.047**
CD (P=0.05)	-

Conclusion

On the basis of the results obtained in the experiment, it could be concluded that Maleic hydrazide @ 500 ppm along with pinching at 30th day after transplanting was better to maximum leaf yield as compared to all other treatment combinations.

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