Original Research Article

Growth and yield of stevia (*Stevia rebaudiana*Bertoni) as influenced by plant density and pinching

ABSTRACT

A field experiment was carried out to standardize spacing and pinching time to enhance the yield and quality of stevia (*Stevia rebaudiana*Bertoni) at the Department of Agronomy, College of Agriculture, Thrissur, Kerala,fromSeptember 2021 to May 2022. The design of the experiment was factorial RBD and replicated thrice. The experimentcomprisedthree levels of spacing (15 cm × 10 cm, 20 cm × 10 cm, and 15 cm × 15 cm) and four pinching intervals (pinching at 20 days after planting (DAP), pinching at 30 DAP, pinching at flower bud initiation and no pinching) in all possible combinations.The plants grown with closer spacing (15 cm × 10 cm) were the tallest at all growth stages. The pinching of the apical bud caused a reduction in plant height and increased the number of branches. Pinched plants recorded higher fresh and dry leaf weights than plants left non-pinched. More leaves were registered under the wider (15 cm × 15 cm) spacing than narrower spacings. Pinching favored the formation of more lateral buds, producing a more significant number of branches and, finally, more leaves per plant. High-density planting of stevia yielded the highest aboveground fresh mass (10925 kg ha^{-1}), whereas lower plant densities yielded the lowest aboveground fresh mass (7330 kg ha^{-1}). The stevia planted at a spacing of 15 cm × 10 cm and pinched at flower bud initiation (11980 kg ha^{-1}) produced higher herbage.

Key words: Stevia, *Stevia rebaudiana*, herbage, spacing and pinching

1. INTRODUCTION

Stevia (*Steviarebaudiana*Bertoni) is an emerging alternative sugar crop and is becoming more popular in various parts of the world. Thestevia leaves mostly contain stevioside, which tastes 300 times sweeter than sucrose [1].It is a natural sweetener that belongs to the sunflower family, Asteraceae, and is native to Paraguay (South America); hence called Paraguay's sweet

herb[2]. Stevia cultivation covers 32,000 hectares worldwide, with China accounting for 75 per cent of the total area. Currently, stevia is widely used in Japan.Due to the huge demand for diabetic products in the market, Indian farmers have also started growing stevia. Stevia is now being grown by farmers in several areas of Rajasthan, Punjab, Uttar Pradesh, West Bengal, Madhya Pradesh, Andhra Pradesh, Karnataka, Chhattisgarh, and other Indian states.Besides its sweetening properties, *Stevia rebaudiana* also contains anti-hyperglycemic, anti-cancer, hepatoprotective, anti-hypertensive, anti-caries, antioxidant, and anti-bacterial properties [3]. It has several common names, like sweet leaf, honey yerba, honey leaf, meetitulsi, sweet chrysanthemum, and candy leaf [4].

Stevia is best known for accumulating a highly sweet-tasting mixture of diterpene glycosides in its leaves, known as steviol glycosides. Eight ent-kaureneglycosides, namely dulcosideA, rebaudiosides A to E, steviolbioside, and stevioside are responsible for the sweet taste sensation [5].Stevioside accumulation varied significantly in different parts of the stevia plant. Since leaves function as the chief organ for synthesizing and accumulating these compounds, their highest concentration was observed in leaves. The decreasing trend of stevioside content follows leaves > shoots > roots >flowers [6].

Plant spacing is an important non-monetary agronomical practice that determines the spatial arrangements of plants that influence canopy structure, light interception and radiation use efficiency and ultimately, biomass production of crops. Pinching, also known as shoot tip removal or decapitation, is an agronomic practice that encourages the production of lateral branches in plants by disrupting apical dominance.

Since the leaf is the economically important component, producing greater leaf biomass is the primary criterion of crop performance. Proper plant spacing is essential for increasing crop yield potential since it influences crop growth and development by altering canopy light interception and interplant competition. Pinching is an agronomic technique that helps to enhance leaf biomass production. Pinched plants achieve bushy growth and dense foliage. Increasing the number of branches and leaves in stevia plants can increase the yield. Pinching apical buds stops apical dominance and increases productive branches, boosting leaf yield and quality.Keeping these points in view, the present study was undertaken to find out the appropriate spacing and pinching time to enhance the yield and quality of stevia.

2. MATERIALS AND METHODS

The trial was conducted at the Agronomy Farm, Department of Agronomy, College of Agriculture, Vellanikarra, Thrissur, Kerala, situated at 13^{o}32'N latitude and 76^{o}26'E longitude. The experimental site was located 40 m above mean sea level. The soil of the experimental site was sandy clay loam in texture, acidic in reaction (pH 4.52), medium in organic carbon (1.33 %),

high in available phosphorus (32.71 kg ha^{-1}), and medium in available potassium (188.16 kg ha^{-1}). The trial was laid out in Factorial Randomized Block Design with three replications.The trial consisted of 12 treatment combinations with three spacing levels as the first factor (15 cm × 10 cm, 20 cm × 10 cm, and 15 cm × 15 cm) and four pinching intervalsas the second factor (pinching at 20 DAP, pinching at 30 DAP, pinching at flower bud initiation and no pinching).

Before planting, the experimental field was ploughed thoroughly with a disc plough twice to bring the soil to a fine tilth. Farmyard manure @ 15 t ha^{-1} was applied uniformly as basal.One-month-old rooted cuttings of about 7 cm in height were transplanted in the main field as per spacing treatments.During the crop establishment phase, the beds were watered twice daily with a rose can in the morning and evening. Following that, 3 mm hose irrigation was applied once daily.The apical buds were removed by pinching manually without causing damage to the plant parts.Plants were trimmed by cutting 5 cm from the top with a sharp scissor. Pinching was done at 20 DAP, 30 DAP, and flower bud initiation as per treatments.The crop was first harvested 90 days after planting. The second harvest occurred 30 days after the first. After four months of planting, the plants were uprooted for the final harvest. The observations on growth parameters were recorded at 20, 30 DAP, and flower bud initiation stages. The data collected were subjected to analysis of variance using the statistical package "GRAPES" [7].

Table 1. Monthly weather data during the cropping period

Month	Max. Temperature (^oC)	Min. Temperature (^oC)	Rainfall (mm)	Mean RH (%)	Mean sunshine hours (hrs/day)
September 2021	30.7	22.4	291.7	83	4
October 2021	31.3	21.5	593.2	86	3.5
November 2021	31	22	364.2	81	2.4
December 2021	32.5	21.9	19.2	67	8.2
January 2022	33.3	22.6	0.0	64	9.1
February 2022	34.8	23.3	0.0	58	8.3
March 2022	36.1	22.4	1.7	74	6.9
April 2022	34.2	25.1	84.3	77	5.9
May 2022	31.1	24	422	85	3.0

3. RESULTS AND DISCUSSION

3.1 Growth parameters

3.1.1 Plant height (cm): Plant height increased significantly with an increase in plant density(Table 2). In general, crops grown under closer spacing (15 cm × 10 cm) recorded the tallest plants at all growth stages, and the plant height observed was 17.00 cm, 19.66 cm, and 38.89 cm at 20 DAP, 30 DAP, and flower bud initiation respectively

The increased plant height in closer spacing may be due to inter-plant competition for light, moisture, space, and aeration, which causes the main stem to lengthen. Plants also tend to grow vertically when crowded because of the shadowing effects of plants on one another [8]. [9 found that the plant height of stevia significantly increased as the spacing got closer. This result agreed with data from [10], who found that closer spacing (50 × 20 cm) encouraged the growth of taller stevia plants. These results also conform with the findings of [11] and [12] in African marigolds, which also belong to the same family as that stevia.

Plant height was significantly reduced as a result of pinching.The pinching of the apical bud caused a reduction in plant height and increased the number of branches. In pinched plants, the loss of apical dominance and the re-translocation of photosynthates from vertical to horizontal growth, as well as the production of more branches per plant, are the leading causes of the reduction in plant height [13].Taller plants were seen in the control plants that were not pinched throughout the observation periods (Table 2). Apical dominance in unpinched plants might have contributed to the higher plant height.

It was also evident from the results that plant height decreased considerably when pinching was performed at earlier stages of stevia. Shorter plants were observed in the plots where pinching was executed at 20 DAP relative to pinching executed at 30 DAP and at the time of flower bud initiation. A decrease in plant height with pinching was also reported by [14] and [12] in African marigolds and [15] in China aster.

3.1.2 The number of branches per plant: Significant variation was observed for the number of branches per plant with different levels of spacing as the plant grows towards the maturity stage. A higher number of branches was noticed under broader spacing (15 cm × 15 cm) as compared to narrower spacing (15 cm × 10 cm) at 30 DAP and the flower bud initiation stage (Table 2). Plants grown at a closer spacing exhibited less luxuriant growth and grew vertically rather than horizontally due to increased competition among plants for resources like nutrients, light, and space [8]. The results are consistent with those of [16] and [17], who found that stevia plants with wider spacing had more branch counts than those with closer spacing.

The number of branches was higher in pinched plants than in non-pinched plants at 30 DAP and flower bud initiation. One month after planting, plants with apical buds nipped at 20 DAP had more branches (5.09) than others. However, more branches (9.02) were seen at 30 DAP-pinched plants compared to 20 DAP-pinched plants (7.03) at the beginning of the flower bud development stage. Due to pinching, more side branches developed below the pinched portion of the main stem. This could be explained by the breaking down of apical dominance and the emergence of axillary buds instead of floral bud initiation. Typically, the plant modifies its system to promote the growth of axiliary buds, which may then develop into lateral branches when apical dominance is removed.

Variations in the number of branches in stevia due to different pinching treatments were reported by [13]. In the case of African marigolds[18], [14] and [12] reported similar results. Similar findings were also reported for other crops,*viz.*, carnation [19] [20], carnation cv. Red corso [21] and fenugreek [22].

3.1.3 The number of leaves per plant: Since leaves are the main economic part of stevia, leaf number significantly impacts yield. As the number of leaves significantly correlated with the plant height in the early growth stages, at 20 DAP, more leaves (42.29) were seen with closer spacing (Table 2). In subsequent growth stages, wider spacing favored stevia leaf production over narrower spacing. For the number of leaves, there was a positive association with the number of branches.

More number of leaves were recorded under the wider (15 cm × 15 cm) spacing when compared to narrower spacings (15 cm × 10 cm and 20 cm × 10 cm) at 30 DAP and the beginning of flower bud development (68.46 and 109.89 respectively). The wider spacing reduces inter-plant competition for space, nutrients, light, and moisture, which may have increased the photosynthetic and metabolic activity of the plant and is the likely cause of the increase in yield-attributing characteristics like the number of branches and leaves per plant [16]. [8]also reported a negative association between plant density and the number of leaves per plant in stevia.

The number of leaves with pinching treatments showed a significant variation as well. One month after planting, plants pinched 20 days after planting had significantly more leaves per plant than plants that were not pinched. While plants that received pinching at 30 DAP had more leaves than plants that received pinching at 20 DAP, unpinched plants, and control at the time of the bud development phase.

Pinching favored the formation of more lateral buds, producing a greater number of branches and, finally, more leaves per plant. [13]reported that at harvest, the number of leaves per plant was substantially higher when the stevia plants were pinched at 30 DAT at 20 cm height

compared to the non-pinched control, pinching at 20, 30, and 40 DAT at 10 cm height and 20 DAT at 20 cm.

3.1.4 Leaf yield per plant (g): During the first month after planting and the bud initiation stage, wider spacing resulted in higher fresh and dry weights (Table 3) of leaves than narrower spacing. Fresh leaf yield per plant at the wider spacing of 15 cm × 15 cm showed 37.82 % and 13.72 % increments over the closest spacing of 15 cm × 10 cm at 30 DAP and bud initiation phase, respectively. Several researchers reported maximum fresh leaf weight per plant at greater spacing on stevia [17]; [8]; [10]. There may be less competition between rows of plants and within rows of plants for the available resources when there is a wider spacing between them, which allows the plant to produce more branches and leaves [23].

Pinched plants were found to have higher fresh and dry leaf weights during the entire crop period than plants left non-pinched. Stevia pinched 20 days after planting had higher fresh and dry weight at 30 DAP. Plants pinched at 30 DAP had a more fresh and dry weight, followed by pinching at 20 DAP at the bud developing phase, whereas plants pinched at bud initiation and control had lower weights.

According to [13], pinching treatments have significantly impacted stevia's fresh leaf biomass per plant. On the observation at 90 DAT, [24] also observed that stevia plants that received pinching one month after transplanting produced the highest fresh and dry leaf yield per plant, while those without pinching treatment had the lowest yield. Similarly, [23] and [25] reported that pinching marigold plants significantly increased fresh and dry weight compared to unpinched plants.

3.1.5 Herbage yield per plant (g): At the 30 DAP and bud development stage of stevia, higher herbage yield was noticed under the wider-spaced condition (15 cm × 15 cm) compared to the closer-spaced condition (15 cm × 10 cm). This increase in plant fresh weight may be due to less limitation for food and water among widely spaced plants. Also, reducing the number of plants per unit area as plant densities decline is counterbalanced by enhancing each plant's productivity.At all growth stages of stevia, higher herbage yield was noticed in pinched plants compared to non-pinched plants. The time of pinching played a significant role in influencing the total herbage yield of stevia. Pinching stevia plants at 30 DAP recorded 71.42 % herbage yield per plant over pinching at 20 DAP.

The proportionate increase in yieldcontributing characteristics, such as the number of branches and leaves per plant and the higher fresh leaf weight (g plant^{-1}), is responsible for the increased herbage yield per plant brought on by apical pinching of plants when compared to no

pinching. According to [25] and [13] reports on marigold and stevia, pinching significantly increased plants' fresh and dry weight compared to no pinching.

3.1.6 Total herbage yield at harvest (kg ha^{-1}): Like all other biometric parameters, closer spacing (15 cm × 10 cm) and pinching at flower bud initiation produced the highest total herbage yield per plot (Table 3). High-density planting of stevia yielded the highest aboveground fresh mass (10925 kg ha^{-1}), whereas lower plant densities yielded the lowest aboveground fresh mass (7330 kg ha^{-1}).

While intense competition between individual plants under closer spacing aims to maximize yield per unit of area, wider plant spacing maximizes the yield potential of each plant. This result is in line with those of [17] and [8], who found that closer spacing produced a higher maximum yield of fresh aboveground biomass per hectare of stevia than wider spacing. A higher plant population density may increase fresh aboveground biomass by increasing the number of plants per unit area, which ultimately results in a higher fresh leaf yield per hectare.

The time of pinching had a significant impact on total herbage yield. Plants that received pinching at flower bud initiation yielded the most, 10167 kg ha^{-1}, comparable to those pinched one month after planting and the control. Pinching at 20 DAP resulted in a yield reduction of 27 % when compared to pinching at bud initiation.Plants that received pinching at the flower bud initiation phase had sufficient time to accumulate photosynthates in their vegetative parts, whereas plants that received pinching at their early vegetative stages (pinching at 20 DAP and 30 DAP) had less time to accumulate photosynthates. It resulted in growth reduction and stunting of those plants, and they failed to recover.

The interaction of spacing and pinching was found significant with respect to total herbage yield per plot (Table 4).At harvest, the stevia planted at a spacing of 15 cm × 10 cm and pinched at flower bud initiation (11980 kg ha^{-1}) produced higher herbage per plot.

Table 2. Effect of spacing and pinching on plant height, number of branches, and number of leaves at different growth stages of stevia.

Treatments	Plant height (cm)			Number of branches			Number of leaves		
	20 DAP	30 DAP	Flower bud initiation (45 DAP)	20 DAP	30 DAP	Flower bud initiation (45 DAP)	20 DAP	30 DAP	Flower bud initiation (45 DAP)
Factor A: Spacing									
15 cm × 10 cm	17.00	19.66	38.89	42.29	56.56	96.28	42.29	56.56	96.28
20 cm × 10 cm	16.22	18.59	37.76	38.18	59.07	103.12	38.18	59.07	103.12
15 cm × 15 cm	15.36	18.30	34.73	34.10	68.46	109.89	34.10	68.46	109.89
CD (0.05)	0.78	0.68	0.97	3.62	5.96	3.63	3.62	5.96	3.63
SE (m) ±	0.27	0.23	0.33	1.24	2.03	1.24	1.24	2.03	1.24
Factor B: Pinching									
No pinching	16.10	20.72	43.91	36.39	58.17	99.62	36.39	58.17	99.62
Pinching at 20 DAP	16.10	12.98	27.42	37.64	67.54	105.21	37.64	67.54	105.21
Pinching at 30DAP	16.26	20.70	33.10	40.40	59.18	110.08	40.40	59.18	110.08
Pinching at flower bud initiation	16.32	21.00	44.06	38.34	60.58	97.48	38.34	60.58	97.48
CD (0.05)	NS	0.78	1.12	NS	3.44	4.19	NS	3.44	4.19
SE (m) ±	0.31	0.27	0.38	1.43	1.17	1.43	1.43	1.17	1.43

Table 3. Effect of spacing and pinching on fresh leaf yield per plant, dry leaf yield per plant, herbage yield per plant and herbage yield (kg ha^{-1}) at different growth stages of stevia

Treatments	Fresh leaf yield (g)			Dry leaf yield (g)			Herbage yield (g)			Herbage yield (kg ha^{-1})
	20 DAP	30 DAP	Flower bud initiation (45 DAP)	20 DAP	30 DAP	Flower bud initiation (45 DAP)	20 DAP	30 DAP	Flower bud initiation (45 DAP)	At harvest
Factor A: Spacing										
15 cm × 10 cm	4.56	6.53	10.86	3.52	5.16	7.01	7.56	12.27	21.68	10925
20 cm × 10 cm	3.84	7.08	11.89	3.17	5.37	7.26	5.87	13.03	22.84	8945
15 cm × 15 cm	3.09	9.00	12.35	2.86	6.48	8.02	5.56	15.49	28.41	7330
CD (0.05)	0.42	0.791	0.86	0.309	0.49	0.55	0.56	1.34	2.48	621
SE (m) ±	0.143	0.27	0.29	0.105	0.17	0.19	0.19	0.46	0.85	211
Factor B: Pinching										
No pinching	3.97	6.81	11.14	3.24	5.28	7.11	6.44	13.41	23.47	8771
Pinching at 20 DAP	4.06	8.84	12.28	3.38	6.42	8.02	5.91	16.03	24.51	7988
Pinching at 30DAP	3.50	7.41	12.84	2.98	5.56	7.59	6.19	11.95	27.48	9340
Pinching at flower bud initiation	3.80	7.08	11.08	3.12	5.42	7.01	6.78	13.00	21.78	10167
CD (0.05)	NS	0.91	0.99	NS	0.56	0.64	NS	1.34	2.86	717
SE (m) ±	0.17	0.31	0.34	0.12	0.19	0.22	0.22	0.46	0.98	244

Table 4.Interction effect of spacing and pinching on total herbage yield (kg h^{-1})

Treatments	Total herbage yield (kg ha^{-1})
15 cm × 15 cm + No pinching	7383
20 cm × 10 cm + No pinching	8093
15 cm × 10 cm + No pinching	10836
15 cm × 15 cm + Pinching at 20 DAP	6013
20 cm × 10 cm + Pinching at 20 DAP	8886
15 cm × 10 cm + Pinching at 20 DAP	9066
15 cm × 15 cm + Pinching at 30 DAP	7416
20 cm × 10 cm + Pinching at 30 DAP	8786
15 cm × 10 cm + Pinching at 30 DAP	11816
15 cm × 15 cm + Pinching at flower bud initiation	8506
20 cm × 10 cm + Pinching at flower bud initiation	10016
15 cm × 10 cm + Pinching at flower bud initiation	11980
CD (0.05)	1242.89

4. CONCLUSION

The present study suggests growing stevia at a closer spacing (15 cm × 10 cm) and pinching at the flower bud initiation stage for higher herbage yield under Kerala conditions and similar agroecological conditions.

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