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Influence of GA_3 and nitrogen on growth, physiological and yield attributes in *Stevia rebaudiana*

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Abstract
Stevia rebaudiana Bertoni is a shrub which is known for non-caloric natural sweetener called stevioside, which is the main component for its sweetness. The present experiment was conducted to analyze effect of concentrations of plant growth hormone (GA_3) and nitrogen on growth, physiological parameters and yield in *Stevia rebaudiana*. The result revealed that at harvest (90 DAT), maximum internodal length and plant spread was recorded at G_3 (500 ppm). The maximum leaf area and leaf area index was recorded at G_2 (300 ppm). Whereas, the highest dry leaf yield per hectare (1.83 t) was recorded at G_1 (Manual deflowering). At harvest, the maximum internodal length, leaf area, leaf area index and dry leaf yield per hectare (1.80 t) was recorded at 120 Kg N per hectare (N_2).

Keywords: growth, yield, days after transplanting, GA_3, nitrogen, deflowering

Introduction
Stevia (*Stevia rebaudiana* Bertoni.) is a natural sweetener plant belongs to family asteraceae. Stevia is commonly known as "Madhuvantha." This was found out by Bertoni, who later studied it and found that the plant was new to science which he named as *Stevia rebaudiana* Bertoni.

In the recent years Stevia is used as a natural low-calorie sweetener as an alternative to chemical sweeteners as well as a dietary supplement in the developed and developing countries. Stevia is likely to become a major source of "calorie-free" highly safe sugar (ideal for diabetic patients) for the worlds growing natural food market in future. Therefore, keeping this in mind the present investigation has made the attempt to study Influence of GA_3 and Nitrogen on growth, physiological parameters and yield in *Stevia rebaudiana.*

Material and methods
A field experiment was conducted at the Department of Plantation, Spice, Medicinal and Aromatic Crops, Kittur Rani Channamma College of Horticulture, Arabhavi, Karnataka. Plants were grown in May, 2015 through vegetative propagation and used for experiment after one month. Gibberellic acid spray was taken up at the concentration of 300, 500 ppm at monthly intervals from 15 days after transplanting up to 90 days. Recommended dose of N, P, K (60: 30: 45 Kg/ha and FYM 10t/ha) were applied to all the treatments. Out of this, 50 per cent of N and full dose of P&K was applied as basal dose to all the treatments followed by drenching the remaining nitrogen in equal split doses at 15 days intervals in between the rows from 45 DAT. The experiment was laid out in split plot with sixteen treatments in two replications, considering nitrogen as main plot and GA_3 as sub plot.

Growth parameters
Observations on growth parameters were recorded on five randomly selected plants in each replication of different treatments at monthly intervals starting from 30 days after transplanting (DAT).

The length between the two nodes was recorded from main shoot of plant at 30, 60 and 90 DAT. Total number of nodes were counted and it is divided with plant height then mean internodal length of five plants in each plot was calculated and expressed in cm. The plant spread was taken in N-S and E-W direction from five tagged plants at monthly intervals from

30DAT. The spread in both the direction was multiplied to calculate total plant spread and average plant spread was calculated and expressed in cm^2.

Leaf area index (LAI)

Leaf area index was computed using the formula suggested by Sestak *et al.* (1971).

$$LAI = \frac{\text{Leaf area of the entire plant } (cm^2)}{\text{Spacing provided } (cm^2)}$$

Where, A = Leaf area

P = Ground area covered by plant or spacing provided

Yield parameters

Dry leaf yield per plant (g)

Freshly harvested leaves of five tagged plants were kept in hot air oven for drying at a temperature of 65 ºC till a constant weight was reached. The leaves were weighed on an electronic balance and the mean was recorded and expressed in grams.

Dry leaf yield per plot (Kg)

The freshly harvested leaves of the plot were cleaned and dried in hot air oven at 65ºC till a constant weight is obtained and expressed in Kg.

Dry leaf yield per hectare (t/ha)

Leaves were dried in hot air oven at 65ºC till a constant weight was obtained and dry weight was recorded and expressed in t per ha.

Table 1: Influence of gibberellic acid and nitrogen on internodal length in stevia (*Stevia rebaudiana* Bertoni).

Treatments	Internodal length (cm)														
	30 DAT					60 DAT					90 DAT				
	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean
G_0	1.49	1.54	1.61	1.61	1.56	2.30	2.40	2.53	2.54	2.44	2.48	2.74	2.58	2.75	2.64
G_1	1.54	1.70	1.86	1.43	1.63	2.40	2.72	3.05	2.56	2.68	2.55	3.13	2.67	2.97	2.83
G_2	2.40	2.33	2.38	2.51	2.41	3.21	3.56	3.16	3.32	3.31	3.62	3.71	3.64	3.13	3.53
G_3	2.60	2.85	2.50	2.70	2.66	3.51	3.75	3.52	3.45	3.56	3.64	3.85	3.70	3.46	3.66
Mean	2.01	2.10	2.09	2.06	2.07	2.85	3.11	3.06	2.97	3.00	3.07	3.36	3.14	3.08	3.16
For comparison of mean															
	S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %		
Nitrogen (N)	0.136		NS			0.010		0.043			0.038		0.173		
Growth regulator (G)	0.115		0.354			0.044		0.134			0.038		0.117		
G at same level of N	0.241		NS			0.076		0.235			0.076		0.244		
G at same or different level of N	0.230		NS			0.087		0.268			0.076		0.233		

NS: Non significant **DAT:** Days after transplanting

Main plot treatments (N) **Sub plot treatments (G)**

N_1: RDN- 60 Kg/ha G_0: Control (No deflowering)

N_2: N_1 + 100% nitrogen (120 Kg /ha) G_1: Deflowering manually

N_3: N_1 + 150% nitrogen (150 Kg /ha) G_2: GA_3-300ppm

N_4: N_1 + 200% nitrogen (180 Kg /ha) G_3: GA_3-500ppm

Table 2: Influence of gibberellic acid and nitrogen on plant spread in stevia (*Stevia rebaudiana* Bertoni).

Treatments	Plant spread (cm^2)														
	30 DAT					60 DAT					90 DAT				
	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean
G_0	10.25	11.56	11.44	12.28	11.39	23.33	25.14	25.43	22.74	24.16	28.05	30.66	30.06	29.53	29.58
G_1	12.69	12.00	12.50	12.30	12.37	24.53	25.87	26.46	25.25	25.53	29.96	32.77	31.79	31.63	31.53
G_2	12.68	12.78	12.45	11.75	12.42	24.58	26.92	26.51	27.59	26.40	32.07	32.80	31.31	32.04	32.05
G_3	12.74	12.99	12.57	12.53	12.71	25.30	27.65	26.65	26.35	26.49	33.90	33.47	32.80	32.42	33.15
Mean	12.09	12.33	12.24	12.21	12.22	24.44	26.39	26.26	25.48	25.64	30.99	32.42	31.49	31.41	31.57
For comparison of mean															
	S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %		
Nitrogen (N)	0.276		NS			0.124		0.559			0.187		0.839		
Growth regulator (G)	0.3515		NS			0.315		0.970			0.191		0.589		
G at same level of N	0.669		NS			0.560		NS			0.383		1.229		
G at same or different level of N	0.704		NS			0.630		NS			0.383		1.178		

NS: Non significant DAT**:** Days after transplanting

Main plot treatments (N) **Sub plot treatments (G)**

N_1: RDN- 60 Kg/ha G_0: Control (No deflowering)

N_2: N_1 + 100% nitrogen (120 Kg /ha) G_1: Deflowering manually

N_3: N_1 + 150% nitrogen (150 Kg /ha) G_2: GA_3-300ppm

N_4: N_1 + 200% nitrogen (180 Kg /ha) G_3: GA_3-500ppm

Table 3: Influence of gibberellic acid and nitrogen on leaf area and leaf area index in stevia (*Stevia rebaudiana* Bertoni).

Treatments	Leaf area (cm^2/plant)										LAI									
	45 DAT					90 DAT					45 DAT					90 DAT				
	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean
G_0	314.18	445.00	327.19	347.36	358.43	629.35	892.55	656.40	696.76	718.77	0.35	0.49	0.36	0.39	0.40	0.70	0.99	0.77	0.73	0.80
G_1	509.63	645.00	568.10	513.37	559.03	1019.74	1292.56	1137.30	1028.72	1119.58	0.57	0.72	0.63	0.57	0.62	1.13	1.44	1.14	1.26	1.24
G_2	586.05	705.50	625.15	648.22	641.23	1180.40	1413.55	1254.30	1297.47	1286.43	0.65	0.78	0.69	0.72	0.71	1.31	1.57	1.44	1.39	1.43
G_3	560.25	677.62	585.66	585.42	602.24	1125.32	1356.28	1175.82	1175.62	1208.26	0.62	0.75	0.65	0.65	0.67	1.25	1.51	1.31	1.31	1.35
Mean	492.53	618.28	526.53	523.59	540.23	988.70	1238.74	1055.96	1049.64	1083.26	0.55	0.69	0.59	0.58	0.60	1.10	1.38	1.17	1.17	1.21
For comparison of mean																				
	S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %		
Nitrogen (N)	15.18		68.36			0.64		2.88			0.017		0.076			0.0007		0.0032		
Growth regulator (G)	10.73		33.06			1.09		3.37			0.012		0.037			0.0012		0.0037		
G at same level of N	24.00		NS			2.00		6.29			0.027		NS			0.0022		0.0070		
G at same or different level of N	21.46		NS			2.18		6.74			0.024		NS			0.0024		0.0075		

NS: Non significant DAT**:** Days after transplanting

Main plot treatments (N) **Sub plot treatments (G)**

N_1: RDN- 60 Kg/ha G_0: Control (No deflowering)

N_2: N_1 + 100% nitrogen (120 Kg /ha) G_1: Deflowering manually

N_3: N_1 + 150% nitrogen (150 Kg /ha) G_2: GA_3-300ppm

N_4: N_1 + 200% nitrogen (180 Kg /ha) G_3: GA_3-500ppm

Table 4: Influence of gibberellic acid and nitrogen on dry leaf yield at harvest in stevia (*Stevia rebaudiana* Bertoni).

Treatments	Dry leaf yield														
	g/plant					Kg/plot					t/ha				
	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean	N_1	N_2	N_3	N_4	Mean
G_0	14.83	17.12	16.15	14.88	15.74	0.71	0.82	0.78	0.71	0.76	1.20	1.39	1.30	1.26	1.27
G_1	16.56	20.16	20.09	18.32	18.78	0.80	0.97	0.96	0.88	0.90	1.64	1.99	1.90	1.81	1.83
G_2	16.26	19.87	19.91	17.43	18.36	0.78	0.95	0.96	0.84	0.88	1.61	1.96	1.97	1.72	1.81
G_3	15.96	19.69	19.45	17.11	18.05	0.77	0.95	0.93	0.82	0.87	1.58	1.94	1.92	1.69	1.78
Mean	15.90	19.21	18.90	16.93	17.74	0.76	0.92	0.90	0.81	0.85	1.50	1.80	1.77	1.62	1.67
For comparison of mean															
	S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %			S.Em ±		CD @ 5 %		
Nitrogen (N)	0.029		0.130			0.001		0.006			0.003		0.011		
Growth regulator (G)	0.075		0.230			0.004		0.011			0.707		2.178		
G at same level of N	0.132		0.412			0.006		0.020			1.224		3.772		
G at same or different level of N	0.149		0.460			0.007		0.022			1.413		4.355		

NS: Non significant DAT**:** Days after transplanting

Main plot treatments (N) **Sub plot treatments (G)**

N_1: RDN- 60 Kg/ha G_0: Control (No deflowering)

N_2: N_1 + 100% nitrogen (120 Kg /ha) G_1: Deflowering manually

N_3: N_1 + 150% nitrogen (150 Kg /ha) G_2: GA_3-300ppm

N_4: N_1 + 200% nitrogen (180 Kg /ha) G_3: GA_3-500ppm

Results and Discussion

The internodal length recorded in GA_3 (500 ppm) was maximum (2.66cm, 3.56cm and 3.66cm) which was significantly higher over G_0 (control) (1.56 cm, 2.44 cm and 2.64 cm) at 30, 60 and 90 DAT, respectively. Similarly, Sen and Maharana (1972) [8] reported that treatment with GA_3 (500 ppm) caused hyper elongation of stem and internode in chrysanthemum. The elongation of internode and stem by the application of GA_3 resulted in increased height of the plant which could be attributed to the action of GA_3, which perhaps softens the cell wall by increasing its plasticity. Another possible reason might be increase in cell division and cell elongation in subapical meristems of dwarf plants. Among different nitrogen levels N_2 (120 Kg N/ha) recorded maximum internodal length (3.36cm) compared to N_1 (60 Kg N/ha) at 90 DAT. The increase in internodal due to nitrogen also helps in the mitotic division of cells at the internodal region. Similar findings were reported by Angelinia *et al*. (2015) [1] and Rakesh *et al*. (2012) [6] in stevia. Significant difference in internodal length was observed due to interaction effect between GA_3 and nitrogen on internodal length except 30 DAT. Among the interactions, the highest internodal length was recorded in N_2G_3 (3.75 cm and 3.85 cm) and lowest internodal length was noticed in N_1G_0 (2.30 cm and 2.48 cm) at 60 and 90 DAT, respectively.

The application of GA_3 (500 ppm) increased the plant spread (33.15 cm^2) compared to G_0 (Control) at 90 DAT. This is due to suppression of apical dominance which has brought functionality of several meristems on the nodal regions at a time leading to more number of branches, thereby increasing the plant spread. Similar findings were observed by Dube (2011) [2], Salama (2008) [7] in stevia.The influence of nitrogen on plant spread in east-west and north-south directions increased significantly with increase in dose of nitrogen (120 Kg N /ha). At harvest, the plants which were supplied with 120 Kg N per hectare (N_2) recorded the maximum plant spread (32.42cm^2), while the plants supplied with 60 Kg N per hectare (N_1) recorded the minimum plant spread (30.99 cm^2). The higher plant spread at this nitrogen level might be due to the robust nature of plant growth as evident by production of more number of branches. As nitrogen doses increased decreasing trend of plant spread was

noticed, this is because of crop requirement of major nutrients was met through this treatment. These findings are in agreement with the results reported by Srinivas *et al*. (2008) [10] in medicinal coleus.

Physiological parameters: Yield variation in terms of growth and development is very complex to explain, as it involves the effect of both internal and external factors on all plant physiological processes. It is well established that the infrastructure of the plant is decided by the growth parameters such as leaf area and leaf area index. At 45 and 90 DAT, the G_2 (300 ppm) recorded significantly maximum leaf area (641.23 and 1286.43 cm^2, respectively) and minimum was observed in G_0 (control) (358.43 and 718.77 cm^2, respectively). Among nitrogen levels, significantly maximum leaf area was observed in N_2 (120 Kg N/ha) (618.28 and 1238.74 cm^2) and least was (492.53 and 988.70 cm^2) noticed in N_1 (60 Kg N /ha) at 45 and 90 DAT, respectively.

The data with respect to the effect of GA_3, nitrogen and their interaction on leaf area index at 45 and 90 DAT are presented in Table 3. At 90 DAT, higher leaf area index (1.43) was recorded at G_2 (300ppm). While, the least (0.80) leaf area index was noticed at G_0 (control). In this experiment, it seems that the plant growth regulator (GA_3) would have increased the physiological activities like cell division and cell elongation, translocation of nutrients and leaf area for photosynthesis which would have caused the maximum increase in the physiological parameters related to in *Stevia rebaudiana*. These findings are in line with Dube, (2011) [2] in stevia, Pawar *et al.* (2005) [5] in Pomegranate.

Among nitrogen levels, significantly maximum (1.38) leaf area index was observed in N_2 (120 Kg N/ha) and the least (1.10) was noticed in N_1 (60 Kg N/ha) at 90 DAT. This could be due to production of more number of leaves, branches due to enhanced availability of nitrogen at the optimum level has increased the leaf area and LAI. Another possible reason was increased leaf area index might be due to more number of broader leaves as a result of better availability of nutrients to the crop. These results are in accordance with the findings of Vijaykumar *et al*. (1988) in china aster, Singh *et al*. (1989) [9] and Izhar *et al*. (2015) [3] in *Mentha arvensis*.

Yield parameters: There was significant difference in dry herbage yield per leaf due to GA_3 application. Among the treatments, G_1 (Manual deflowering) recorded higher dry leaf yield per hectare (1.83 t) but it was found to be on par (1.81 t) with G_2 (300ppm). The lowest dry leaf yield per hectare (1.27 t) was noticed in G_0 (control).

Application of nitrogen had significant effect on dry leaf yield per hectare. Among the different nitrogen levels, application of 120 Kg N per hectare (N_2) recorded the maximum dry leaf yield per hectare (1.80 t) and minimum (1.50 t) was noticed in N_1 (60 Kg N/ha). The increase in yield may be attributed to the fact that due to optimum levels of nitrogen, there would be improved growth of the plant, which leads to production of more number of leaves, branches and ultimately resulting in highest fresh leaf yield. This is in conformity with the results of Murayama *et al*. (1980) [4] in stevia who reported that the application of optimum doses (100 Kg N/ha) of nitrogen produced better growth rate and dry leaf yield than the application of lower dose. The data indicates that interaction of GA_3 and nitrogen exhibit significant effect on dry leaf yield per hectare. However, maximum (1.99 t) and minimum (1.20 t) dry leaf yield per hectare was recorded in N_2G_1 and N_1G_0 treatment combination, respectively.

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