

Original Research Article

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## Effect of Nitrogen and GA<sub>3</sub> on Suppression of Flowering for Enhancement of Vegetative Phase in Stevia (*Stevia rebaudiana* Bertoni.)

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### ABSTRACT

#### Keywords

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A field study was conducted during 2015 to investigate the effect of nitrogen and GA<sub>3</sub> on suppression of flowering for enhancement of vegetative phase in stevia (*Stevia rebaudiana* Bertoni.). The result revealed that maximum plant height, number of branches per plant and fresh leaf yield per hectare was recorded with the application of nitrogen at 120 Kg per hectare at 90 DAT. At 90 DAT application of GA<sub>3</sub> 500 ppm recorded significantly higher plant height and number of branches per plant. Manual deflowering recorded highest fresh leaf yield per ha, while the minimum fresh leaf yield per ha was observed in control.

### Introduction

*Stevia rebaudiana* (Bert.) belongs to family asteraceae is a slender short day perennial plant native to Paraguay and South-West Brazil. It is widely distributed in USA, Brazil, Japan, Korea, Taiwan and South-East Asia. Stevia (*Stevia rebaudiana* Bertoni.) is a natural sweetener plant with zero calorie content, becomes an inevitable alternative to cane sugar especially with over 346 million diabetic population across the world and this figure might increase to 592 million by 2035 (Madhumitha kumara, 2014).

The sugar obtained from stevia is considered to be the best natural alternate source for artificial sugars like aspartame and saccharine for diabetic sufferers. Although, there are more than 180 species of the stevia plant, only *Stevia rebaudiana* gives the sweetest essence due to the fact that these leaves accumulate sweet diterpene glycoside having a sweetness of 250-350 times that of sucrose. This plant has an extraordinary sweetness in its natural form of dried leaves; it is about 10 to 15 times sweeter than white sugar. Various studies have revealed that the stevia leaf contains proteins, fiber, carbohydrates, iron, phosphorus,

calcium, sodium, magnesium, zinc, vitamin A, vitamin C and oil which contain 53 other constituents. Stevia has no toxicity. Stevia is safe for use by both diabetics and hypoglycemic due to its low glycaemic index (Singh and Rao, 2005).

### **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 during 2015, to enhance the vegetative phase by suppressing flowering in stevia (*Stevia rebaudiana* Bertoni.) by nitrogen and GA<sub>3</sub>. The experiment was laid out in split plot with sixteen treatments in two replications, considering nitrogen as main plot and GA<sub>3</sub> as sub plot. Thirty days old seedlings were transplanted by ridge and furrow method at the spacing of 45×20 cm. 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 eight equal split doses at 15 days intervals in between the rows from 45 DAT. 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. The crop was harvested after 3 months of transplanting.

Plant height was measured from the ground level to the growing tip of the plant at monthly interval starting from 30 days after planting (DAT) and it was expressed in centimetre. The number of flowers per plant was counted from five randomly selected plants at 30, 60 and 90 DAT and mean was expressed as number of flowers 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 labelled plants were harvested and weighed by using the electronic balance and the mean was worked out.

### **Results and Discussion**

The plant height was significantly influenced by nitrogen levels at 30, 60 and 90 DAT. At harvest, those plants applied with N<sub>2</sub> (120 Kg N/ha) recorded maximum plant height (53.59 cm) compared to lower nitrogen level (N<sub>1</sub>:60 Kg N/ha). But above this level of nitrogen hinder the growth and plant height decreased in stevia. Similar results were reported by Chung *et al.*, 1992 in tomato. The positive influence of nitrogen on plant height might be due to the fact that nitrogen is required for cell division and cell elongation which triggers the growth of meristamatic tissue and the efficient utilization of this by the plants manifested in production of taller plants. Similar observations were also made by Raghuraja (1992) in *Gailardia pulchella*. The results are in agreement with the findings of Laura *et al.*, (2011) in french basil (Table 1).

The plant height was significantly higher (56.85 cm) with GA<sub>3</sub> (500 ppm) at 90 DAT. Increased 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). This clearly indicates that the mode of action of these GA<sub>3</sub> differed between the PGRs and their concentrations. Another possible reason was, the increased plant height by GA<sub>3</sub> application due to enhanced cell division and cell elongation. 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) in davana, who showed increase in plant height, due to application of GA<sub>3</sub>.

**Table.1** Influence of gibberellic acid and nitrogen on plant height in stevia (*Stevia rebaudiana* Bertoni)

Treatments	Plant height (cm)														
	30 DAT					60 DAT					90 DAT				
	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean
<b>G<sub>0</sub></b>	22.50	23.50	20.76	20.24	<b>21.75</b>	42.55	44.29	44.09	42.04	<b>43.24</b>	44.60	48.50	44.72	45.11	<b>45.73</b>
<b>G<sub>1</sub></b>	24.50	25.00	21.50	24.50	<b>23.87</b>	46.55	47.50	44.50	44.65	<b>45.80</b>	46.80	52.33	50.20	51.48	<b>50.20</b>
<b>G<sub>2</sub></b>	23.50	25.75	23.50	24.67	<b>24.35</b>	47.89	48.08	47.99	44.56	<b>47.13</b>	54.00	55.12	53.50	53.99	<b>54.15</b>
<b>G<sub>3</sub></b>	23.25	23.64	26.60	26.25	<b>24.93</b>	48.00	48.44	47.50	47.55	<b>47.87</b>	56.45	58.44	56.90	55.62	<b>56.85</b>
<b>Mean</b>	<b>23.44</b>	<b>24.47</b>	<b>23.09</b>	<b>23.92</b>	<b>23.73</b>	<b>46.24</b>	<b>47.07</b>	<b>46.02</b>	<b>44.70</b>	<b>46.00</b>	<b>50.46</b>	<b>53.59</b>	<b>51.33</b>	<b>51.55</b>	<b>51.73</b>
<b>For comparison of mean</b>															
					<b>S.Em ±</b>	<b>CD @ 5 %</b>						<b>S.Em ±</b>	<b>CD @ 5 %</b>		
<b>Nitrogen (N)</b>					0.618	NS						0.195	0.879	0.301	
<b>Growth regulator (G)</b>					0.790	NS						0.285	0.878	0.341	
<b>G at same level of N</b>					1.501	NS						0.531	NS	0.662	
<b>G at same or different level of N</b>					1.579	NS						0.570	NS	0.681	

NS: Non significant

**Main plot treatments (N)**

N<sub>1</sub>: RDN- 60 Kg/ha

N<sub>2</sub>: N<sub>1</sub> + 100% nitrogen (120 Kg /ha)

N<sub>3</sub>: N<sub>1</sub> + 150% nitrogen (150 Kg /ha)

N<sub>4</sub>: N<sub>1</sub> + 200% nitrogen (180 Kg /ha)

DAT: Days after transplanting

**Sub plot treatments (G)**

G<sub>0</sub>: Control (No deflowering)

G<sub>1</sub>: Deflowering manually

G<sub>2</sub>: GA<sub>3</sub>-300ppm

G<sub>3</sub>: GA<sub>3</sub>-500ppm

**Table.2** Influence of gibberellic acid and nitrogen on number of flowers in stevia (*Stevia rebaudiana* Bertoni)

Treatments	Number of flowers														
	30 DAT					60 DAT					90 DAT				
	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean
<b>G<sub>0</sub></b>	34.22	35.00	34.06	34.44	<b>34.43</b>	784.11	770.70	785.60	790.49	<b>782.73</b>	1005.00	985.50	987.51	985.83	<b>990.96</b>
<b>G<sub>1</sub></b>	32.63	30.83	32.00	31.01	<b>31.61</b>	715.00	725.11	735.00	775.00	<b>737.53</b>	800.26	800.55	835.25	810.39	<b>811.61</b>
<b>G<sub>2</sub></b>	35.22	30.46	32.76	32.00	<b>32.61</b>	767.00	733.00	725.00	785.23	<b>752.55</b>	820.44	813.00	840.11	849.12	<b>830.67</b>
<b>G<sub>3</sub></b>	35.96	32.94	30.44	33.55	<b>33.22</b>	775.89	759.00	775.00	799.00	<b>777.20</b>	848.05	855.51	862.61	857.86	<b>856.01</b>
<b>Mean</b>	<b>34.51</b>	<b>32.31</b>	<b>32.31</b>	<b>32.75</b>	<b>32.97</b>	<b>760.50</b>	<b>747.00</b>	<b>755.15</b>	<b>787.43</b>	<b>762.50</b>	<b>868.44</b>	<b>863.64</b>	<b>881.37</b>	<b>875.80</b>	<b>872.31</b>
<b>For comparison of mean</b>															
	<b>S.Em ±</b>		<b>CD @ 5 %</b>		<b>S.Em ±</b>		<b>CD @ 5 %</b>		<b>S.Em ±</b>		<b>CD @ 5 %</b>				
<b>Nitrogen (N)</b>	2.45		NS		7.279		NS		2.352		10.585				
<b>Growth regulator (G)</b>	2.20		NS		11.847		NS		5.393		16.618				
<b>G at same level of N</b>	4.53		NS		21.77		NS		9.63		NS				
<b>G at same or different level of N</b>	4.40		NS		23.69		NS		10.79		NS				

NS: Non significant

**Main plot treatments (N)**

N<sub>1</sub>: RDN- 60 Kg/ha

N<sub>2</sub>: N<sub>1</sub> + 100% nitrogen (120 Kg /ha)

N<sub>3</sub>: N<sub>1</sub> + 150% nitrogen (150 Kg /ha)

N<sub>4</sub>: N<sub>1</sub> + 200% nitrogen (180 Kg /ha)

DAT: Days after transplanting

**Sub plot treatments (G)**

G<sub>0</sub>: Control (No deflowering)

G<sub>1</sub>: Deflowering manually

G<sub>2</sub>: GA<sub>3</sub>-300ppm

G<sub>3</sub>: GA<sub>3</sub>-500ppm

**Table.3** Influence of gibberellic acid and nitrogen on number of branches in stevia (*Stevia rebaudiana* Bertoni)

Treatments	Number of branches														
	30 DAT					60 DAT					90 DAT				
	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mea n	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mea n	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mea n
<b>G<sub>0</sub></b>	2.50	2.90	2.75	3.10	<b>2.81</b>	10.6 5	11.1 0	10.4 5	10.7 5	<b>10.7 4</b>	12.0 0	13.2 0	12.4 0	12.3 5	<b>12.4 9</b>
<b>G<sub>1</sub></b>	2.75	3.10	2.90	2.55	<b>2.83</b>	11.2 5	12.2 5	11.5 5	12.1 5	<b>11.8 0</b>	14.6 3	15.8 5	15.5 5	15.6 5	<b>15.4 2</b>
<b>G<sub>2</sub></b>	2.90	3.16	3.10	3.10	<b>3.07</b>	11.4 0	13.1 0	11.7 5	12.8 5	<b>12.2 8</b>	15.8 5	16.4 5	16.7 0	16.0 4	<b>16.2 6</b>
<b>G<sub>3</sub></b>	3.25	3.25	3.15	2.85	<b>3.13</b>	12.2 0	13.9 0	12.4 0	12.9 0	<b>12.8 5</b>	16.7 5	17.7 5	17.4 0	17.3 9	<b>17.3 2</b>
<b>Mean</b>	<b>2.85</b>	<b>3.10</b>	<b>2.98</b>	<b>2.90</b>	<b>2.96</b>	<b>11.3 8</b>	<b>12.5 9</b>	<b>11.5 4</b>	<b>12.1 6</b>	<b>11.9 2</b>	<b>14.8 1</b>	<b>15.8 1</b>	<b>15.5 1</b>	<b>15.3 6</b>	<b>15.3 7</b>
<b>For comparison of mean</b>															
	<b>S.Em ±</b>		<b>CD @ 5 %</b>			<b>S.Em ±</b>		<b>CD @ 5 %</b>			<b>S.Em ±</b>		<b>CD @ 5 %</b>		
<b>Nitrogen (N)</b>	0.121		NS			0.141		0.635			0.123		0.554		
<b>Growth regulator (G)</b>	0.108		NS			0.142		0.437			0.116		0.357		
<b>G at same level of N</b>	0.223		NS			0.283		NS			0.235		NS		
<b>G at same or different level of N</b>	0.216		NS			0.284		NS			0.232		NS		

NS: Non significant  
**Main plot treatments (N)**  
 N<sub>1</sub>: RDN- 60 Kg/ha  
 N<sub>2</sub>: N<sub>1</sub> + 100% nitrogen (120 Kg /ha)  
 N<sub>3</sub>: N<sub>1</sub> + 150% nitrogen (150 Kg /ha)  
 N<sub>4</sub>: N<sub>1</sub> + 200% nitrogen (180 Kg /ha)

DAT: Days after transplanting  
**Sub plot treatments (G)**  
 G<sub>0</sub>: Control (No deflowering)  
 G<sub>1</sub>: Deflowering manually  
 G<sub>2</sub>: GA<sub>3</sub>-300ppm  
 G<sub>3</sub>: GA<sub>3</sub>-500ppm

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

Treatments	Fresh leaf yield														
	g/plant					Kg/plot					t/ha				
	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Mean
<b>G<sub>0</sub></b>	29.55	36.00	32.97	30.94	<b>32.36</b>	1.42	1.73	1.58	1.49	<b>1.55</b>	2.92	3.56	3.26	3.06	<b>3.20</b>
<b>G<sub>1</sub></b>	31.93	43.05	38.81	34.15	<b>37.00</b>	1.53	2.07	1.86	1.64	<b>1.77</b>	3.15	4.25	3.83	3.37	<b>3.65</b>
<b>G<sub>2</sub></b>	30.25	39.10	34.32	32.41	<b>34.02</b>	1.45	1.88	1.65	1.56	<b>1.63</b>	2.99	3.86	3.39	3.20	<b>3.36</b>
<b>G<sub>3</sub></b>	30.00	38.95	35.06	31.85	<b>33.96</b>	1.44	1.87	1.68	1.53	<b>1.63</b>	2.96	3.85	3.46	3.15	<b>3.36</b>
<b>Mean</b>	<b>30.43</b>	<b>39.27</b>	<b>35.29</b>	<b>32.33</b>	<b>34.33</b>	<b>1.46</b>	<b>1.88</b>	<b>1.69</b>	<b>1.55</b>	<b>1.64</b>	<b>3.01</b>	<b>3.88</b>	<b>3.49</b>	<b>3.20</b>	<b>3.39</b>
<b>For comparison of mean</b>															
	<b>S.Em ±</b>		<b>CD @ 5 %</b>		<b>S.Em ±</b>		<b>CD @ 5 %</b>		<b>S.Em ±</b>		<b>CD @ 5 %</b>				
<b>Nitrogen (N)</b>	0.488		2.195		0.023		0.105		0.048		0.217				
<b>Growth regulator (G)</b>	0.384		1.183		0.018		0.057		0.038		0.117				
<b>G at same level of N</b>	0.824		NS		0.040		NS		0.081		NS				
<b>G at same or different level of N</b>	0.768		NS		0.037		NS		0.076		NS				

NS: Non significant  
**Main plot treatments (N)**  
 N<sub>1</sub>: RDN- 60 Kg/ha  
 N<sub>2</sub>: N<sub>1</sub> + 100% nitrogen (120 Kg /ha)  
 N<sub>3</sub>: N<sub>1</sub> + 150% nitrogen (150 Kg /ha)  
 N<sub>4</sub>: N<sub>1</sub> + 200% nitrogen (180 Kg /ha)

DAT: Days after transplanting  
**Sub plot treatments (G)**  
 G<sub>0</sub>: Control (No deflowering)  
 G<sub>1</sub>: Deflowering manually  
 G<sub>2</sub>: GA<sub>3</sub>-300ppm  
 G<sub>3</sub>: GA<sub>3</sub>-500ppm

Reduction in plant height due to maleic hydrazide appears because of its reduction in cell division and cell expansion due to its inhibitory action in the biosynthetic pathway of GA<sub>3</sub> (Moore, 1980).

With regard to number of flowers per plant, significant differences were manifested between different nitrogen levels at 90 DAT. Among nitrogen treatments, N<sub>2</sub> (120 Kg N/ha) recorded minimum number of flowers (863.64) per plant. Nitrogen recorded significantly less number of flowers and also caused delay in flowering, due to increase in the vegetative growth in this treatment (Table 2).

At 90 DAT, the minimum number of flowers (811.61) was observed in the treatment G<sub>1</sub> (Manual deflowering) and maximum number of flowers (990.96 and 856.01) were noticed in G<sub>0</sub> (control) followed by G<sub>3</sub> (500ppm). Rakesh *et al.*, (2014) reported that, pinching treatments significantly reduced the number of flowers per plant (40.2), as compared with the control (95.6) at 45 DAT in stevia. Although gibberellin not flowering hormones, but when these are present in proper concentrations, florigen (flowering hormone) may be synthesized. GA<sub>3</sub> might also activate genes, which control the synthesis of florigen and thus induced the early flowering. It is in conformity with Salisbury and James bonner, 1985 in cockelbur.

The production of number of branches per plant increased significantly with the optimum level of nitrogen dose. Among different levels of nitrogen, N<sub>2</sub> (120 Kg N/ha) had the highest (15.81) number of branches per plant and the minimum (14.81) was noticed in treatment N<sub>1</sub> (60 Kg N/ha) at harvest. The production of more number of branches might be due to the fact that in the initial stages of growth the number of branches produced per plant was minimum and as the growth proceeds, production of

more branches occurs. This could be ascribed to the availability of optimum quantity of nitrogen during vegetative growth and split application influenced the availability of nitrogen. At higher doses of nitrogen decreasing trend of branches was noticed. This might be due to the fact that stevia responds well at optimum levels of nitrogen compare to higher doses of nitrogen. Similar trend was observed by Singh and Singh, 1977 in *Mentha arvensis*. The application of GA<sub>3</sub> (500 ppm) increased number of branches per plant (17.32) compared to G<sub>0</sub> (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 Salama, 2008 in stevia (Table 3 and 4).

Fresh leaf yield differed significantly due to nitrogen levels. Application of 120 Kg N per hectare (N<sub>2</sub>) resulted in production of maximum fresh leaf yield per plant (39.27g), per plot (1.88 Kg) and per ha (3.88 t), while the minimum fresh leaf yield per plant (30.43 g), per plot (1.46 Kg) and per ha (3.01 t) was observed in plants supplied with N<sub>1</sub> (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 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.

Among the treatments, G<sub>1</sub> (Manual deflowering) recorded highest fresh herbage yield per plant (37.00 g), per plot (1.77 Kg) and per ha (3.65 t), while the minimum fresh

herbage yield per plant (32.36 g), per plot (1.55 Kg) and per ha (3.20 t) was observed in G<sub>0</sub> (control). Among interactions, the maximum plant height (58.44 cm) was noticed in the treatment combination N<sub>2</sub>G<sub>3</sub> (120 Kg N/ha+ GA<sub>3</sub>-500ppm) and N<sub>1</sub>G<sub>3</sub> (60 Kg N/ha + GA<sub>3</sub>-500ppm). The positive influence of nitrogen and GA<sub>3</sub> might be due to the fact that combination of nitrogen and GA<sub>3</sub> cause the enormous increase in cell division and cell elongation. There was no significant difference in number of flowers and number of branches per plant due to interaction effect of GA<sub>3</sub> and nitrogen.

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