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Effect of spacing and biofertilizers on growth and nutrients of stevia (*Stevia rebaudiana* Bertoni)

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ABSTRACT

A field experiment on effect of spacing and bio fertilizers on growth and nutrients of stevia (*Stevia rebuadiana* Bertoni.) was conducted during *kharif* seasons of 2006 and 2007. The experiment was laid out in split plot design with main plot with four spacing levels (30 cm × 20 cm, 30 cm × 30 cm, 45 cm × 20 cm and 45 cm × 30 cm) and sub-plot treatments included six bio-fertilizer based nutritional trials (100% NPK (recommended dose: 60:30:45 kg/ha) 75% N + PK + *Azotobacter* (Azoto), 100% N + PK + Azoto, 75% N + PK + *Azospirillum* (Azosp), 100% N + PK + Azosp; and control (no fertilizers). The experiment was laid out in split plot design with three replications and 24 treatment combinations. Significantly, higher spread was recorded in the spacing of 30 cm × 30 cm, over other spacing levels, at 60 and 90 DAP. However, the spacing of 30 cm × 20 cm, 45 × 20 cm and 45 × 30 cm recorded comparable plant spread at 30 DAP. The spacing of 30 cm × 30 cm recorded significantly higher number of leaves, than other spacing levels, at 30, 60 and 90 DAP. Nitrogen and phosphorus (P) content did not differed significantly due to spacing levels during both the years of experimentation. Significantly higher potash content of 1.36% was recorded in the spacing of 45 cm × 30 cm. Higher plant height, plant spread(24.06 cm^2/plant), more number of leaves and number of branches were recorded in the treatment that received 100% N + PK + *Azotobacter* and 100% N + PK + *Azospirillum* at all the growth stages. Higher nitrogen content was recorded in the treatment that received 100% N + PK + *Azotobacter* (1.56%) than other treatments. Significantly, higher phosphorous content was recorded in the treatment that receives 100% N + PK + *Azotobacter* (0.074%) and higher potash content (1.42%) than other treatments. Significantly, higher N content of 1.64% and K content of 1.49% was recorded in the treatment combination 30 cm × 20 cm with 100% N + PK + *Azotobacter* than other treatment combinations.

Key words: *Stevia rebaudiana*, spacing, stevioside, nutrients.

INTRODUCTION

Stevia rebaudiana (Bertoni.) officially discovered by Dr M.S. Bertoni in 1905, belonging to the family Asterceae is a recent high demanding medicinal crop in herbal world. Health causing diseases by natural caloric sweetener as well as by chemical sweeteners (like saccharin and aspartame) make the life risk. The plant contains Stevioside, Rebaudiside, Rebaudioside C, Dulcoside A, with strong sweet taste but with very few calories (Grassi *et al*, 12). Consequently, stevia is potentially extremely useful for food industry and dietary treatment. Hence, focus shifted on stevia, which is completely natural and non-caloric plant. The plant is native to South America (Paraguay and Brazil) but recently domesticated in India for its large scale cultivation. Above all, recently *Stevia* is gaining momentum due to its natural sweetener properties and an alternative sweetener source for the diabetics. (Kohda *et al.*, 5; Kobayashi *et al.*, 6; Soejarto *et al.*, 11).

Since, it is a newly adopted crop there is almost no information available on its proper production techniques, which may be one of cause for the non-availability of its quality raw materials. The modern and intensive agriculture methods are not only costly, but also cause soil and water pollution along with diminishing the quality of the raw materials. Thus, in this situation, the recent concept of eco-friendly technology, application of bio-fertilizers in combination with inorganic fertilizers substitutes may prove to be necessary for this wonder crop. In stevia, leaves are the economic part of the plant. The agronomic manipulations and practices aimed at improving the yield of leaves through optimizing source-sink ratio are of more practical significance. Optimum spacing provided to each plant helps to utilize growth resources optimally resulting in better yields. Hence, keeping in view the above facts the present investigation was carried out.

MATERIALS AND METHODS

The experiment was carried out at the Experimental Farm of Department of Horticulture, Chaudhary Chhotu Ram P.G College, and Muzaffarnagar, Uttar

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Pradesh during the 2006 -2007. The experimental site is situated at 29° 28° N, longitude of 77° 44°' East and at an altitude of 245.82 m above mean sea level. The experiment was laid out in split plot design with three replications and 24 treatment combinations. The treatments in each plot (3.78 m^2) were allotted randomly. Details of treatments were as factor one spacing (S_1 = 30 × 20 cm, S_2 = 30 × 30 cm, S_3 = 45 × 20 cm, and S_4 = 45 × 30 cm) and different combinations S_1F_1= 30 × 20 (cm) spacing with 100% NPK; S_1F_2 = 30 × 20 (cm) spacing with 75% N+PK + Azoto; S_1F_3 = 30 × 20 (cm) spacing with 100% N+PK + Azoto; S_1F_4= 30 × 20 (cm) spacing with 75% N+PK + Azosp; S_1F_5= 30 × 20 (cm) spacing with 100% N+PK + Azosp; S_1F_6= 30 × 20 (cm) spacing with no fertilizer (control); S_2F_1 = 30 × 30 (cm) spacing with 100% NPK ; S_2F_2 = 30 × 30 (cm) spacing with 75% N+PK + Azoto; S_2F_3 = 30 × 30 (cm) spacing with 100% N+PK + Azoto; S_2F_4 = 30 × 30 (cm) spacing with 75% N+PK + Azosp; S_2F_5 = 30 × 30 (cm) spacing with 100% N+PK + Azosp; S_2F_6 = 30 × 30 (cm) spacing with no fertilizer (control); S_3F_1 = 45 × 20 (cm) spacing with 100% NPK; S_3F_2 = 45 × 20 (cm) spacing with 75% N+PK + Azoto, S_3F_3 = 45 × 20 (cm) spacing with 100% N + PK + Azoto; S_3F_4 = 45 × 20 (cm) spacing with 75% N+PK + Azosp; S_3F_5 = 45 × 20 (cm) spacing with 100% N+PK + Azosp; S_3F_6 = 45 × 20 (cm) spacing with no fertilizer (control); S_4F_1 = 45 × 20 (cm) spacing with 100% NPK; S_4F_2 = 45 × 20 (cm) spacing with 75% N+PK+ Azoto; S_4F_3 = 45 × 20 (cm) spacing with 100% N + PK+Azoto; S_4F_4 = 45 × 20 (cm) spacing with 75% N+PK + Azosp; S_4F_5 = 45 × 20 (cm) spacing with 100% N+PK + Azosp; and S_4F_6 = 45 × 20 (cm) spacing with no fertilizer (control).

The nutrients were applied in the form of straight fertilizers, i.e. nitrogen in the form of urea, phosphorus in the form of single super phosphate and potassium in the form of muriate of potash. Thirty-day-old healthy and uniform rooted cuttings of stevia from Horticulture division of University of Agriculture Sciences, Bangalore were planted as per design and treatments in their respective plots. Before planting the rooted cuttings were treated well with bioinoculum of *Azotobacter* and *Azospirillum* in the month of July 2006 and August 2007. The recommended dose of nutrients (60 kg nitrogen, 30 kg phosphorus and 45 kg potassium per hectare) was applied as per the treatments as suggested by Chalapathi *et al*. (1). Full dose of phosphorus and potassium along with one fifth of nitrogen were applied as basal dose and the remaining nitrogen was given in four splits doses as top dressing after 15 days of each harvesting. The crop was harvested at the stage of pre-flowering stage at 60-90 days interval by cutting at ground level and dried under shade for 2-5 days. Observation on growth and soil nutrient content in soil of stevia were recorded at 30 days of planting and after harvest respectively and the data were statistically analyzed as procedure using standard procedures given by Panse and Sukhatme (9).

RESULTS AND DISCUSSION

Plant height at 90 DAP, was significantly higher at wider spacing of 45 cm × 20 cm (54.60 cm) and 30 cm × 30 cm (53.98 cm) than closer spacing of 30 cm × 20 cm (48.67 cm). Plant height was also higher at closer spacing levels (Table 1). Higher plant height was due to more linear growth of plants as a result of higher density of planting per unit area. Lee *et al.*, (4). reported that plant height, number of branches and number of nodes were unaffected by planting density (50-70 cm between and 10-30 cm within rows). The number of branches in the treatment combinations 30 cm × 30 cm with application of 100% N+PK + *Azotobacter* (S_2F_3) were significantly higher than other treatment combinations. Similarly, number of leaves in 30 cm × 30 with 100% N+PK + *Azotobacter* (S_2F_3) and 30 cm × 30 cm with 100% N+PK + *Azospirillum* (S_2F_5) was significantly higher than other treatment combinations (Table 3). Higher plant spread and higher number of branches and leaves per plant could have contributed for higher fresh and dry leaf and stem weight. Higher fresh and dry weight of leaf in the above treatment combinations was the result of higher number of leaves and branches per plant.

The spacing levels did not influenced the nitrogen and phosphorus content in stevia, but potash content was influenced significantly by different spacing levels (Table 5). Higher content of potash was observed at wider spacing than closer spacing levels and it could be due to higher biomass yield per plant basis. These results are in line with those of Kuntaldas *et al.* (7) in stevia. The differences in leaf and stem yield among different bio-fertilizer levels, which led to significant differences in biomass accumulation in top portion, could be attributed to differences in growth parameters such as plant height and its spread and number of leaves and branches per plant. The plant height (60.17 and 57.33 cm), number of branches per plant (64.23 and 65.94), plant spread (635.28 and 613.27) recorded at 90 DAP, increased significantly with application of 100% N+PK + *Azotobacter* and 100% N+PK + *Azospirillum* per hectare, respectively (Tables 1, 2 & 4). This might be due to the fact that *Azotobacter* and *Azospirillum* produces a variety of growth promoting substances like indole acetic acid, gibberellins, vitamin-B and antifungal substances. Another important characteristic of *Azotobacter* associated with crop improvement is excretion of

Table 1. Effect of spacing and biofertilizers on plant height in stevia.

Sub-treatment	30 DAP					60 DAP					90 DAP				
	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
F_1	23.33	21.83	22.58	25.08	23.73	37.34	39.17	33.33	25.08	37.17	48.83	50.83	51.25	51.17	50.52
F_2	23.33	23..91	24.08	29.83	25.14	39.00	37.84	35.50	29.83	38.37	50.00	48.50	51.00	51.58	50.27
F_3	26.50	23.33	26.58	25.83	27.85	41.17	47.17	44.83	25.83	44.35	53.00	70.00	63.00	54.67	60.17
F_4	23.50	32.50	24.08	25.18	24.08	37.17	36.67	40.50	25.17	38.95	45.50	45.17	58.00	50.67	49.83
F_5	22.08	23.58	23.750	25.83	25.25	35.67	45.83	41.33	25.83	41.12	50.00	67.83	58.67	52.83	57.33
F_6	21.83	29.33	21.17	22.00	21.58	32.50	32.67	29.83	22.00	31.25	44.67	41.50	44.83	40.50	42.87
Mean	23.43	21.33	23.71	25.67		37.14	39.89	37.56	39.57		48.67	53.98	54.60	50.24	
CD at 5% A	0.69					1.24					1.18				
B	0.67					0.38					0.84				
AxB	1.39					2.05					1.98				

S_1= 30 × 20 cm, S_2 = 30 × 30 cm, S_3 = 45 × 20 cm, S_4 = 45 × 30 cm, F_1 = 100% NPK (recommended dose-60:30:45 kg/ha), F_2 = 75% N + PK + Azoto, F_3 = 100% N + PK + Azoto, F_4 = 75% N + PK + Azosp, F_5 = 100% N + PK + Azosp, F_6 = control (no fertilizer) and NS = Non significant

Table 2. Effect of spacing and biofertilizers on number of branches in stevia.

Sub-treatment	30 DAP					60 DAP					90 DAP				
	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
F_1	27.50	29.50	30.67	30.83	29.63	46.00	50.67	44.83	51.50	48.25	53.83	69.00	58.50	63.50	61.21
F_2	26.50	31.17	31.33	30..33	29.76	49.00	56.33	45.17	52.50	50.75	53.83	70.03	59.91	64.00	61.95
F_3	25.83	45.50	32.00	31..50	33.71	50.33	61.00	49.33	55.50	54.04	56.16	75.97	60.33	64.50	64.23
F_4	23.50	35.00	35.67	32.50	31.54	50.67	51.50	48.50	54.83	51.38	57.83	68.08	62.16	66.00	63.52
F_5	23.00	41.83	36.67	34.33	33.83	52.17	59.00	45.83	55.50	53.12	60.16	73.03	63.50	67.00	65.94
F_6	20.83	22.17	24.67	25.67	23.33	39.17	40.17	41.17	44.41	41.23	49.33	50.33	52.07	54.00	51.43
Mean	24.53	34.19	31.62	30.86		47.89	53.11	45.81	52.38		55.19	67.75	59.41	63.17	
CD at 5% A	0.78						0.83				0.97				
B	0.59						0.68				1.24				
A × B	1.33						1.48				2.46				

S_1 = 30 × 20 cm, S_2 = 30 × 30 cm, S_3 = 45 × 20 cm, S_4 = 45 × 30 cm, F_1 = 100% NPK (recommended dose-60:30:45 kg/ha), F_2 = 75% N+PK+Azoto, F_3 = 100% N+PK+Azoto, F_4 = 75%N+PK+Azosp, F_5 = 100%N+PK+Azosp, F_6 = control (no fertilizer) and NS: Non significant

Table 3. Effect of spacing and bio-fertilizers on number of leaves (per plant) in stevia.

Sub-treatment	30 DAP					60 DAP					90 DAP				
	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
F_1	170.33	217.67	161.00	175.16	179.58	382.00	368.33	430.00	401.67	395.50	447.67	609.67	614.33	501.67	543.33
F_2	176.83	230.00	164.00	178.000	186.50	398.33	387.33	439.00	420.00	411.17	452.17	624.67	618.00	510.00	551.21
F_3	180.33	240.00	166.00	185.333	191.08	410.66	574.67	484.33	490.33	490.00	527.00	775.17	690.60	548.33	635.28
F_4	182.33	231.17	169.17	192.333	192.00	416.00	540.33	450.00	459.67	466.50	462.67	650.67	670.00	539.67	580.75
F_5	202.33	235.67	169.83	161.667	200.04	427.33	561.67	444.67	474.67	477.08	470.00	765.73	672.67	544.67	613.27
F_6	134.33	141.00	151.33		147.08	280.33	295.00	305.33	321.00	300.42	329.67	334.67	344.67	349.67	339.68
Mean	174.42	215.91	163.56	176.97		385.77	454.56	425.55	427.89		448.19	626.76	601.71	499.00	
CD at 5% A	3.567					7.839					22.627				
B	2.770					6.779					19.184				
A × B	6.16					14.60					41.56				

S_1 = 30 × 20 cm, S_2 = 30 × 30 cm, S_3 = 45 × 20 cm, S_4 = 45 × 30 cm, F_1 = 100% NPK (recommended dose-60:30:45 kg/ha), F_2 = 75% N+PK+Azoto, F_3 = 100% N+PK+Azoto, F_4 = 75% N+PK+Azosp, F_5 = 100% N+PK+Azosp, F_6 = control (no fertilizer) and NS: Non significant

ammonia in the rhizosphere in presence of root exudates. *Azotobacter* also produces fixation of thiamine, riboflavin, nicotine, indole acetic acid and gibberellins. When *Azospirillum* also produces growth promoting substances like indole acetic acid, gibberellins, pantothenic acid, thiamine and niacin and promotes root proliferation. It increases the rootlet density and root branching resulting in increased uptake of mineral and water. These substances improve the plant growth and yield. Increase in plant height with increased N levels was also reported by many workers. In a field trial with gundumalli (*Jasminum sambac*), Manonmani (8) observed that inoculation of *Azospirillum* along with application of nitrogenous fertilizer an increase in the plant height, number of tertiary branches, shoot and leaf area, dry weight, root bio-mass, flower weight and yield.

Azotobacter and *Azospirillum* supply is related to production of growth promoting substances which allows the plants to grow faster, increase rate of metabolism, cell division, cell elongation and thereby stimulated apical growth as well as formation of leaves. This is evident from the increased number of branches and leaves per plant with application of *Azotobacter* and *Azospirillum* levels at all the stages. Thus, it can be inferred that planting the stevia crop at 30 cm × 30 cm with 100% N+PK + *Azotobacter* (S_2F_3) and 100% N+PK + *Azospirillum* (S_2F_5) were equally superior in terms of growth parameters and soil nutrients.

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Table 4. Effect of spacing and biofertilizers on plant spread (cm) in stevia.

Sub-treatments	30 DAP					60 DAP					90 DAP				
	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
F_1	27.50	23.61	22.50	22.63	22.59	29.50	40.81	39.08	40.23	39.49	30.67	55.88	54.36	57.15	55.06
F_2	26.50	26.41	22.85	22.21	23.41	31.17	45.01	39.75	41.50	41.13	31.33	60.15	55.15	56.50	56.24
F_3	25.83	27.21	22.05	23.90	23.86	45.50	51.33	40.25	41.76	43.09	32.00	66.13	55.33	57.38	58.25
F_4	23.50	26.36	22.15	23.25	23.54	35.00	46.46	40.91	41.85	42.37	35.67	61.40	56.00	56.96	57.39
F_5	23.00	26.90	23.35	23.48	24.06	41.83	47.50	40.90	42.43	42.84	36.67	62.48	56.76	57.35	58.03
F_6	20.83	23.33	22.80	21.63	22.56	22.17	40.46	42.53	43.06	41.57	24.67	55.61	57.56	53.03	55.48
mean	22.21	25.64	22.62	22.63		37.85	40.82	40.57	41.81		54.43	60.28	55.86	56.39	
CD at 5%	0.645					0.16					0.34				
b	0.39					0.18					0.34				
A × B	0.97					0.37					0.70				

S_1 = 30 × 20 cm, S_2 = 30 × 30 cm, S_3 = 45 × 20 cm, S_4 = 45 × 30 cm, F_1 = 100% NPK (recommended dose-60:30:45 kg/ha), F_2 = 75% N+PK+Azoto, F_3 = 100% N+PK+Azoto, F_4 = 75% N+PK+Azosp, F_5 = 100% N+PK+Azosp, F_6 = control (no fertilizer) and NS: Non significant

Table 5. Effect of spacing and bio-fertilizers on NPK content (%) in stevia at pre-flowering stage.

Sub-treatment	N					P					K				
	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
F_1	1.34	1.33	1.32	1.33	1.33	0.065	0.066	0.066	0.061	0.064	1.25	1.23	1.23	1.25	1.24
F_2	1.36	1.35	1.38	1.39	1.37	0.070	0.069	0.069	0.068	0.069	1.28	1.30	1.27	1.28	1.28
F_3	1.62	1.38	1.64	1.59	1.56	0.072	0.075	0.075	0.073	0.074	1.49	1.53	1.30	1.36	1.42
F_4	1.33	1.60	1.33	1.28	1.39	0.068	0.070	0.070	0.069	0.069	1.31	1.29	1.53	1.48	1.40
F_5	1.56	1.50	1.49	1.53	1.52	0.066	0.069	0.069	0.072	0.069	1.40	1.30	1.34	1.36	1.35
F_6	1.28	1.30	1.31	1.30	1.29	0.057	0.060	0.060	0.052	0.056	1.21	1.42	1.43	1.38	1.35
Mean	1.42	1.41	1.41	1.40		0.067	0.066	0.068	0.066		1.32	1.35	1.35	136	
CD at 5% A	NS					NS					0.01				
B	0.019					0.004					0.403				
A × B	0.043					NS					0.074				

S_1 = 30 × 20 cm, S2 = 30 × 30 cm, S_3 = 45 × 20 cm, S_4 = 45 × 30 cm, F_1= 100% NPK (recommended dose-60:30:45 kg/ha), F_2 = 75% N+PK+Azoto, F_3 = 100% N+PK+Azoto, F_4 = 75% N+PK+Azosp, F_5 = 100% N+PK+Azosp, F_6 = control (no fertilizer) and NS: Non significant

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