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Influence of crop geometry on yield, yield attributes and glycoside yield of *Stevia rebaudiana* Bertoni

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Abstract: Field experiments were conducted in a loamy sand soil during 2006 and 2007 at Punjab Agriculture University, Ludhiana to evaluate the performance of *Stevia rebaudiana* Bertoni under varying planting geometry. The studies revealed that in case of row to row spacing, highest number of leaves per plant (533.0, 447.6), leaf area (8113.1, 6322.6 cm^2/plant), leaf area index (5.1, 8.7) and dry matter accumulation per plant (86.0, 76.9 g/plant) during 2006 and 2007, respectively were found under wider row spacing of 75 cm. Which was significantly higher than narrower row spacing of 60 cm and 45 cm. Whereas, the fresh biomass yield (9861 and 11801 kg/ha), dry biomass (2080 and 2550 kg/ha), leaf yield (6129 and 4414 kg/ha) and stem yields (7611 and 5447 kg/ha) during 2006 and 2007, respectively and glycoside yield were registered higher under closer row spacing of 45 cm than the wider row spacing of 60 cm and 75 cm. In plant to plant spacings, the maximum number of leaves (5681.3 cm^2/plant) was recorded under plant spacing of 45 cm which was statistically at par with plants spaced at 37.5 cm and 30.0 cm but significantly higher than 22.5 cm and 15.0 cm plant spacing, leaf area and dry matter accumulation per plant were highest with than other closer plant spacings.Whereas, the fresh and dry biomass, leaf and stem yields and glycoside yield were recorded highest under closer plant spacing of 15 cm which was statistically at par with 22.5 cm plant spacing.

Keywords: Glycoside yield, Spacing Yield, Stevia

INTRODUCTION

Stevia (*stevia rebaudiana* Bertoni) is an important zero -calorie natural sweetener plant belonging to the family Asteraceae. It is grown as a crop in many countries including Japan, China, India, Korea, USA, Canada, Mexico, Russia, Indonesia, Tanzania, Brazil, Paraguay, Canada and Argentina (Ramesh *et al*., 2007). The leaves of stevia have commercial importance due to the presence of non-caloric diterpenes and sweet glycosides, especially stevioside and rebaudioside - A which are ~300 times sweeter than sugar without any side -effects. . It is of immense value due to its adaptability to wide climatic range, the high sweet content and its significant contribution to the welfare of human life. This offers a solution for complex diabetic problems and obesity in human life being calorie free. Stevia derives its sweetness from diterpene glycosides, eight in number (steviol glycosides). Among eight steviol glycosides, the principal glycosides are stevioside and rebaudioside-A. The worldwide demand for high potency sweeteners with low energy is increasing day -by-day because of considerable increase in the diabetic patients and change in life style i.e."slim is beautiful". Presently people are using low calorie table-top sweeteners such as Saccharin, Aspartame, Asulfam-K etc, which are called as "nutritional terrorists". These are not only expensive but have bitter after taste and side effects. Under such situation the demand for sweetener produced from stevia which is known as natural non- calorie sweetener, has great scope in the national and international markets (Anonymous 2005). Steviol glycoside content in stevia leaves greatly depends on the package of practices for cultivation of stevia (Kumar *et al*., 2012; Kumar *et al*., 2013). Stevia is still a plant of recent domestication in India. If the cultivation of stevia is standardized, it may replace the table top sweeteners. There is, however, a dearth of information on the influence of spacing and organic mulch on stevia. Therefore, field experiments were conducted to evaluate the effect of spacings on growth, yield and quality of *Stevia rebaudiana* Bertoni

MATERIALS AND METHODS

Field experiments were conducted during 2006 and 2007 at the Student's Research Farm of the Department of Agronomy, Punjab Agricultural University, Ludhiana (247 amsl, 30^0 56` N latitude, $75^0$ 52` E longitude),

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Punjab, India. It has sub-tropical semi-arid type of climate with hot summer and very cold winters. The mean monthly temperature during growing seasons ranged between 16.8^{0}C and 39.0^{0}C in 2006 and between 11.3^{0}C and 34.2^{0}C in 2007. Maximum temperature being in the month of May and minimum in January during 2006 whereas the maximum was in June and minimum in January during 2007. The physic-chemical analysis of the soil was done by collecting soil samples from 4-5 randomly selected spots in the experimental fields before the conduct of the experiments. Soil at the experimental field was loamy sand in texture with organic carbon content (0.28 and 0.33 %) and pH of 7.9 and 7.32, respectively in the years 2006/2007. The available nitrogen in the field was (200 and 263 kg/ha), available phosphorus was (21.2 and 15.4 kg/ha) and available potash was (235 and 150 kg/ha) during 2006 and 2007, respectively. Mechanical analysis was done with the international pipette method (Piper, 1966). pH was determined with the help of Beckman's glass electronic pH meter in 1:2 soil: water suspension (Jackson, 1967), organic carbon was analyzed by Walkley and Black method (Piper, 1966), Available Nitrogen was analyzed by Modified alkaline permanganate method (Subbiah and Asija ,1956), Available Phosphorus by 0.5 M $NaHCO_3$ extractable method (Olsen *et al*, 1954) and Available potassium by Ammonium acetate K method (Jackson, 1967). The experiment was laid out in completely Randomized Block Design under factorial arrangement with fifteen combinations of three row to row spacings (45.0 cm, 60.0 cm and 75.0 cm) and five plant to plant spacings (15.0 cm, 22.5 cm, 30.0 cm, 37.5 cm and 45.0 cm) with four replications. The nursery was sown on 30th January during 2006 and on 25th January during 2007. The field was prepared by giving preparatory tillage with disc harrow twice. After pre-sowing irrigation a fine seed bed was prepared by giving two ploughings with tractor- drawn cultivator followed by planking each time during both the years. Farm yard manure was applied at the rate of 30 t/ha on 50% moisture basis by spreading the material uniformly few days before transplanting of the crop. Three month old seedlings were transplanted on ridge and furrow method and the spacing was kept as per treatment. Basal dose of nitrogen, phosphorus and potassium at the rate of 40 Kg/ha each were applied through urea, DAP and muriate of potash, respectively. Half the dose of nitrogen and full dose of phosphorus and potassium were applied at the time of transplanting. The crop was irrigated immediately after transplanting of the crop. Thereafter light and frequent irrigations were given as per requirement of the crop. In order to maintain the plant stand gap filling was done 20 DAT during both the years. Weed control was done by hand weeding whenever necessary and plant protection measures were taken as and when required. Chloropyriphos 20 EC at 5 litres per hectare was applied along with irrigation to control termites and Indofil at 0.3% (30 g in 10 litres of water) was sprayed to control the leaf spot disease caused by *Septoria steviae.* The crop was harvested manually with the help of sickle and the plants were cut 15 cm above the ground level.

Observations were recorded for yield attributes viz., number of branches per plant, number of leaves per plant, leaf area, leaf area index, leaf : stem ratio for each replication. Leaf area per plant was calculated by measuring leaf area of two randomly selected plants from each plot with the help of leaf area meter. The leaves graded in categories of small, medium and large. The leaves from each category were counted and area of a representative leaf from each plot was measured and it was multiplied by number of leaves of each category. Then mean was taken to calculate leaf area per plant. The leaf area was used to compute leaf area index. Leaf area index was calculated by dividing leaf area by respective ground area. Fresh biomass and dry biomass yield were recorded at the time of harvesting. Statistical analysis were done by Cochran and Cox (1967) and adapted by Cheema and Singh (1991) in statistical package CPCS-1. All the comparisons were made at 5 per cent level of significance.

RESULTS AND DISCUSSION

Effect of crop geometry on yield attributes:

Number of branches per plant: The number of branches is a good parameter which determines the crop yield. The perusal of the data evinced that there was increase in number of branches per plant with each increase in row spacings from 45 to 75 cm. The crop planted in rows at 75 cm apart produced the maximum number of branches per plant (18.97 and 17.33 during 2006 and 2007, respectively) which was statistically at par (17.96 and 16.42 during 2006 and 2007, respecively) with plants spaced at 60 cm apart but significantly higher than 45 cm row spacing. In plant to plant spacing, number of branches per plant was not significantly influenced by different plant spacings. However, the highest number of branches per plant was observed with wider plant spacing of 45 cm. Higher number of branches per plant at wider spacing could be due to low planting density and less interplant competition resulting in more metabolic activities through proper utilization of moisture, light and nutrient etc which in turn increased the number of branches per plant.

Number of leaves per plant: The green leaves are important plant growth indices, determining the capacity of plant to trap solar energy for photosynthesis. Row spacing of 75 cm observed highest leaves count (533.0) per plant which was significantly higher than 60 cm and 45 cm row spacing during the first year of experimentation. Whereas during second year, the highest leaves count (447.6) per plant was accrued with row spacing of 75 cm which was statistically at par with 60 cm row spacing but significantly higher than 45 cm row spacing.

Leaves count increased significantly with increase in

Table 1. Effect of crop geometry on yield attributes of *Stevia rebaudiana* Bertoni.

Treatment	No. of branches/plant		No. of leaves/plant		Leaf Area		Leaf Area Index		Dry Matter/plant		Leaf: Stem	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Row to Row Spacing (cm)												
45	16.25	14.95	275.3	241.4	3260.9	3107.8	3.9	5.7	74.7	62.7	0.620	0.560
60	17.96	16.42	362.5	426.8	4676.9	5735.0	4.3	6.7	79.1	69.0	0.623	0.597
75	18.97	17.33	533.0	447.6	8113.1	6322.6	5.1	8.7	86.0	76.9	0.631	0.599
Plant to Plant Spacing (cm)												
15.0	16.52	15.13	334.5	330.8	4319.0	4429.7	4.1	3.4	52.8	58.5	0.616	0.560
22.5	17.54	15.73	375.8	344.7	5098.1	4577.8	4.4	5.1	73.9	70.3	0.624	0.595
30.0	17.70	16.55	388.4	382.0	5376.8	5316.9	4.5	7.0	74.0	70.8	0.624	0.596
37.5	17.91	16.77	426.3	391.6	5903.7	5333.0	4.5	8.8	97.8	74.1	0.628	0.597
45.0	18.95	17.00	426.9	411.0	6054.1	5618.3	4.6	10.7	101.4	74.6	0.635	0.598

plant spacing only up to 37.5 cm whereas with further increase in plant spacing up to 45 cm the leaves count increased with non-significant differences and both the spacings produced higher number of leaves per plant than other closer spacings during 2006. Whereas during 2007, the maximum number of leaves per plant (411.0) was recorded with plants spaced at 45 cm which was statistically at par with 37.5 cm and 30.0 cm plant spacing but significantly higher than 22.5 cm and 15 cm plant spacing. The yield attributing characters like number of branches per plant and number of leaves per plant Table 1 were higher under wider spacing. The reason for increase in yield attributing characters may be that the wider spacings result in less inter-plant competition for space, nutrient, light and moisture which in turn might have increased the photosynthetic and metabolic activity of the plant.

Leaf area: Leaf area is governed by number of leaves per plant and size of leaves and it plays a vital role for plant growth and crop yield. In general, more of the leaf area more is the contribution of the leaf to the yield, as leaf is the economic part of the stevia crop. The leaf area, being the most important yield determinant and was greatly influenced by different row to row spacings. The leaf area produced during both the years with row spacing of 75 cm was significantly superior than other closer row spacings of 60 cm and 45 cm .The leaf area produced with 75 cm row spacing gave 148.8, 73.4 and 103.4, 102 per cent higher than 60 cm and 45 cm row spacing during 2006 and 2007, respectively. Data in the Table 1 further revealed that leaf area increased significantly with the increase in plant spacing from 15.0 cm to 45.0 cm. During 2006, the highest leaf area (6054.1 cm^2/plant) was recorded with 45.0 cm plant spacing which was statistically at par (5903.7 cm^2 /plant) with plants spaced at 37.5 cm apart but significantly higher than other closer plant spacing of 30.0 cm, 22.5 cm and 15.0 cm apart. During 2007, the leaf area increased with increase in plant spacing significantly up to 37.5 cm and with further increase in plant spacing to 45.0 cm the leaf area increased with non-significant differences. However, the maximum leaf area (5618.3cm^2/plant) was recorded under plant spacing of 45.0 cm which was statistically at par with plants spaced at 37.5 cm (5333.0cm^2/plant) and 30.0 cm (5316.9cm^2/plant) but significantly higher than 22.5 cm and 15.0 cm plant spacing. Increase in leaf area with increase in spacing might be due to the fact that with increase in spacing there was more space for growth of the plant as compared to closer spacing.

Leaf area index: Leaf area index is the ratio of leaf area to ground area. Maximum leaf area is obtained when sufficient amount of sunlight is just able to pass through the canopy. The different row to row spacing influenced the leaf area index significantly at all the growth stages during both the years. The maximum leaf area index was recorded at row spacing of 75 cm (1.2 and 1.1during 2006 and 2007, respectively) which

Table 2. Influence of crop geometry on yield and quality of *Stevia rebaudiana* Bertoni.

Treatment	Dry Leaf Yield (Kg/ha)		Total Biomass Yield (Kg/ha)		Total stevioside & rebaudioside-A Content (%)	
	2006	2007	2006	2007	2006	2007
Row to Row Spacing (cm)						
45	1458	1060	3019	2080	6.3	7.1
60	822	947	1739	1851	7.6	7.5
75	737	843	1545	1646	7.1	7.0
Plant to Plant Spacing (cm)						
15.0	1195	1204	2502	2358	7.6	6.9
22.5	1153	1035	2405	2028	6.5	8.8
30.0	949	903	1999	1766	6.9	6.6
37.5	902	811	1870	1585	7.7	9.2
45.0	830	797	1729	1558	6.3	6.7

was closely followed by leaf area index recorded at row spacing of 60 cm and 45 cm. the leaf area index recorded at row spacing of 75 cm apart was 33.3, 16.6 and 22.2, 22.2 per cent higher over 45 cm and 60 cm row spacings during 2006 and 2007, respectively.

Similarly different plant to plant spacing had significant effect on leaf area index throughout the crop growth period during both the years except at harvest during first year. The highest values for leaf area index recorded at harvest during second year were under plant spacing of 45.0 cm which was 241.7, 109.8, 52.8 and 23.0 per cent higher over plant spacing of 15.0 cm, 22.5 cm, 30.0 cm and 37.5 cm, respectively. In the first year, there was no significant difference in leaf area index was observed with each increase in plant spacing from 15.0 cm to 45.0 cm and numerically higher leaf area index was recorded at plant spacing of 45.0 cm

Leaf stem ratio: Different treatments did not significantly affect leaf stem ratio during both the years of experimentation. However, the highest leaf to stem ratio (0.629 and 0.599 during 2006 and 2007, respectively) was recorded with row spacing of 75 cm which was marginally higher than other closer row spacing of 60 cm and 45 cm. These results are in conformity with R. Kumar *et al.,* 2012.

The perusal of the data in Table 1 further reflected that different plant spacing did not affect the leaf:stem ratio. Highest leaf to stem ratio (0.635 and 0.589 during 2006 and 2007, respectively) was observed when plants were spaced at 45.0 cm apart which was followed by plants spaced at 30.0 cm, 37.5 cm, 22.5 and 15.0 cm apart.

Dry matter accumulation: Dry matter production serves a reliable measure of the relative influence of different treatments on the plant growth and ultimately the crop yield. Data presented in Table 1 on dry matter accumulation by the crop indicated that the dry matter tended to increase with increase in row spacing from 45 cm to 75 cm. The crop planted at 75 cm apart in rows obtained significantly higher dry matter than row spacing of 60 cm and 45 cm during both the years. The highest dry matter (86.0 and 76.9 g/plant) was obtained with row spacing of 75 cm apart which was significantly higher than other row spacing of 60 cm and 45 cm during both the years. The increase in dry matter produced under 75 cm row spacing was 15.1, 22.6, 8.7 and 11.4 per cent higher than 45 cm and 60 cm row spacing during 2006 and 2007, respectively.

The increase in plant spacings significantly increased the dry matter per plant. Data in the Table 1 showed that during the first year, dry matter produced at plant spacing of 45.0 cm was statistically at par with plant spacing of 37.5 cm but increase was significantly higher than other closer plant spacing. In the second year, highest dry matter per plant (74.6 g/plant) recorded at 45.0 cm was statistically at par with plants spaced at 37.5 cm, 30.0 cm and 22.5 cm but significantly higher than 15.0 cm. Plant geometry helps to modify the microclimate. Narrow row spacing results in higher leaf photosynthesis (Dwyer *et al.* 1991). Adjusting plant geometry to narrow row spacing results in higher radiation use efficiency which further contributes to higher dry matter yield (Tollenear and Aguilera 1992).

Effect of crop geometry on yield: The sweetness in stevia is attributed to ent-kaurene diterpene glycosides (stevioside and rebaudiosides) which are water soluble (Duke and de Cellier 1993) and are 300 times sweeter than cane sugar. Because of this, stevia is considered to provide no calories (much like the artificial sweeteners saccharin and aspartame). Stevioside and Rebaudioside -A are the important constituents which determines the quality of the crop and are extracted from dried leaves in the form of a white amorphous powder. The leaf extract of this plant has anti-diabetic properties (Mishra *et al.* 2011).

Dry biomass yield: Data on dry biomass presented in table 2 amply purported that the dry biomass yield was significantly affected by different row spacings during both the years. In the first year of experiment, highest biomass yield (3019 kg/ha) was produced with crop planted at 45 cm row spacing which was significantly higher than 60 cm and 75 cm row spacings. whereas, during second year, the data showed that the biomass produced by the crop planted at 45 cm and 60 cm apart in rows were statistically at par with each other but significantly higher than the row spacing of 75 cm.

Ramesh *et al* 2007 reported that a spacing of 45 cm x 45 cm recorded higher fresh and dry biomass yield than 60 cm x 45 cm. Higher biomass yield under closer spacing could be due to higher plant population.

Plant spacing of 15.0 cm produced highest (2502 kg/ha) dry biomass which was statistically at par with 22.5 cm and 30.0 cm plant spacing but significantly superior to other plant spacing of 37.5 cm and 45.0 cm during 2006. Whereas in the year 2007, highest (2357 kg/ha) dry biomass registered with plants spaced at 15.0 cm apart was significantly higher than all other wider spacings. The increase in dry biomass yield under plant spacing of 15.0 cm was 16.22, 33.46, 48.70 and 51.28 % higher over plant spacing of 22.5 cm, 30.0 cm, 37.5 cm and 45.0 cm

Dry leaf yield: Leaf is the main economic part of stevia plant hence production of more leaf biomass is the main criteria for crop performance. Data presented in Table 2 revealed that significant differences were observed for dry leaf yield with different row to row spacing. During Ist year, the highest leaf yield (1458 kg/ha) was produced with 45 cm row spacing which was significantly higher than 60 cm and 75 cm row spacing. During second year, 45 cm row spacing was again superior over the others and produced the highest dry leaf yield (1060 kg/ha) which was statistically at par with row spacing of 60 cm (947 kg/ha) but significantly higher than 75 cm row spacing. The results are in conformity with the results of (Donalisio *et al* 1982).The lower yield at wider spacings was mainly due to the fact that decreased plant population with increase in spacings. Data in table 2 further opined that maximum dry leaf yield (1195 kg/ha) was registered at 15 cm plant spacing which was statistically at par with 22.5 cm and 30 cm plant spacing (only 3.6 and 25.9 per cent reduction). Similarly during 2007, highest dry leaf yield (1204 kg/ha) was recorded at 15 cm plant spacing which was significantly superior over all other wider plant spaccings of 22.5 cm, 30.0 cm, 37.5 cm and 45.0 cm. The increase in dry leaf yield with closer spacing are in agreement with that of Murayama *et al.,* (1980).

Effect of crop geometry on quality: A perusal of the data presented in Table 2 manifested that the stevioside and rebaudioside-A content recorded highest (7.6%) with row spacing of 60 cm which was followed by 75 cm and the lowest (6.3%) was found with 45 cm row spacing. However, the stevioside and rebaudioside –A yield registered with 45 cm row spacing may be due to higher dry leaf yield.

Perusal of the data Table 2 further evinced that the highest stevioside and rebaudioside-A content (7.7% and 9.2% during 2006 and 2007, respectively) was obtained with plants spaced at 37.5 cm which gave 1.3, 18.5, 11.6 and 33.3, 45.5, 39.4 per cent higher over 15 cm , 22.5 cm and 30 cm spacing during 2006 and 2007, respectively.

Conclusion

As concluded that planting Stevia with a row spacing of 45.0 cm and plant spacing of 22.5 cm yielded higher dry leaf weight and glycoside yield. The farming community may obtain additional benefit by adopting stevia in intercropping with cereals, oilseed, or pulses. Further stevia being a new introduction to India, several confirmatory agronomic practices to enhance dry leaf yields are required to be under taken under varied agro-climatic situations

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