

# Effects of Planting Density and Harvesting Time on Productivity of Natural Sweetener Plant (*Stevia rebaudiana* Bertoni.) in Larache Region, Morocco

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**Abstract** *Stevia* (*Stevia rebaudiana* Bertoni) has achieved economic importance due to the different natural steviol glycosides (SG) which are obtained from its leaves which are many times sweeter than sugarcane and sugarbeet. The productivity of stevia depends on many agronomical factors such as planting density. A field experiment was conducted from 10th May to 23rd October 2014 in a sandy soil under irrigated conditions to study the effect of plant spacing D1 (70 cm x 30 cm), D2 (70 cm x 20 cm), and D3 (70 cm x 10 cm) on growth, yield, and quality of stevia. Spacing significantly affected plant height, stem diameter, fresh biomass yield, fresh and dry leaf yield, stevioside (STV), rebaudioside A (Reb A), and total SG yield in all the harvests. Higher plant height (79.67 cm) and stem diameter (6.73 mm) were obtained in the wider spacing D1 as compared to closer spacing D3 (65.00 cm and 6.09 mm, respectively) in first harvest (95 days after transplanting). While, highest fresh biomass, dry leaf and total SG yields (cumulative of two harvests) were obtained with D3 (37.23, 4.95, and 0.69 t ha<sup>-1</sup>, respectively). SG contents were not significantly affected by planting density. However, higher STV and total SG contents were obtained with closer plant spacing D3 (11.27 and 17.20%, respectively) in the first harvest. At the second harvest (72 days after first harvest) significantly lower dry leaf and SG yields were obtained in all the 3 planting densities. The study revealed that under Larache, Moroccan conditions, the higher dry leaf yield and better quality of stevia were obtained in the narrow spacing when plants were harvested during summer season.

**Keywords** *Stevia*, Density, Harvesting time, Steviol glycosides, Dry leaf yield, Morocco

## 1. Introduction

*Stevia* (*Stevia rebaudiana* Bertoni) has achieved economic importance due to the different natural sweet compounds called steviol glycosides (SG) which are obtained from its leaves with taste sweet but with zero calories. Hence, SG can be used as a natural alternative to artificial sweeteners viz., saccharine, aspartame, asulfam-K, sucralose that are available in the market to the diet of obese and diabetic people (Yadav *et al.*, 2011; Aladakatti *et al.*, 2012). The main sweet components in stevia dry leaves are stevioside (STV) (4–13%) which is about 200-300 times sweeter than sucrose (Crammer and Ikan, 2003) and rebaudioside A (Reb A) (2–6%), with Reb B and F, as well

as dulcoside A, as minor SG (Tavarini and Angelini, 2013). Cultivation of stevia made significant impact in countries like Japan, China, Brazil, Paraguay, Mexico, Russia, Indonesia, Korea, United States, India, Tanzania, Canada, and Argentina (Brandel *et al.*, 1998; Pal *et al.*, 2015). Though China is the largest stevia producer in the world market, Japan and Korea are the main consumers (Pal *et al.*, 2015). SG content in stevia leaves greatly depends on the package of agronomical factors of stevia such as planting densities (Kumar *et al.*, 2012a; Kumar *et al.*, 2013).

Planting density is an important factor for higher production and gives equal opportunity to plants for their survival and best use of other inputs. Spacing has critical effects on quantitative and qualitative characteristics of plants (Badi *et al.*, 2004). For stevia, several authors reported that spacing significantly affected the yield attributes and dry leaf biomass (Murayama *et al.*, 1980; Basuki and Sumaryono, 1990; Ramesh *et al.*, 2006; Taleie *et al.*, 2012; Kumar *et al.*, 2014b). In Japan plant densities from 40000 to 400000 ha<sup>-1</sup>

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were tried and it was found that the leaf yield increased with increasing density up to 83000 to 111000 plants ha<sup>-1</sup> (Katayama *et al.*, 1976). In similar, Donalisio *et al.* (1982) had recommended a plant population of 80000-100000 ha<sup>-1</sup>, and harvested four ratoon crops in a year. Field experiment conducted at Bangalore, India during summer season on red sandy clay loam soil showed that plant density up to 96618 plants ha<sup>-1</sup> produced also higher dry leaf yield and STV yield (Chalapathi *et al.*, 1997). While, Colombus (1997) recommended high densities up to 160000 ha<sup>-1</sup> for higher yields in Canada. Aladakatti *et al.* (2012) reported that closer spacing of 30 cm x 20 cm resulted in higher plant height and dry leaf yield which was comparable with 45 cm x 30 cm. Also, Kumar *et al.* (2012b) have reported that annual crop of stevia under mid hill conditions of north-western Himalayas recorded significantly higher leaf dry biomass at narrow spacing. Narrow spacing may also result in better control of weeds. By contrast, Lee *et al.* (1980) reported that plant height, number of branches and number of nodes plant<sup>-1</sup> were unaffected by a planting geometry of 50-70 cm x 10-30 cm. Kumar *et al.* (2014a) reported also that SG were not significantly affected by different treatments. However, Angkapradipta *et al.* (1986) found that a narrow spacing of 25 cm x 25 cm was not optimum considering the root spread of the crop. While, Gvasaliya *et al.* (1990) obtained the highest stevia yield with a wider spacing at Abkhazia. In addition, some studies reported that stevia dry leaf weight was highest in the wider spacing than in the narrow spacing (Filho *et al.*, 1997; Singh and Kaul, 2005; Kumar *et al.*, 2013). Hence, optimal plant density for stevia varies considerably depending upon climatic conditions of the growing area and fertility status of the soil.

Under north- western Moroccan conditions, the effect of planting density on growth, yield, and quality parameters for annual crop of stevia has not been yet reported. Therefore, the present research was undertaken to determine the optimal planting density for stevia to get higher dry leaf yield and SG content (%) and their accumulation (t ha<sup>-1</sup>).

## 2. Material and Methods

### 2.1. Experimental Site

The trial was performed from 10th May to 23rd October, 2014 in the Experimental Station of Larache (35.11 N, 6.40 E, 6°07'W, 47 m above mean sea-level) which belongs to Regional Centre of Agronomic Research (RCAR) of Tangier in Morocco (INRA). The climate is Mediterranean sub humid, with mean maximum temperature of 27.9 °C in August and mean minimum of 14.9 °C in May, and the total rainfall received during the crop growth season was 481mm (Table 1). The soil of the experimental site was collected from 0 to 30 cm soil depth before commencement of the experiment and was analysed in laboratory of Research unit on Environment and Conservation of Natural Resources INRA, RCAR of Rabat. The soil contained 1.1% clay, 12.9 silt, and 86% sand. The organic matter content was 2.1 %, the pH was 6.6 and the P and K contents were 97.7 and 48.2 ppm, respectively. Soil moisture at field capacity was 8.3%. No mineral fertilization was applied for correcting soil. Soil properties and climatic data indicate the range of conditions of our trial.

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**Table 1.** Meteorological conditions from transplanting to harvest of stevia

Month	Mean temperature	Mean maximum temperature	Mean minimum temperature	Precipitation
	°C	°C	°C	(mm month <sup>-1</sup> )
May	20.6	26.1	14.9	29
June	21.5	26.4	16.7	17
July	22.4	26.6	18.4	0
August	22.9	27.9	17.9	0
September	22.3	26.6	18.1	7
October	22.5	27.7	17.3	34

### 2.2. Experimental Details

The INRA variety is tested. The sowing was performed into plug trays filled with land and commercial substrate on March 1<sup>st</sup>, 2014 and watered to field capacity (FC) by tap water. The experiment consisted planting densities of D1 (70cm x 30cm), D2 (70cm x 20 cm), and D3 (70 cm x 10 cm) with a plant population of 47619, 71428, and 142857 plants ha<sup>-1</sup>, respectively. The experiment was laid out in a randomized complete block design with three replicates. 71 days old stevia seedlings were transplanted at respective spacing on May 10th, 2014. The experimental unit was defined as a plot size of 6 m x 5 m. Irrigation was applied with a system of drip irrigation which functioned according to the soil moisture conditions. Drip irrigation consisted of a line of tape with a flow of 2 L h<sup>-1</sup>. Between the main line and the irrigation lines interpolated a manual valve for controlling the flow in the system. All main plots were separated by a 2 m wide alley. Gap filling with planting new seedling was undertaken after 14 days of transplanting to maintain required plant. First crop was harvested at 95 days after transplanting on August 11, 2014, whereas the second crop was harvested at 72 days after first cutting on October 23, 2014.

### 2.3. Measurements

Plant height and stem diameter were measured for five selected plants in the two center rows of each plot, just before harvest of the crop. The plant height was measured with a meter ruler from ground to the base of the fully opened leaf and the stem diameter was measured with slide calipers up to 0.01 mm accuracy. For each plot, the average of the 5 plant height measurements was used for further data analysis.

Biomass yield (total fresh leaf and stem yield), fresh leaf yield, and dry leaf yield were determined in each harvest. The whole stevia plants in one center 6-m-long row of each plot were cut manually 10 cm above the base of the stem (Megeji *et al.*, 2005), just prior to flowering when steviol

glycoside (SG) contents in the leaves is maximum (Serfaty *et al.*, 2013; Guzman, 2010). Leaves and stems were separated from all stevia plants on the day of harvest and fresh and dry leaf yield was recorded as total biomass yield and expressed in  $t\ ha^{-1}$ . Leaves were dried at 50°C temperature in hot air dryer for 6 hours and stored in clean gunny bags. At this temperature, the quality of dried leaves produced in terms of colour, sweetness, and nutrient content was better compared with drying at 70°C (Samsudin and Aziz, 2013). Then, dry leaves were ground in a laboratory grinding mill to produce powder particles of 0.10 mm in size, and were kept at ambient temperature until they were used for the analysis to assess the contents of stevioside (STV), rebaudioside A (Reb A), and total SG (STV; Reb, A, B, C, D and F; steviolbioside; rubudioside, and dulcoside A) as influenced by planting density. STV (%), Reb A (%), and total SG (%) were determined in the powdered stevia leaves sent to the STEVIA NATURA Company of France. The SG yield was calculated by multiplying dry leaves yield by the concentration of SG in leaves.

## 2.4. Statistical Analysis

Data were processed using analysis of variance (ANOVA). When the hypothesis of equal means is rejected, homogeneous groups of planting densities were compared using the Least Significant Difference (LSD) at the 0.05 significance level. Statistical Analysis System software ver. 9.1 (SAS Institute Inc., Cary, NC., USA) was used.

# 3. Results

## 3.1. Growth Parameters

Planting density had significant effect on plant height and stem diameter in each harvest and in mean values (Table 2). Wider spacing D1 (70 cm x 30 cm) recorded significantly higher plant height (62.17 cm) as compared to closer spacing of D2 (70 cm x 20 cm) (53.83 cm) and D3 (70 cm x 10 cm) (52.00 cm) in mean values. Stem diameter showed the same trend as plant height. Mean values revealed that planting density D1 recorded significantly higher stem diameter (5.27 mm), followed by planting density D2 (4.86 mm). Lower stem diameter was recorded with planting density D3 (4.57 mm). The plant height and stem diameter in each harvest varied significantly due to planting density which followed the similar trend as in mean values. At first harvest, significantly higher plant height and stem diameter were obtained. However, for the second harvest, lower plant height and stem diameter were found in all the 3 planting densities.

## 3.2. Yield Parameters

Different planting densities resulted in significant variation with respect to fresh biomass yield, fresh leaf yield, and dry leaf yield in all harvests as well as in cumulative total (Table 3). Planting density D3 (70 cm x 10 cm) with a plant

population of 142857 plants  $ha^{-1}$  accumulated 35.24% and 50.20% more total fresh biomass than planting densities D2 (70 cm x 20 cm) with a plant population of 71428 plants  $ha^{-1}$  and D1 (70 cm x 30 cm) with a plant population of 47619 plants  $ha^{-1}$ , respectively. Similar trend of fresh biomass yield was observed in each harvest. Fresh leaf yield and dry leaf yield were significantly influenced by the planting density in each harvest and in cumulative total. Data on cumulative total fresh and dry leaf yields revealed that planting density D3 recorded higher fresh leaf yield (18.69  $t\ ha^{-1}$ ) and dry leaf yield (4.95  $t\ ha^{-1}$ ) which was followed by planting density D2 (12.07 and 3.33  $t\ ha^{-1}$ , respectively). The planting density D1 resulted in significantly lower fresh leaf yield and dry leaf yield of 9.25 and 2.82  $t\ ha^{-1}$ , respectively. In addition, the results showed that yield parameters of stevia were influenced by time of harvest. The greater fresh biomass yield, fresh, and dry leaf yield were obtained at the first harvest compared with the second harvest in all treatments.

## 3.3. Steviol Glycoside Content

Total steviol glycoside (SG) content of dry leaves did not change due to different planting densities at both harvests (Table 4). Marginally higher SG content was obtained with closer plant spacing D3 (70 cm x 10 cm) (17.20 and 8.10%), closely followed by D2 (16.00 and 7.57%) and D1 (14.57 and 6.97%) in the first and second harvests, respectively. But in mean values, the total SG content was significantly influenced by the planting density, the highest value of total SG content was obtained with the planting density D3 (12.65%), closely followed by D2 (11.78%), while the lowest value was obtained with the planting density D1 (10.77%). STV content values showed the same trend as total SG content. STV content in dry leaves was not significantly influenced by the planting density in both harvests. However, marginally higher STV content was obtained with planting density D3 (11.27 and 4.67%), closely followed by D1 (9.10 and 4.17 %) and D2 (8.63 and 3.80%) in the first and second harvests, respectively. While in mean values, the STV content was significantly influenced by the planting density, the highest value of STV content was obtained with the planting density D3 (7.97%), while the lowest value was obtained with the planting density D2 (6.22%). By contrast, Reb A content was significantly influenced by the planting density in the first harvest. However, higher Reb A content was obtained with the planting density D2 (4.93, 1.97, and 3.45%) which was followed by D3 (3.33, 1.87, and 2.60%) and D1 (2.53, 1.40, and 1.97%) in the first harvest, the second harvest and the mean values, respectively. Lower STV, Reb A, and total SG contents in leaves were observed in the second harvest as compared to the first harvest due to environmental conditions.

## 3.4. Steviol Glycoside Yield

Total SG yield varied significantly due to the planting density in both harvests and cumulative total (Table 5).

Significantly higher total SG yield of  $0.55 \text{ t ha}^{-1}$  was recorded with planting density D3 in the first harvest, followed by D2 ( $0.35 \text{ t ha}^{-1}$ ) which was closely followed by D1 ( $0.28 \text{ t ha}^{-1}$ ). Similar trend was also observed in the second harvest and for the cumulative total values.

Stevioside yield values showed the same trend as total SG yield (Table 5). STV yield varied significantly due to the planting density in both harvests and for the cumulative total. Significantly higher STV yield of  $0.36 \text{ t ha}^{-1}$  was recorded with planting density D3 in the first harvest, followed by D2 ( $0.19 \text{ t ha}^{-1}$ ) which was closely followed by D1 ( $0.17 \text{ t ha}^{-1}$ ).

Similar trend was also observed in the second harvest and for the cumulative total values.

Rebaudioside A yield was significantly influenced by the planting density in the second harvest and for the cumulative total, but it had non-significant effect on Reb A yield in the first harvest (Table 5). Planting density D3 recorded the highest Reb A content ( $0.11 \text{ t ha}^{-1}$ ) which was on par with D2 in the first harvest. Significantly lower Reb A yield was obtained with D1 ( $0.01 \text{ t ha}^{-1}$ ) in the second harvest. Similar trend was seen in cumulative total.

**Table 2.** Effect of planting density on growth parameters of stevia at harvest

Planting density	Plant height (cm)			Stem diameter (mm)		
	First harvest	Second harvest	Mean	First harvest	Second harvest	Mean
D1	79.67 <sup>a</sup>	44.67 <sup>a</sup>	62.17 <sup>a</sup>	6.73 <sup>a</sup>	3.82 <sup>a</sup>	5.27 <sup>a</sup>
D2	67.67 <sup>b</sup>	40.00 <sup>b</sup>	53.83 <sup>b</sup>	6.14 <sup>b</sup>	3.57 <sup>a</sup>	4.86 <sup>b</sup>
D3	65.00 <sup>b</sup>	39.00 <sup>c</sup>	52.00 <sup>b</sup>	6.09 <sup>b</sup>	3.04 <sup>b</sup>	4.57 <sup>c</sup>
LSD (5%)	4.20	1.69	1.87	0.28	0.30	0.20

\* Values in each column with different letters are significantly different at the 5% significance level.

**Table 3.** Effect of planting density on yield parameters of stevia

Planting density	Fresh biomass yield ( $\text{t ha}^{-1}$ )			Fresh leaf yield ( $\text{t ha}^{-1}$ )			Dry leaf yield ( $\text{t ha}^{-1}$ )		
	First harvest	Second harvest	Total	First harvest	Second harvest	Total	First harvest	Second harvest	Total
D1	11.68 <sup>c</sup>	6.85 <sup>b</sup>	18.54 <sup>c</sup>	5.34 <sup>c</sup>	3.91 <sup>b</sup>	9.25 <sup>c</sup>	1.92 <sup>b</sup>	0.91 <sup>b</sup>	2.82 <sup>b</sup>
D2	15.92 <sup>b</sup>	8.19 <sup>b</sup>	24.11 <sup>b</sup>	7.05 <sup>b</sup>	5.02 <sup>b</sup>	12.07 <sup>b</sup>	2.18 <sup>b</sup>	1.15 <sup>ab</sup>	3.33 <sup>b</sup>
D3	22.71 <sup>a</sup>	14.52 <sup>a</sup>	37.23 <sup>a</sup>	10.74 <sup>a</sup>	7.95 <sup>a</sup>	18.69 <sup>a</sup>	3.21 <sup>a</sup>	1.75 <sup>a</sup>	4.95 <sup>a</sup>
LSD (5%)	3.85	1.75	2.74	1.15	1.51	1.42	0.61	0.62	0.59

\* Values in each column with different letters are significantly different at the 5% significance level.

**Table 4.** Effect of planting density on steviol glycoside contents of stevia

Planting density	STV (%)			Reb A (%)			Total SG (%)		
	First harvest	Second harvest	Mean	First harvest	Second harvest	Mean	First harvest	Second harvest	Mean
D1	9.10 <sup>a</sup>	4.17 <sup>a</sup>	6.63 <sup>ab</sup>	2.53 <sup>b</sup>	1.40 <sup>a</sup>	1.97 <sup>b</sup>	14.57 <sup>a</sup>	6.97 <sup>a</sup>	10.77 <sup>b</sup>
D2	8.63 <sup>a</sup>	3.80 <sup>a</sup>	6.22 <sup>b</sup>	4.93 <sup>a</sup>	1.97 <sup>a</sup>	3.45 <sup>a</sup>	16.00 <sup>a</sup>	7.57 <sup>a</sup>	11.78 <sup>ab</sup>
D3	11.27 <sup>a</sup>	4.67 <sup>a</sup>	7.97 <sup>a</sup>	3.33 <sup>ab</sup>	1.87 <sup>a</sup>	2.60 <sup>ab</sup>	17.20 <sup>a</sup>	8.10 <sup>a</sup>	12.65 <sup>a</sup>
LSD (5%)	3.81	2.67	1.70	2.10	1.11	1.15	3.54	3.21	1.88

\* Values in each column with different letters are significantly different at the 5% significance level.

**Table 5.** Effect of planting density on steviol glycoside yields of stevia

Planting density	STV yield ( $\text{t ha}^{-1}$ )			Reb A yield ( $\text{t ha}^{-1}$ )			Total SG yield ( $\text{t ha}^{-1}$ )		
	First harvest	Second harvest	Total	First harvest	Second harvest	Total	First harvest	Second harvest	Total
D1	0.17 <sup>b</sup>	0.04 <sup>b</sup>	0.21 <sup>b</sup>	0.05 <sup>a</sup>	0.01 <sup>b</sup>	0.06 <sup>b</sup>	0.28 <sup>b</sup>	0.07 <sup>b</sup>	0.35 <sup>b</sup>
D2	0.19 <sup>b</sup>	0.04 <sup>b</sup>	0.23 <sup>b</sup>	0.11 <sup>a</sup>	0.02 <sup>ab</sup>	0.13 <sup>ab</sup>	0.35 <sup>b</sup>	0.09 <sup>ab</sup>	0.43 <sup>b</sup>
D3	0.36 <sup>a</sup>	0.08 <sup>a</sup>	0.44 <sup>a</sup>	0.11 <sup>a</sup>	0.03 <sup>a</sup>	0.14 <sup>a</sup>	0.55 <sup>a</sup>	0.14 <sup>a</sup>	0.69 <sup>a</sup>
LSD (5%)	0.09	0.03	0.08	0.08	0.02	0.07	0.13	0.05	0.11

\* Values in each column with different letters are significantly different at the 5% significance level.

## 4. Discussion

In the present investigation, the narrow spacing of 70 cm x 10 cm with a plant population of 142857 plants ha<sup>-1</sup> resulted in lower plant height and stem diameter as compared to 70 cm x 30 cm with a plant population of 47619 plants ha<sup>-1</sup> due to competition for nutrition and light. The results are in accordance with the findings of Basuki and Sumaryono (1990) in Brazil who reported that a high plant density resulted in poor crop growth and lower leaf to stem ratio due to higher competition for light. Taleie *et al.* (2012) also reported that maximum plant height (80 cm) was obtained in the low plant density (50 cm x 20 cm) when plants were transplanted on the 15 March. Murayama *et al.* (1980) reported also that higher plant height when low plant density was adopted (60 cm x 20 cm) in Okinawa, Brazil. However, Angkapradipta *et al.* (1986) found that a narrow spacing of 25 cm x 25 cm was not optimum considering the root spread of the crop. By contrast, Aladakatti *et al.* (2012) in India found that the closer spacing of 30 cm x 20 cm resulted in higher plant height (61.16 cm) as compared to wider spacing of 45 cm x 30 cm (54.64 cm), while Lee *et al.* (1980) reported that the plant height, number of branches, and number of nodes were unaffected by planting density of 50-70 cm between and 10-30 cm within rows under Korean conditions.

Different planting densities resulted in significant variation with respect to fresh biomass yield, fresh leaf yield, and dry leaf yield in both harvests as well as in cumulative total. The wider spacing of 70 cm x 30 cm could not produce the dry leaf and biomass yield as that of narrow spacing of 70 cm x 10 cm due to lower plant population. Decreasing spacing may also increase the competitive ability of a crop and limit the period of time that weeds can compete with crops (Basuki and Sumaryono, 1990). The competitive advantage of narrow spacing may contribute to increased crop yield (Basuki and Sumaryono, 1990; Kumar *et al.*, 2012b). Murayama *et al.* (1980) reported also higher dry leaf yield with higher density (60 cm x 10 cm) in Okinawa, Brazil. Chalapathi *et al.* (1997) found that stevia planted at 45 cm x 22.5 cm (96618 plants ha<sup>-1</sup>) produced higher fresh leaf yield (13.8 t ha<sup>-1</sup>) and dry leaf yield (3.29 t ha<sup>-1</sup>). Colombus (1997) recommended high densities up to 160000 ha<sup>-1</sup> for higher yields in Canada. Aladakatti *et al.* (2012) reported that planting geometry of 30 cm x 20 cm recorded the highest cumulative total dry leaf yield (11.12 t ha<sup>-1</sup>) which was comparable with the dry leaf yield obtained with the planting geometry of 30 cm x 30 cm (10.89 t ha<sup>-1</sup>) and planting geometry of 45 cm x 30 cm (8.73 t ha<sup>-1</sup>). Kumar *et al.* (2012b) have reported that annual crop of stevia under mid hill conditions of north western Himalayas recorded significantly higher leaf dry biomass at a spacing of 45 cm x 10 cm. Kumar *et al.* (2014b) found that plants spaced in 30 x 30 cm accumulated more dry leaf yield and total dry biomass than 60 x 45 cm and dry leaf yield was ranged from 1.1 to 1.9 t ha<sup>-1</sup>. While Gvasaliya *et al.* (1990) obtained the highest stevia yield with a spacing of 70 cm x 25 cm at Abkhazia.

However, Filho *et al.* (1997) obtained higher biomass yield at a planting geometry of 50 cm x 20 cm. Observations made at Palampur, India, indicated that plant population of 50000 ha<sup>-1</sup> was found optimum with a planting density of 45 cm x 45 cm (Singh and Kaul, 2005). Therefore, it seems that plant spacing could be used as a management tool for maximizing crop growth and yield.

In addition, data on growth and yield parameters of stevia across both harvests revealed that the yields were lower in all the treatments in the second harvest as compared to the first harvest. This was due to low temperature and shorter photoperiod that prevailed during the crop growth period. Research carried out at Egypt revealed that temperature, length, and intensity of photoperiod significantly affected stevia biomass production as evident from the remarkable increase in yield during summer cuttings than that of winter cuttings (Allam *et al.*, 2001). The sensitivity of stevia to day length, photoperiod and temperature was also reported by Lester (1999) in Australia and Valio and Rocha (1977) in Japan. Ramesh *et al.* (2007) illustrated that the time of harvest is closely related to yield.

The total SG and STV contents of stevia leaves were unaffected by different planting densities in both harvests. Similarly, Alladakatti (2011) reported that different planting densities did not influence the STV and Reb A contents in leaves at harvest significantly in all the cuttings. Kumar *et al.* (2014b) reported also that SG content were not significantly affected by different planting densities; however, closer plant spacing (30 x 30 cm) recorded higher SG accumulation compared to wider row spacing (60 x 45 cm).

In this study, the STV content was in the range of 3.80 to 11.27% and the Reb A was in the range of 1.40 to 4.93% across both harvests. However, Alladakatti (2011) found that the STV content was in the range of 7.54 to 11.66% and the Reb A was in the range of 4.28 to 5.68% across different cuttings. Singh and Kaul (2005) found that STV content in leaf varied from 3.17 to 12 %.

The lower contents of STV and Reb A were obtained in leaves during the second harvest, which was due to lower temperature and shorter photoperiod that prevailed during the crop growth compared to the first harvest. Similarly, Brandle *et al.* (1998) had reported higher STV content in leaves at Ontario, Canada where long days were experienced during growing season relative to the subtropical regions of the world. Singh and Kaul (2005) reported also lower content of SG in the leaves grown under winter conditions than during summer conditions under agro-climatic conditions of Palampur in India. Alladakatti *et al.* (2012) indicated that the climatic factors like minimum temperature and photoperiod had greater impact on the glycoside content in the leaves. Also, Pereira *et al.* (2016) found that the highest concentration of STV was 12.16% while Reb A was 7.01% in December.

Higher STV and Reb A yield was possible with closer spacing 70 cm x 10 cm. Optimum plant population in these treatments resulted in higher leaf yield compared to wider plant spacing with lower plant population. In similar,

Alladakatti *et al.* (2012) found higher STV yield (116.4 and 114.6 kg ha<sup>-1</sup>) and Reb A yield (65.2 and 68.5 kg ha<sup>-1</sup>) with closer spacing 30 cm x 30 cm and 30 cm x 20 cm, respectively. While, Chalapathi *et al.* (1997) reported that higher STV yield (303 kg ha<sup>-1</sup>) with 45 cm x 22.5 cm planting density at Bangaluru. Lower STV and Reb A yields in all the treatment of the second harvest were due to the lower temperatures and shorter day length which hampered the growth of stevia with lower number of leaves plant<sup>-1</sup>.

## 5. Conclusions

It is concluded, through this study, that closer spacing of 70 cm x 10 cm (142857 plants ha<sup>-1</sup>) resulted in higher fresh biomass yield, fresh leaf yield, dry leaf yield, steviol glycoside content, and steviol glycoside yield of stevia as compared to wider spacing of 70 cm x 20 cm (71428 plants ha<sup>-1</sup>) and 70 cm x 30 cm (47619 plants ha<sup>-1</sup>). But the optimal result depends on harvesting time. Stevia dry leaf yield and steviol glycoside yield were significantly increased when harvesting was made during summer (August 11, 2014) than that of autumn harvesting (October 23, 2014). From the findings of the experiment, it can be inferred that under Larache, Moroccan conditions, to obtain higher dry leaf yield and better quality of stevia, it is beneficial to follow closer spacing (70 cm x 10 cm) or maximum plant density and stevia would preferably be harvested during summer. Thus, this study provided basic information with respect to some agronomy of stevia useful for further investigation.

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