International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2012/4-6/298-302.
ISSN 2227-670X ©2012 IJACS Journal

IJACS

Effects of plant density and transplanting date on herbage, stevioside, phenol and flavonoid yield of *Stevia rebaudiana* Bertoni

Negar Taleie[1], Yousef Hamidoghli[1*], Babak Rabiei[1], Saeid Hamidoghli[2]

1. Department of Horticultural Sciences, University of Guilan, Rasht, Iran
2. Department of Horticultural Sciences, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran

Corresponding author email: Hamidoghli@guilan.ac.ir

ABSTRACT: *Stevia rebaudiana* Bertoni is a natural sweetener plant. The leaves of Stevia are the source of glycosides. Even more, stevia contains a high percentage of phenols, flavonoids and antioxidant activity. Planting density and transplanting date are the most important agronomical factors that may affect the quality and quantity of yield. This study was conducted in Guilan province, on the base of a split-plot field experiment in a randomized complete block design for two cultivation seasons (2009-2010 and 2010-2011). Tissue culture-derived stevia plantlets were transplanted on 15 March, 30 March and 15 April in rows 50-cm apart with an inter-row spacing of 20, 25, 35, and 50 cm. Transplanting date and spacing significantly ($P < 0.05$) affected plant height, herbage (leaf and stem) fresh and dry weight and also stevioside, phenol and flavonoid yield. Maximum plant height (80 cm), total fresh (2017.21 g/m^2) and dry (588.69 g/m^2) herbage weight, and the highest stevioside (34.51 g/m^2), phenol (1.5 g/m^2) and flavonoid (1.97 g/m^2) yield were obtained in the 50×20 cm spacing when plants were transplanted on the 15 March.

Key words: Cultivation, Dry matter, Steviol glycosides, antioxidant activity, Stevia

INTRODUCTION

Stevia rebaudiana Bertoni is a perennial herbaceous plant native to between 22° to 24° South and 53° to 56° west in Paraguay and Brazil (Sivaram and Mukundab, 2003; Jain et al., 2009). It is a natural sweetener plant and is grown commercially in many parts of Brazil, Paraguay, Central America, Thailand, Korea and China (Elkins, 1997; Megeji et al., 2005).

The leaves of Stevia are the source of steviol glycosides, stevioside and rebaudioside, which estimated to be 300 times sweeter than sugar but also have no effect on blood sugar, so it is helpful for hypoglycaemia and type 2 diabetes (Soejorto, 2002; Ramesh et al., 2006). It nourishes pancreas and thereby helps to restore its normal function. Furthermore, stevia contains high percentage of phenol, flavonoid which causes stevia to have a high antioxidant activity (Tadhani et al., 2002; Shukla et al., 2009). Phenols are the secondary metabolites that cause the cardiac and cancer diseases to be decreased (Dragovi-Uzelac, 2010).

Agricultural factors such as spacing and transplanting date have critical effects on quantitative and qualitative characteristics of plants (Naghdi badi et al., 2004).

In the another study, was examined 3 planting date (January, October and November) in Stevia yield and was reported, under Dharwad condition, higher plant height, total dry matter production, higher biomass yield, Biomass yield, fresh leaf yield and dry leaf yield were observed during January planting (Maheshwar, 2005).

Al-Dein and AL-Ramamneh (2009) cultivated thyme to determine the effects of three intra-row spacing (15, 30 and 45 cm) and reported that thyme plants grown using 15cm planting had the highest fresh and dry weight of shoots.

Morteza et al., (2009) examined three sowing date (10th August, 1st September and 20th September) and three planting density (4, 8 and 12 plants/m2) on vegetative and reproductive characteristics of valerian (*Valeriana*

officinalis L.) and reported sowing date and planting density are the main factors influencing the quantity and quality characteristics of valerian.

Among the different factors affected on plant growth and development, climatic conditions play an important role in the efficiency of crops. For prosperous cultivation of any production, crop should be exposed to optimum climatic conditions during the growing cycle, so as to get maximum growth and yield of Stevia plant (Maheshwar, 2005).

The aim of this study is to estimate the proper planting density and transplanting date of stevia plantlets to field in Guilan province located in the north of Iran. Evaluation of herbage fresh and dry weight, stevioside, total phenol and flavonoid yield were also calculated.

MATERIAL AND METHOD

A split plot field experiment has been conducted in a randomized complete block design with four replications, three transplanting dates and four planting densities for two cultivation seasons (2009-2011) in Guilan (37˚, 12´ N and 49˚, 39´ E, 7 m below sea level), Iran. *In vitro* acclimaized plantlets had been transplanted into experimental field in three planting dates (15 March, 30 March and 15 April) in rows of 50 cm apart with inter-row spacing of 50, 35, 25 and 20 cm. Irrigation and other field practices has been done the same in 2 cultivation seasons. Before harvesting, plant height was measured. After harvesting the leaves were separated from stem and dried in an automatic herb dryer at 55 ^{o}C for 10 h. Fresh and dry weight of plants aerial parts, the stevioside value, total phenol and flavonoid compound have been determined.

The stevioside value were estimated by Makapugay et al., (1984) method, and HPLC Agilent column NH2 systems chromatography equipped with a pump HPLC Model 1311A G, along with the milk injected Rheodyne model 7725 (I) with loop 1 micro liter, detector (Diode array and multiple wavelength detector). and Agilent chemistations software installed on the Windows operating system capabilities for integration of the area under the peak, correct line and field automation functionality was used.

The flavonoides were analyzed spectro-photometrically using Yong et al., (2008) method with some modifications. For measuring the total phenol of leaves, Folin-Ciocalteu method (Du et al., 2009) was used. The absorption of extract was read using spectrophotometer (Model PG Instruments ltd - T80 + UV / VIS).

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System [SAS v.9.1 for windows; Statistical Analysis System]. The means were compared by Tukay's significant test at $P < 0.05$.

RESULTS

No significant differences were observed among measured traits in 2 cultivation seasons (2009-2011) while the effect of transplanting date, plant spacing and their interactions were significant on plant height, fresh and dry leaves and stem weight. The highest plant (80 cm) was achieved by the closer spacing (50×20 cm) and transplanting date in 15 March (Table 1). Transplanting date and spacing affected fresh and dry herbage. The maximum fresh leaves weight, stem weight, dry leaves weight was obtained by plants planted on 15 March with spacing of 50×20 cm that showed significant differences with other treatments (Table 1).

Table1. Effects of transplanting date and spacing on stevia plant height, total herbage, leaf and stem fresh and dry weight.

Treatment		Plant height (cm)	Total herbage weight (g/m^2)		Leaf weight (g/m^2)		Stem weight (g/m^2)	
Transplanting date	Spacing		Fresh	Dry	Fresh	Dry	Fresh	Dry
15March	50×50	70.31 ± 0.4 d*	1012.93±9.6h	289.16±3.4gh	552.19±12.7h	103.65±0.8gh	264.21±4.8h	186.65±2.7ef
15March	50×35	60±0.2 h	1300.34±12e	365.49±4.2^{d}e	713.49±6.4e	131.06±1.1e	586.57±5.9e	232.22±3.3cd
15March	50×25	76.2 ±0.4b	1799.37±23.46b	505.59±7.1b	983.28±24b	180.06±2.3b	814.6±11.5b	326.8±5.09b
15March	50×20	80± 0a	2017.21±21.1a	558.69±7.9a	1094.25±11.1a	199.52±1.2a	921.53±10.8a	366.37±6.4a
30March	50×50	66.56±0.3e	1111.78±11.1g	313.8±3.7fg	606.36±5.9g	110.57±1.2g	503.48±5.4g	203.25±2.9def
30March	50×35	69.38±0.2d	1196.4±16.3f	336.12±5.1ef	655.98±8.8f	119.82±1.6f	531.77±7.4fg	217.94±3.8de
30March	50×25	66.18±0.1e	1631.81±23.9c	457.49±6.6c	897.19±12.4c	163.41±2.3c	730.77±12.2c	292.98±4.5b
30March	50×20	77±0.2 b	1762.71±27.7b	498.91±9.5b	954.96±15.02b	177.28±2.7b	799.81±12.5a	322.15±7.1b
15 April	50×50	66.56±0.5b	703.25±7.7i	195.52±2.8i	389.01±3.9i	71.66±0.7i	314.96±4i	125.13±2.2g
15 April	50×35	61.5±0.5g	964.18±15.4h	263.61±4.9h	524.85±7.9h	97.17±1.5h	423.48±6.5h	167.22±3.6f
15 April	50×25	71.39±0.1c	1230.01±11.5f	344.34±5.1def	676.02±6f	129.83±1.9e	563.9±6.4ef	214.37±3.4cde
15 April	50×20	63.62±0.3f	1398.06±16.8e	383.26±5.3d	766.08±8.8f	141.01±1.6d	639.7±8.9d	254.64±4c

* For each parameter significant difference between mean among the sites are indicated by different letters (Tukey test, alpha = 0.05).

The spacing and transplanting date had no significant effect on stevioside, phenol and flavonoid content (%) but due to the increase of dry matter yield in per unit area, the yield of stevioside, phenol and flavonoid has been significantly increased in lower spacing and sooner transplanting date. However, inter-row spacing of 50×20 cm with transplanting date on 15 March have been suggested to give the highest stevioside (34.51 g/m^2), phenol (1.5 g/m^2) and flavonoid (1.97 g/m^2) yield per unit area (Table 2).

Table 2. Effects of transplanting date and spacing on Stevioside, phenol and flavonoid yield of stevia dry leaf

Factors		Stevioside (g/m^2)	Phenol (g/m^2)	Flavonoid (g/m^2)
Transplanting date	Spacing			
15March	50×50	17.93±0.2 g*	0.78±0.01 gh	1.02 ±0.01gh
15March	50×35	22.67±0.15 e	0.98±0.03 c	1.29±0.02 e
15March	50×25	31.15±0.1 b	1.35± 0.01b	1.78±0.07 b
15March	50×20	34.51±0.08 a	1.5±0.04 a	1.97±0.09 a
30March	50×50	19.12± 0.07g	0.83±0.05 g	1.09 ±0.06 g
30March	50×35	20.73±0.09 f	0.9 ±0.06 f	1.18±0.08 f
30March	50×25	28.27±0.03 c	1.23± 0.01 c	1.61±0.04 c
30March	50×20	30.67±0.06 b	1.33±0.09 b	1.75±0.07 b
15 April	50×50	12.39±0.02 h	0.54±0 i	0.7±0 i
15 April	50×35	16.81±0.01 g	0.73 ±0.03h	0.96±0 h
15 April	50×25	22.46±0.04 e	0.97±0.05 e	1.28±0.04 e
15 April	50×20	24.39±0.03 d	1.06 ±0.07d	1.39±0.03 d

* For each parameter significant difference between mean among the sites are indicated by different letters (Tukey test, alpha = 0.05).

DISCUSSION

Stevia plants are sensitive to water tension and it is the most effective factor on stevia growth. Plant height is a quantitative genetic trait which could be influenced by environmental factors. It is reported that increasing the growth period, is associated with increasing with plant height (Salahi et al., 2006). Stevia plantlets, those transplanted in 15 March have more height than those transplanted in 30th March and 15th April and this may be due to having more time to grow. These results have agreement with Shalby and Razin (1992), Salahi et al., (2006) and Goldani et al., (2007) studies. They reported that plant density and earlier planting date results in enhancing plant height.

The increased number of leaves in early planting dates could be directly correlated to the fact that early planting had increased number of leaf bearing points in terms of increased number of branches per plant (Maheshwar, 2005). The reasons for higher dry leaf yield in case of 15th March transplanting, probably due to higher number of leaf bearing points in terms of increased number of branches per plant on 15th March compared to 30th March and 15th April transplanting.

Delayed transplanting date decreases the product quantity, because the time between transplanting and harvesting reduces. Also, long-day conditions with high temperature and light intensity during vegetative growth resulted in vigorous growth, which enabled them to produce more yeild (Maheshwar, 2005).

On the other hand, the plants were transplanted on 15 March with the density of 50×20 cm had more time for growth and greater density of plants enhanced yield. This increasing density not only raises plants performance per m^2 but also reduces the weed growth.

Leaf yield increased with increasing density up to 83000 and 111000 plant/ha in stevia for the first years of production (Madan et al., 2009).

Naghdi Badi et al., (2004) cultivated thyme plants in 3 harvesting stages and rows of 50 cm apart with inter-row spacing of 15, 30 or 45 cm. They founded that 15 cm spacing and harvesting in the beginning of blooming was the best treatment in respect of yield of dry matter, oil and thymol.

Ganjali et al., (2010) cultivated *Calendula officinalis* in 3 levels sowing date (1th,15th and 30th of April) with plant density in 3 levels (6×50, 8×50, 12×50 cm^2) and founded that sowing date on April 1st, with (12 ×50) plant density increase yield, yield components and essence of calendula.

Morteza et al., (2009) were examined the effects of transplanting dates and plant density on quantity and quality characteristics of valerian. They reported, transplanting date and planting density are the main factors influencing the vegetative and reproductive characteristics of valerian.

Evaluation of dry matter production and dispensation to various plant parts is important for designation of total yield of crop (Maheshwar, 2005). Our results showed that delayed planting date or putting forward and plant density have no effect on the rate of leaf to stem ratio in stevia plants. Consequently, the performance increase due to increase in leaf weight did not increase in stem weight.

The spacing and transplanting date had no significant effect on stevioside, phenol and flavonoid content (%) but had significant effect on stevioside, phenol and flavonoid yield. This results have agree with Nagddi badi et al., (2004) that reported spacing had no significant effect on oil content of thymus, but oil yield significantly increased in lower spacing.

It was indicated that in *Agasstache foeniculum* (pursh) kuntz the effect of transplanting date on yields of herbage and essential oil per plant was significant (Shamskia et al., 2006).

CONCLUSION

Stevia is not grown in Iran originally but in this experiment we found that it has a good industrial performance in experimental field in Guilan province. Our investigation showed that planting density and transplanting date significantly influence most of the productive characteristics of stevia. From these investigations, it can be inferred that under Guilan, Iran conditions, to maximize the herbage yield (199.52 g/m^2), stevioside (34.51 g/m^2), phenol (1.5 g/m^2) and flavonoid (1.97 g/m^2) transplanting in the month of 15th March with planting density (50×20 cm) was suitable.

REFERENCES

Al-Dein E, Al-Ramamneh M. 2009. Plant growth strategies of *Thymus vulgaris* L. in response to population density. Industrial Crops and Products. 30: 389–394.

Dragovi-Uzelac V, Savi Z, Brala A, Levaj B, BursaKovaevi D, Bisko A. 2010. Evaluation of phenolic content and antioxidant capacity of blueberry cultivars (*Vaccinium corymbosum* L.) grown in the northwest Croatia. Food Technol. Biotechnol 48 (2): 214–221.

Du G, Li M, Ma F, Liang D. 2009. Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. Food Chemistry 113: 557–562.

Ganjali HR, Ayeneh Band A, Heidari Sharif Abad H, Moussavi Nik M. 2010. Effects of sowing date, plant density and nitrogen fertilizer on yield, yield components and various traits of *Calendula officinalis.* American-Eurasian J. Agric. & Environ. Sci. 9 (2): 149-155.

Goldani m, Rezvani moghadam p. 2007. The effect of different treatments of humidity and sowing date on phonological and growth characters of three pisum varieties in mashhad. The journal of agriculture and environment (14): 1–8.

Jain P, Kachhwaha S, Kothari SL. 2009. Improved micropropagation protocol and enhancement in biomass and chlorophyll content in *Stevia rebaudiana* Bert. Bertoni by using high copper levels in the culture medium. *Scientia Horticulturae*. 119: 315–319.

Madan S, Ahmad S, Singh GN, Kohli K, Kumar Y, Singh R, Garg M. 2010. *Stevia rebaudiana* (Berr.) Bertoni- A review. Indian journal of natural products and resources 1(3): 267-286.

Maheshwar HM. 2005. Effect of different levels of nitrogen and dates of planting on growth and yield of Stevia *(Stevia rebaudiana* Bert.). MSc Thesis. Department of horticulture college of agriculture, Dharwad university of agricultural sciences, Dharwad, 66 pp.

Makapugay HC, Nanayakkara NPD, Kinghorn AD. 1984. Improved high performance liquid chromatographic separation of the *Stevia rebaudiana* sweet diterpene glycosides using linear gradient elution. Journal of Chromatography 283: 390-395.

Megeji NW, Kumar JK, Sing V, Kaul VK, Ahuja PS. 2005. Introducing *Stevia rebaudiana*, anatural zero-calorie sweetener. Current Science 88(10): 801-804.

Morteza E, Akbari GA, Modares Sanavi SAM, Hossein Aliabadi Farahani H. 2010. Determination of the vegetative and reproductive characteristics of valerian (*Valeriana officinalis* L.) under sowing dates and planting densities at Iran. Journal of Medicinal Plants Research 4(10): 857-861.

Naghdi Badi H, Yazdani D, Mohammad Ali S, Nazari F. 2004. Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, *Thymus vulgaris* L. Industrial Crops and Products 19: 231–236.

Ramesh K, Singh V, Megeji NW. 2006. cultivation of *Stevia rebaudiana* (BERT.) Bertoni a comprehensive review.

Rita Elkins MH. 1997. Stevia nature's sweetener (ed) Stevia. Woodland Publishing, Inc. 160: 1-29.

Salahi FN, Amjadian m. 2006. The effect of sowing date on yield of Soya viliam cultivar in Gorgan, Iran. The journal of agriculture and environment. (13). Special issue, Agronomy and Plant Breeding.

Shalby AS, Razin AM. 1992. Dense cultivation and fertilization for higher yield of thyme (*Thymus vulgaris* L.). J. Agronomy. Crop Science 168: 243–248.

Shamskia F, omidbeigi R, Sadrayei Manjili K. 2006. The Effect of sowing date on growth, development, herbage yield and extract of *Agasstache foeniculum* (Pursh) kuntz. In 1rd regional conference on medicinal plants, spices and aromatic- Islamic Azad University, Shahrekord , Iran 312.

Shukla S, Mehta A, Bajpai VK, Shukla S. 2009. *In vitro* antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. Food and Chemical Toxicology 47: 2338–2343.

Sivaram L, Mukundan U (2003) *In vitro* culture studies on *Stevia rebaudiana.* In Vitro Cellular and Developmental Biology - Plant 39(5): 520-523.

Soejarto DD. 2002. Botany of Stevia and Stevia rebaudiana. In Kinghorn AD (ed) Stevia. Department of Medicinal Chemistry and Pharmacognosy University of Illinois at Chicago USA.18-39.

Tadhani MB, Patel VH, Subhash R. 2007. *In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus. Journal of Food Composition and Analysis 20: 323–329.

Yong SP, Soon TJ, Seong GK, Buk GH, Patricia A, Fernando T. 2008. Antioxidants and proteins in ethylene-treated kiwi fruits. Food Chemistry 107(2): 640–648.