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Influence of growing conditions and date of planting on the yield and quality of stevia (*Stevia rebaudiana* Bertoni)

S. SUHAS, *P. V. SINDHU, C. BEENA AND M. V. MENON
College of Agriculture, Kerala Agricultural University, Vellanikkara, Kerala

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ABSTRACT

A field study was carried out from May to November 2020, to assess the effect of growing conditions and planting date on herbage yield and quality of stevia (Stevia rebaudiana Bertoni). *The design of the experiment was split plot with two main plots (open and 50 per cent shade) and four sub plots (May 15th, June 15th, July 15th, and August 15th). Higher plant height was observed under 50 per cent shaded conditions. Leaf weight, herbage yield, and steviol glycoside contents were higher under open growing conditions. The herbage yield was higher in the treatment combination of planting in May under open conditions (7800 kg ha^{-1}) and was on par with the August planting (7212 kg ha^{-1}) under open conditions. Lower herbage yield was recorded in June planting (3872 kg ha^{-1}) under 50 per cent shade and was on par with the July planting (4077 kg ha^{-1}) under 50 per cent shade. Under open conditions, the crop planted in May recorded the highest total steviol glycoside content (9.05 percent). A strong positive correlation was observed between growth parameters of stevia with maximum and minimum temperatures, whereas the correlation with rainfall was negative.*

Keywords: Date of planting, growing condition, light intensity, shade levels, *Stevia rebaudiana*

Stevia (*Stevia rebaudiana* Bertoni) is a perennial herb belonging to Asteraceae. It is a natural sweetener commonly recognized as candy leaf, honey leaf, sweet leaf, sweet herb, or sweet herb of Paraguay. The plant contains steviol glycosides (SVglys), which are of high interest in the human diet as a low-calorie and high potency sweetener in the face of the increasing severity of diabetes and obesity. The plant is indigenous to Rio Monday in the highlands of northeastern Paraguay in South America. Paraguayans have used the leaves to sweeten bitter beverages for centuries. Stevia was earlier known as *Eupatorium rebaudianum*. In 1905, Dr. M. S Bertoni officially discovered stevia and was renamed *Stevia rebaudiana* (Mondaca *et al*., 2012).

The leaves of stevia are the primary reserve of steviol glycosides. Stevia leaves have a high percentage of phenols and flavonoids, and have antioxidant activity. The two main glycosides - Stevioside (5-10 per cent of the dry weight of the leaves) and Rebaudioside A (2-4 per cent of the dry weight of the leaves) are the sweetest compounds that give sweetness to the plant. The plant contains secondary compounds such as Rebaudioside C, Dulcoside A and C. (Khiraoui *et al*., 2017).

Since the leaf is the main economic part of stevia, the production of more leaf biomass with higher steviol glycosides is the main criteria for assessing crop performance. Secondary metabolism is affected by various environmental conditions that affect development, photosynthesis, and other aspects of primary metabolism (Pant *et al*., 2015). Environmental factors such as light, temperature, and atmospheric CO_2 can influence secondary metabolites in plants (Fini *et al*., 2017). Light is a physical component that can enhance plant growth and development. Both insufficient and excessive light intensities can harm plants, affecting their growth, development, and yield. Studies on crop growth responses to various light intensities help to determine the best conditions for crop cultivation. In any crop, the time of sowing is critical for better vegetative growth and eventual yield outputs. Sowing too early or too late might affect the crop's growth, yield, and quality. The planting time influences the phenological development and effective conversion of biomass into economic output.

Taking into account the foregoing, an experiment to assess the influence of the growing conditions and the date of planting on the yield and quality of stevia (*Stevia rebaudiana* Bertoni) was done at the Department of Agronomy, College of Agriculture, Kerala Agricultural University, Vellanikkara.

The trial was conducted from May to November 2020 at the Agronomy Farm, Department of Agronomy, College of Agriculture, Vellanikarra, Thrissur, Kerala. The field was at 13°32'N latitude and 76°26'E longitude, at 40 m above mean sea level. The texture of the experimental site was sandy clay loam and was strongly acidic, with a pH of 4.76. The available nutrient contents of the soil tested before planting were 193.41kg ha^{-1} N, 27.64 kg ha^{-1} P, and 169.76 kg ha^{-1}.

The experiment followed a statistical design Split plot with three replications. The main plots (Open and

Short Communication
Email: pv.sindhu@kau.in

50 per cent shade) were the two growing conditions, and the planting date (May 15th, June 15th, July 15th and August 15th) were the subplots. Fine tilling and leveling prepared the planting beds as per the planting schedule. Well rotten farmyard manure was applied @ 15 t ha^{-1} at bed preparation. Thirty days old, two noded rooted stem cuttings were planted at a spacing of 30 cm x 30 cm under open and 50 per cent shaded growing conditions as per the planting schedule. Green colored shade nets were erected to regulate light intensity to 50 per cent. Hand weeding was carried out at 30 and 45 days after planting. The beds were irrigated twice a day, in the morning and the evening, with a rose can during the crop establishment period, and later 3 mm hose irrigation was given once a day. The harvesting was done after three months of planting, with the initiation of flower buds. Observations on plant height, fresh leaf weight and total herbage yield (kg ha^{-1}), and steviol glycosides (total) in the leaves were recorded at the harvest stage. Steviol glycoside (total) content in the leaves was estimated using the method given by Parhi and Mohapatra (2012). The data collected were subjected to analysis of variance using the statistical package "GRAPES" (Gopinath *et al*., 2020).

Data on weather parameters such as surface air temperature (maximum and minimum), rainfall, and bright sunshine hours during the experiment period were obtained from the Meteorological Observatory attached to the College of Agriculture Vellanikkara. A simple linear correlation between mean monthly meteorological weather parameters and crop observations was worked out.

The growing conditions and the planting date significantly influenced the plant height of stevia (Table 1). The taller plants were observed under shaded conditions. Plant height followed the trend of May>August>June>July among different planting dates. The interaction between growing condition and planting dates was significant and taller plants were observed in treatment combination May planting under shaded growing condition (40.30 cm). It was followed by planting in August under 50 per cent shade (34.17 cm) and May planting in open condition (32.25 cm), which were on par. The shorter plants were noticed when planting was done either in June or July under open conditions. The high temperature under open conditions might have favored the formation of more lateral buds, resulting in a more significant number of branches. In contrast, the shaded environment favored apical dominance and increased plant height. Changes in the number of primary and secondary branches in stevia due to differences in planting dates were reported by Khan *et al*. (2012). According to them, the maximum number of branches was observed when planting was done in April. They attributed it to warm environmental conditions like clear sunshine and high April temperatures.

A higher fresh weight of leaves per plant was noticed in the treatment combination of planting in May under open conditions (89.91g $plant^{-1}$). Lower fresh weight of leaves was recorded in June planting (39.08 g $plant^{-1}$) under 50 per cent shade and was on par with the July planting (39.16 g $plant^{-1}$) under 50 per cent shade. Khan *et al*. (2012) reported a higher fresh weight of leaves in stevia crops planted during April and May. Variation in fresh weight of leaves due to changes in planting dates could be attributed to variations in weather parameters experienced by the crop during their various life stages.

Significant variation was observed for fresh herbage yield with different dates of planting. Higher herbage yield was noticed in the treatment combination May planting under open condition (7800 kg ha^{-1}) and was on par with the August planting (7212 kg ha^{-1}) under open condition. The herbage yield difference between the crop planted in May under open and shaded conditions was 1374 kg ha^{-1} (17.62 per cent). Lower herbage yield was recorded in June planting (3872 kg ha^{-1}) under 50 per cent shade and was on par with the July planting (4077 kg ha^{-1}) under 50 per cent shade. A yield reduction of 50.36 per cent as compared to May planting was noticed in June planted crop. A strong positive correlation was observed between fresh herbage yield and maximum temperature, whereas the correlation with rainfall was negative (Table 2). June planted crop received 103.15 mm rainfall during its seedling stage (Table 3) and a negative correlation between total biomass and rainfall was observed. Better production of herbage in May planted crop under open growing conditions could be correlated with the availability of ample sunlight and favorable temperature experienced by the crop in the early establishment stage. Allam *et al*. (2001) observed a remarkable increase in stevia yield during the summer (May-August) due to favorable climatic conditions, such as temperature and duration and intensity of photoperiod, compared to winter. Orazco *et al.* (2020) also reported heavy rainfall as the main reason for stevia's low relative growth rate.

Data on the steviol glycoside content revealed the significant influence of environmental conditions on enhancing the quality of a crop. Planting in May under open conditions resulted in the highest steviol glycoside content of 9.05 per cent. It was followed by August planting in open condition (8.19 per cent). July planting in shaded conditions had lower steviol glycoside content (3.64 per cent) and was on par with June planting in shady conditions (3.93 per cent). The highest steviol glycoside content was obtained under open growing conditions. Compared to crops planted under the open condition in May, the crop grown in 50 per cent shade in

Table 1: Interaction effect of the growing condition and the planting dates on the plant height, herbage yield and steviol glycosides (total) of stevia at harvest

	Plant height (cm)		Leaf weight (fresh) (g plant^{-1})		Herbage yield (fresh) (kg ha^{-1})		Steviol glycosides (total) (%)	
	Open	50 per cent shade	Open	50 per cent shade	Open	50 per cent shade	Open	50 per cent shade
May 15th	32.25	40.30	89.91	68.33	7800	6426	9.05	7.60
June 15th	22.38	26.06	48.25	39.08	4218	3872	5.86	3.93
July 15th	18.76	28.36	44.00	39.16	4307	4077	5.20	3.64
August 15th	28.14	34.17	64.16	53.75	7212	6144	8.19	5.20
SEm (±)	**1.50**		**4.33**		**161.59**		**0.46**	
LSD(0.05)	**6.36**		**22.43**		**572.23**		**1.64**	

Table 2: Correlation between the weather parameters and the yield and yield attributes of stevia

	Temperature (Maximum)	Temperature (Minimum)	Rainfall	Bright sunshine hours
Plant height	0.879**	0.522*	-0.792**	0.463
Fresh leaf weight	0.842**	0.631**	-0.705**	0.347
Fresh herbage yield	0.895**	0.424	-0.852**	0.553*
Steviol glycosidecontent (Total)	0.874**	0.492	-0.790**	0.461

Table 3: Monthly weather data during the cropping period

Months	Maximum temperature (oC)		Minimum temperature (oC)		Forenoon relative humidity (%)		Afternoon relative humidity (%)		Rainfall (mm)	Bright Sunshine hours
	Open	Shade	Open	Shade	Open	Shade	Open	Shade		
May	33.76	32.56	25.10	24.90	91.66	93.66	68.33	71.33	80.3	108.3
June	31.10	29.90	23.60	23.40	94.50	96.50	75.25	78.25	412.6	66.4
July	30.26	29.06	23.30	23.10	96.00	98.00	79.00	82.00	728.7	85.4
August	30.55	29.35	23.17	22.97	95.50	97.50	75.25	78.25	512.8	105.9
September	29.80	28.60	22.37	22.17	80.75	82.75	88.50	91.50	576.4	57.9
October	31.20	30.00	21.68	21.48	95.00	97.00	68.60	71.60	310.3	185.5
November	33.30	32.10	22.30	22.10	82.50	84.50	57.50	60.00	55.6	91.7

May recorded 19 per cent reduction in steviol glycoside content. According to Tateo *et al.* (1998), environmental and agronomic factors significantly influenced stevioside production in stevia. Kumari *et al.* (2016) studied the effect of growing conditions (open field and shade) on the quality of stevia. They reported higher rebaudioside-A content (7 per cent) in open area grown plants than plants grown in shaded conditions. As per Ramesh *et al.* (2006), stevia is extremely sensitive to the day length and requires 12-16 hours of sunlight to maximize the accumulation of stevioside in the leaves.

The results indicated a positive correlation of maximum and minimum temperatures with plant height, leaf weight, herbage yield, and steviol glycoside content. Temperature plays a vital role in crop plants' biological processes. It is one of the most important climatic factors affecting crops' growth, development, and yield (Kumar *et al.,* 2013). A positive correlation between growth, yield, quality, and weather parameters of stevia was reported by Clemente *et al*. (2021). The correlation of rainfall with plant height, leaf weight, biomass yield, and steviol glycoside contents was negative. Tavarini and Angelini (2013) reported negative relation between rainfall and the quality of stevia.

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