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Kumari Shivani
M.Sc. Scholar, Department of Agronomy, Naini Agricultural Institute, SHUATS, Prayagraj, Uttar Pradesh, India

Ghosh Gautam
Professor and Dean, Naini Agricultural Institute, SHUATS, Prayagraj, Uttar Pradesh, India

Ghosh Sukany
PGDM-FPBM, Indian Institute of Plantation Management, Bengaluru, Karnataka, India

Mesharm MR
Ph.D. Scholar, Department of Agronomy, Naini Agricultural Institute, SHUATS, Prayagraj, Uttar Pradesh, India

Correspondence
Kumari Shivani
M.Sc. Scholar, Department of Agronomy, Naini Agricultural Institute, SHUATS, Prayagraj, Uttar Pradesh, India

Impact of spacing and levels of nitrogen on growth and yield of stevia (*Stevia rebaudiana* Bertoni)

Kumari Shivani, Ghosh Gautam, Ghosh Sukany and Mesharm MR

Abstract
A field experiment was conducted during *Zaid* season 2018 to investigate "Impact of spacing and levels of Nitrogen on the growth and yield of stevia (*Stevia rebaudiana* Bertoni)" under eastern Uttar Pradesh conditions. The research was conducted using Randomized Block Design (RBD) replicated thrice, which comprised of two factors. The first factor was dosage of the nitrogen with three levels *i.e.,* 50, 75, 100 kg N ha^{-1}. The second factor was different inter row spacing *i.e.,* 30cm x 20cm, 40cm x 20cm and 50cm x 20cm. The results showed that the growth and yield attributes have been found to be significantly affected by different treatments. Application of 100 kg ha^{-1} Nitrogen with 50cm x 20cm spacing recorded significantly higher plant height and number of branches. The total fresh leaf yield and fresh stem yield per hectare increased significantly with application of 100 kg ha^{-1} Nitrogen with 30cm x 20cm spacing.

Keywords: Stevia, nitrogen, fresh leaf yield

Introduction
Stevia (*Stevia rebaudiana* Bertoni) is a herbaceous and perennial plant of Asteraceae family. It is native to Paraguay (Yadav *et al.*, 2010) [15]. It has achieved economic importance due to the different natural sweet compounds called steviol glycosides (SG) which are obtained from its leaves. The normal proportions of the four major glycosides observed in stevia are: stevioside (5-10%), rebaudioside-A (2-4%), rebaudioside-C (1-2%) and dulcoside (0.5-1%). Stevia is the sweetest gift from mother nature called as sweet leaf of Paraguay, sweet herb, honey leaf, honey yerba, candy leaf etc. Stevia is also known as the "sweetest plant of the world" (Joshi *et al.*, 2006) [3]. Since longtime, the main source of sugar is sugarcane and sugar beet contributing calories which are not to be consumed by diabetics. The dried leaves are about 30-45 times sweeter than sugar importantly without any Calories and extracted stevioside is 300-350 times sweeter than sugar but rebaudioside-A is nearly 450 times sweeter than sugar (Das *et al.*, 2006) [2]. These compounds are pH stable, heat stable, not fermentable and posses health promoting potential. Along with sweetness, stevia has some bitter aftertaste due to the presence of some essential oils, tannins and flavonoids (Phillips, 1987) [8]. Stevia is grown as a crop in many countries including Japan, China, India, Korea, USA, Canada, Mexico, Russia, Indonesia, Tanzania, Brazil, Paraguay, Canada and Argentina. Global stevia market is rapidly increasing, in 2014, the global consumption of stevia as food ingredient was estimated at 5,100 tonnes and it is projected to reach 8,507 tonnes by 2020. Stevia can play an important role in India which tops the diabetic population in the world with 30 million patients, and this is expected to increase to 80 million by 2025 as per the reports of world health organization. Though, China is the largest stevia producer in the world market, Japan and Korea are the main consumers).

The Indian farmers have also started taking up stevia cultivation following the large demand for diabetic market here. In India, farmers have started growing stevia in some parts of Rajasthan, Punjab, Uttar Pradesh, West Bengal, Madhya Pradesh, Andhra Pradesh, Karnataka, Chhattisgarh and West Bengal etc. Since the production potential of stevia in India is 2-3 t ha^{-1} of dry leaves as against 1-2 t ha^{-1} in China, it has definite advantage over China. The climatic conditions in most parts of India are quite favorable for stevia cultivation. Stevia is safe for use by both diabetics and hypo glycaemics due to its low glycaemic index. The leaf extract of this plant has anti-diabetic property (Misrha *et al.*, 2011) [6]. Stevia products and extracts are now used in a variety of food products. The steviol glycosides obtained from stevia leaves are used as an alternative to sugars in variety of foods and beverages. It is used as table sugar, in soft drinks, pastry, pickles, tobacco products, candy, jam, yoghurt, chewing gum, sorbets etc. stevia plants consist of 1.4% nitrogen (N), 0.3% phosphorus (P),

and 2.4% potassium (K) at maximum dry matter accumulation. Nutritional requirements of stevia are reported to be low to moderate since this crop is adapted to poor quality soils in its natural habitat at Paraguay.
To date, little is known about the effect of levels of nitrogen (N) fertilization on stevia growth (Ramesh *et al*. 2006; Aladakatti *et al*. 2012) [9, 1]. Stevia is a new crop for India so there is a lack of practical experiences on its cultivation and agronomy. At present not much information is available on the cultivation and agronomic requirements of stevia *viz*., plant population, planting geometry, fertilizer doses, irrigation requirement etc. Therefore, in view of the above, the present investigation were undertaken to find out N requirement and planting density of stevia is an important tool in order to achieve high crop yields, high quality level, balanced use of fertilizers, low environmental impact, suitable adaptation and mitigation strategies able to encourage responsible sustainable development under eastern Uttar Pradesh condition.

Materials and Methods

The experiment was carried out during *Zaid* season 2018, at the Crop Research Farm, Department of Agronomy, Naini Agricultural Institute, SHUATS, Prayagraj (U.P.) which is located at 25° 39’ 42’’ N latitude, 81° 67’ 56” E longitude and 98 m altitude above the mean sea level (MSL). In the experiment, impact of different levels of nitrogen and spacing were evaluated on the clayey loam soil which was basic in reaction (pH 7.8), low in organic carbon content (0.3%), available nitrogen (183.50 Kg ha^{-1}), available phosphorus (15.63 Kg ha^{-1}) and available potassium (197.63 Kg ha^{-1}). The experiment was laid out in randomized block design with three replication and nine treatments. Details of treatment were as first factor levels of nitrogen (N_1 = 50 kg ha^{-1}, N_2 = 75 kg ha^{-1}, N_3 = 100 kg ha^{-1}) and second factor three spacing (S_1 = 30 x 20cm, S_2 = 40 x 20cm, S_3 = 50 x 20cm) and their treatment combinations as N_1S_1 = 50 kg ha^{-1} nitrogen at 30 cm x 20cm; N_1S_2 = 50 kg ha^{-1} nitrogen at 40cm x 20cm; N_1S_3 = 50 kg ha^{-1} nitrogen at 50 cm x 20 cm; N_2S_1 = 75 kg ha^{-1} nitrogen at 30cm x 20cm; N_2S_2 = 75 kg ha^{-1} nitrogen at 40cm x 20cm; N_2S_3 = 75 kg ha^{-1} nitrogen at 50cm x 20cm; N_3S_1 = 100 kg ha^{-1} nitrogen at 30cm x 20cm; N_3S_2 = 100 kg ha^{-1} nitrogen at 40cm x 20cm; N_3S_3 = 100 kg ha^{-1} nitrogen at 50cm x 20cm. One month old seedlings were used for transplanting as per designs in their respective plots. Inorganic nutrients were applied at the time of transplanting *viz*; urea (N 46%), single super phosphate (P_2O_5 16%), and muriate of potash (K_2O 60%). Half dose of Nitrogen at the time of transplanting and the remaining half was applied in two equal splits; 1st half 30 days after transplanting and 2nd half 60 days after transplanting, whereas, the full doses of phosphorus and potassium at the rate of 40 kg ha^{-1} were applied at the time of transplanting. Need based irrigation was supplied at an interval of 5-7 days. In the experiment biometric observation were recorded at 20 days interval upto 80 DAT. Three plants/plot were randomly selected for recording observations. The crop was harvested at 90 DAT from the bottom leaving 5 cm up to the ground level and dried under shade for 4-5 days. Observation on growth during the experimental period includes plant height (cm), number of branches $plant^{-1}$, number of leaf $plant^{-1}$. Observation at the harvest was done to measure the yield components (Fresh leaf yield, fresh stem yield) as well as harvest index. The data were statistically analyzed as procedures given by Panse and Sukhatme (1978) [7].

Results and Discussion

Effect of Spacing and Levels of Nitrogen

Plant height

Plant height is an important growth factor in stevia. Data was recorded at an interval of 20, 40, 60 and 80 DAT is presented in Table 1. At 20 and 40 DAT the effect of treatments were non-significant. At 60 and 80 DAT, significantly maximum plant height was observed in treatment T_9 *i.e.*, 100 kg ha^{-1} nitrogen at (50cm x 20cm) spacing, whereas the minimum plant height was recorded from treatment T_1 *i.e.*, 50 kg ha^{-1} nitrogen at (30cm x 20cm) spacing. However, treatments T_6 *i.e.*, 75 kg ha^{-1} nitrogen at (50cm x 20cm) spacing, T_7 *i.e.*, 100 kg ha^{-1} nitrogen at (30cm x 20cm) spacing and T_8 *i.e.*, 100 kg ha^{-1} nitrogen at (40cm x 20cm) spacing were found to be statistically at par to treatment T_9 *i.e.*, 100 kg ha^{-1} nitrogen at (50cm x 20cm) spacing at 60 and 80 DAT. The positive influence of nitrogen on plant height might be due to the fact that nitrogen is required for cell division and cell elongation which triggers the growth of meristematic tissue and the efficient utilization of this by the plants manifested in production of taller plants. The results are in conformity with the findings of Taleie *et al.* (2012) [13].

Number of Branches per plant

The variation in plant height is responsible for the variation in number of branches/plant. Data was recorded at an interval of 20, 40, 60 and 80 DAT is presented in Table 2. At 20 and 40 DAT the effect of treatments were found non-significant. At 60 and 80 DAT, treatment T_9 *i.e.*, 100 kg ha^{-1} nitrogen at (50cm x 20cm) spacing produced significantly maximum number of branches whereas the minimum number of branches was recorded in treatment T_1 *i.e.*, 50 kg ha^{-1} nitrogen at (30cm x 20cm) spacing. However, treatments T_3 *i.e.*, 50 kg ha^{-1} nitrogen at (50cm x 20cm) spacing, T_5 *i.e.*, 75 kg ha^{-1} nitrogen at (40cm x 20cm) spacing, T_6 *i.e.*, 75 kg ha^{-1} nitrogen at (50cm x 20cm) spacing, and T_8 *i.e.*, 100 kg ha^{-1} nitrogen at (40cm x 20cm) spacing were found to be statistically at par with treatment T_9 *i.e.*, 100 kg ha^{-1} nitrogen at (50 cm x 20 cm) spacing. Rashid *et al.* (2013) [11] found that N fertilization in stevia influenced the number of branches per plant significantly. The results are in conformity with the findings of Tavarini *et al.* (2015) [14]. No. of branches per plant was increased in wider spacing. The results are in conformity with the findings of Maheshwar (2005) [5], Rashid *et al.* (2015) [10] and Tadesse Btru *et al.* (2017) [12] who reported higher branches number $plant^{-1}$ at the wider spacing than the closest in stevia.

Number of Leaves per plant

Number of leaves is an important factor which determines the yield of stevia. Data was recorded at an interval of 20, 40, 60 and 80 DAT is presented in Table 3. Data at 20 DAT, number of leaves was not affected by different treatments. At 40 DAT, treatment T_9 *i.e.*, 100 kg ha^{-1} nitrogen at (50 cm x 20 cm) spacing demonstrated significantly higher number of leaves while at 60 and 80 DAT, treatment T_8 *i.e.*, 100 kg ha^{-1} nitrogen at (40cm x 20cm) spacing produced maximum number of leaves. Maheswar reported that the increasing dosage of nitrogen from 60 kg N ha^{-1} to 105 kg N ha^{-1} could increase number of leaf $plant^{-1}$ for about 49.13%. Higher nitrogen nutrition increases the number of branches and leaves $plant^{-1}$ of stevia. Similar findings were reported by Maheshwar (2005) [5]. The green leaves are important plant growth indices which determine the capacity of plant to trap solar energy for photosynthesis. The yield attributing characters like number of branches per plant and number of leaves per plant were higher under wider spacing. The results are in conformity with those of Rashid *et al.* (2015) [10].

Yield

Observation regarding yield and yield attributes are presented in the table 4.

Fresh leaf yield (t ha^{-1})

At harvest, the fresh leaf yield was influenced significantly under various treatments. Treatment, T_7 *i.e.*, 100 kg ha^{-1} nitrogen at (30cm x 20cm) spacing recorded higher fresh leaf yield and treatment T_4 *i.e.*, 75 kg ha^{-1} nitrogen at (30cm x 20cm) spacing was found at par with treatment T_7. However the lowest fresh leaf yield was observed under treatment T_3 *i.e.*, 50 kg ha^{-1} nitrogen at (50cm x 20cm) spacing. Higher number of branches and leaves attributed to higher fresh leaf yield of stevia. The role of nitrogen on the dry leaf yield of stevia is increasing the levels of nitrogen increases yield. The lower yield at increased planting geometry was mainly due to the fact that decreased plant population with increase in spacing. The results are in conformity with the findings of Rashid *et al.* (2015) [10].

Fresh stem yield (t ha^{-1})

Treatment T_7 *i.e.*, 100 kg ha^{-1} nitrogen at (30cm x 20cm) spacing demonstrated significantly higher fresh stem yield and treatment T_4 *i.e.*, 75 kg ha^{-1} nitrogen at (30cm x 20cm) spacing was found at par with treatment. Further the lowest fresh stem yield was observed under treatment T_3 *i.e.*, 50 kg ha^{-1} nitrogen at (30cm x 20cm) spacing. The results are in line with the findings of Aladakatti *et al.* (2012) [1] who found the similar findings with planting geometry of 30cm x 20cm.

Harvest Index (%)

The maximum harvest index (43.67%) was recorded in treatment T_4 *i.e.*, 75 kg ha^{-1} nitrogen at (30cm x 20cm) spacing. This was significantly superior to all other treatments except T_3 *i.e.*, 50 kg ha^{-1} nitrogen at (50cm x 20cm) spacing, T_5 *i.e.*, 75 kg ha^{-1} nitrogen at (40cm x 20cm) spacing and T_7 *i.e.*, 100 kg ha^{-1} nitrogen at (30cm x 20cm) spacing which were at par with treatment T_4. The lowest harvest index (39.33%) was recorded under treatment T_1 *i.e.*, 50 kg ha^{-1} nitrogen at (30cm x 20cm) spacing. The possible reason for lowering harvest index may be due to the less amount nutrient supplied which results in lower leaf yield which is the economic part of stevia because of lower translocation of photosynthates. The results are in conformity with the findings of Aladakatti *et al.* (2012) [1].

Table 1: Impact of Spacing and levels of Nitrogen on Plant height of Stevia

Treatment No.	Treatment Combinations	Plant height (cm)			
		20 DAT	40 DAT	60 DAT	80 DAT
T_1	50 kg ha^{-1} nitrogen at 30cm x 20cm spacing	20.92	26.56	34.17	39.11
T_2	50 kg ha^{-1} nitrogen at 40cm x 20cm spacing	18.94	31.61	38.28	41.42
T_3	50 kg ha^{-1} nitrogen at 50cm x 20cm spacing	25.00	34.75	39.86	43.97
T_4	75 kg ha^{-1} nitrogen at 30cm x 20cm spacing	20.60	32.02	38.88	43.73
T_5	75 kg ha^{-1} nitrogen at 40cm x 20cm spacing	24.78	30.57	40.40	48.00
T_6	75 kg ha^{-1} nitrogen at 50cm x 20cm spacing	25.58	34.50	42.83	48.13
T_7	100 kg ha^{-1} nitrogen at 30cm x 20cm spacing	23.74	31.16	41.51	47.71
T_8	100 kg ha^{-1} nitrogen at 40cm x 20cm spacing	26.55	35.65	43.06	48.31
T_9	100 kg ha^{-1} nitrogen at 50cm x 20cm spacing	25.81	32.80	45.10	51.24
	SEm±	2.36	3.41	1.35	1.15
	CD (P = 0.05)	NS	NS	4.04	3.44

Table 2: Impact of Spacing and levels of Nitrogen on Number of Branches of Stevia

Treatment No.	Treatment Combinations	Number of Branches plant^{-1}			
		20 DAT	40 DAT	60 DAT	80 DAT
T_1	50 kg ha^{-1} nitrogen at 30cm x 20cm spacing	6.07	10.16	15.15	16.09
T_2	50 kg ha^{-1} nitrogen at 40cm x 20cm spacing	7.63	13.88	17.14	18.17
T_3	50 kg ha^{-1} nitrogen at 50cm x 20cm spacing	7.88	14.11	18.99	21.00
T_4	75 kg ha^{-1} nitrogen at 30cm x 20cm spacing	7.58	13.77	16.83	18.93
T_5	75 kg ha^{-1} nitrogen at 40cm x 20cm spacing	6.75	12.89	17.07	21.61
T_6	75 kg ha^{-1} nitrogen at 50cm x 20cm spacing	10.44	16.11	19.84	22.26
T_7	100 kg ha^{-1} nitrogen at 30cm x 20cm spacing	6.99	10.55	17.85	19.13
T_8	100 kg ha^{-1} nitrogen at 40cm x 20cm spacing	7.83	15.22	20.81	22.25
T_9	100 kg ha^{-1} nitrogen at 50cm x 20cm spacing	6.46	12.54	20.99	23.37
SEm±		0.81	1.64	1.10	1.30
CD (P = 0.05)		NS	NS	3.31	3.91

Table 3: Impact of Spacing and levels of Nitrogen on Number of Leaves of Stevia

Treatment No.	Treatment Combinations	Number of leaves plant^{-1}			
		20 DAT	40 DAT	60 DAT	80 DAT
T_1	50 kg ha^{-1} nitrogen at 30cm x 20cm spacing	60.53	107.50	201.40	267.18
T_2	50 kg ha^{-1} nitrogen at 40cm x 20cm spacing	58.56	110.12	203.61	270.26
T_3	50 kg ha^{-1} nitrogen at 50cm x 20cm spacing	65.57	118.67	214.38	276.48
T_4	75 kg ha^{-1} nitrogen at 30cm x 20cm spacing	69.43	112.38	208.87	284.83
T_5	75 kg ha^{-1} nitrogen at 40cm x 20cm spacing	78.01	114.39	218.15	287.78
T_6	75 kg ha^{-1} nitrogen at 50cm x 20cm spacing	67.38	121.53	221.65	295.75
T_7	100 kg ha^{-1} nitrogen at 30cm x 20cm spacing	71.79	121.13	216.42	310.02

T_8	100 kg ha^{-1} nitrogen at 40cm x 20cm spacing	73.62	120.48	225.99	314.55
T_9	100 kg ha^{-1} nitrogen at 50cm x 20cm spacing	72.74	123.36	224.26	313.29
	SEm±	4.13	3.43	4.52	6.82
	CD (P = 0.05)	NS	10.28	13.54	20.44

Table 4: Impact of Spacing and levels of Nitrogen on yield and yield attributes of Stevia

Treatment No.	Treatment Combinations	Fresh leaf yield (t ha^{-1})	Fresh stem yield (t ha^{-1})	Harvest index (%)
T_1	50 kg ha^{-1} nitrogen at 30cm x 20cm spacing	7.04	10.86	39.33
T_2	50 kg ha^{-1} nitrogen at 40cm x 20cm spacing	6.10	8.91	40.67
T_3	50 kg ha^{-1} nitrogen at 50cm x 20cm spacing	5.15	7.02	42.33
T_4	75 kg ha^{-1} nitrogen at 30cm x 20cm spacing	9.98	12.87	43.67
T_5	75 kg ha^{-1} nitrogen at 40cm x 20cm spacing	6.55	9.16	41.67
T_6	75 kg ha^{-1} nitrogen at 50cm x 20cm spacing	5.37	7.95	40.33
T_7	100 kg ha^{-1} nitrogen at 30cm x 20cm spacing	10.54	13.81	43.34
T_8	100 kg ha^{-1} nitrogen at 40cm x 20cm spacing	7.18	10.79	40.00
T_9	100 kg ha^{-1} nitrogen at 50cm x 20cm spacing	6.28	9.28	40.33
	SEm±	0.25	0.39	0.94
	CD (P = 0.05)	0.75	0.19	2.84

Conclusion

As per findings from experiment it is concluded that for better plant growth treatment T_9 *i.e.*, 100 kg ha^{-1} with 50 cm x 20 cm spacing gave maximum growth response, however maximum yield was recorded with application of treatment T_7 *i.e.*, 100 kg ha^{-1} with 30cm x 20cm spacing due to maximum plant population. Hence, for better economic return application of 100 kg ha^{-1} with 30 cm x 20 cm spacing, treatment T_7 can be adopted to get maximum yield/economic return.

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