PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(4): 219-225
https://doi.org/10.14719/pst.3263

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Synergistic effects of nitrogen and naphthalene acetic acid on growth, yield and nutritional content of *Stevia* (*Stevia rebaudiana* Bertoni)

Md. Shariful Islam[1], Md. Riaz [2], Md. Sohag Hossain[2], Mehedi Amin[2], Md. Sirajul Islam Khan[2] & Mohammed Ariful Islam[2]*

[1]Bangladesh Sugarcrop Research Institute, Thakurgaon -5102, Bangladesh
[2]Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka -1207, Bangladesh

*Email: * arif.agch@sau.edu.bd, arif.sau.agch@gmail.com

OPEN ACCESS

ARTICLE HISTORY

Received: 01 March 2024
Accepted: 20 May 2024

Available online
Version 1.0 : 20 September 2024
Version 2.0 : 01 October 2024

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

CITE THIS ARTICLE

Islam M S, Riaz M, Hossain M S, Amin M, Khan M S I K, Islam M A. Synergistic effects of nitrogen and naphthalene acetic acid on growth, yield and nutritional content of *Stevia* (*Stevia rebaudiana* Bertoni) . Plant Science Today. 2024; 11(4): 219-225. https://doi.org/10.14719/pst.3263

Abstract

Stevia farming in Bangladesh is increasingly significantly due to limitations in cane sugar production and the rise in diabetic cases. This study examined how nitrogen and naphthalene acetic acid (NAA) affect *Stevia* development, production and nutritional content on the experimental farm of the regional station of the Bangladesh Sugar crop Research Institute, Thakurgaon. Various nitrogen treatments (N_1: untreated, N_2: 105 kg ha^{-1}, N_3: 140 kg ha^{-1}, N_4: 175 kg ha^{-1}, N_5: 210 kg ha^{-1}) and NAA applications (H_1: untreated, H_2: 50 mg/L, H_3: 100 mg/L, H_4: 150 mg/L) were administered employing a randomized complete block design (RCBD). Results showed that N_3 and H_3 treatments produced the highest yield, averaging 10.06 % more than other doses. *Stevia* leaf nutrients like nitrogen (3.47 %), potassium (0.19 %) and magnesium (0.15 %) were also influenced by N_3H_3 treatment. The study suggests that for *Stevia* cultivation in northern Bangladesh, an optimal nitrogen application rate of 140 kg ha^{-1} and an NAA concentration of 100 mg/L are recommended.

Keywords

Stevia; nitrogen; NAA; yield; nutrient content

Introduction

Stevia, botanically classified as *Stevia rebaudiana* Bertoni, is a member of the Asteraceae family and is categorized as an herbaceous plant. Originally hailing from Paraguay (1), commercial *Stevia* cultivation began in 1964 in this region (2). Known for its inherent sugariness without any calories, this plant has greatly impacted the economics of many countries, including Canada, the United States, Brazil, Korea, Mexico, Indonesia and Tanzania (3). While *Stevia*'s pure extract, stevioside, has no calories and a sweetness level roughly 300 times greater than sugar, the dried leaves of the plant are about 30 times sweeter than sugar but have no calories (4). *Stevia* is a popular natural sweetener used in foods and beverages. It stands out from other non-caloric sugar alternatives because it can withstand heat, resist acid hydrolysis and does not ferment (5). Because of its many beneficial properties, *Stevia* is useful for purposes more than only as a sweetener. These include supporting to management of obesity, encouraging weight loss, improving dental health, controlling hypertension, enhancing oral hygiene, lowering cravings for carbohydrates, improving skin tone, accelerating wound healing, lowering cravings for alcohol and tobacco and having antihyperglycemic effects in diabetics (6). The global market for medicinal plant materials, including *Stevia*, currently stands at around 1 trillion US dollars and is projected to exceed 5 trillion US dollars by the year 2050 (7).

Nitrogen stands out as a crucial macronutrient essential for robust plant development, particularly in stimulating prolonged vegetative growth and fostering expansive leaf growth. In the case of *Stevia*, a plant prized for its vegetative productivity, nitrogen supplementation plays a pivotal role (8). Inadequate nitrogen levels have been observed to have a significant inhibitory effect on vegetative development, resulting in a reduction in leaf biomass and lowering the total yield potential of *Stevia* for commercial uses. While prior studies on *Stevia's* nutrient requirements have primarily been conducted abroad (9-12), Nasrin's research (13) underscores a positive correlation between nitrogen levels and various growth parameters, leaf yield and overall yield attributes in *Stevia* cultivation. Furthermore, in agricultural practices, the utilization of plant growth regulators (PGRs) has emerged as a strategy to boost crop yields (14-17). Lee's investigation (18) specifically explored the benefits of foliar application of NAA (naphthaleneacetic acid), highlighting its positive impacts on plant height, leaf count per plant and fruit size across different crops, leading to enhanced seed yields. Consequently, Lee suggests leveraging PGRs as a means to optimize production outcomes.

In Bangladesh, research has delved into various nitrogen and NAA combinations across different crops. One study observed that employing 60 kg of combined nitrogen per ha, along with 50 mg/L of NAA, led to a sesame yield of 1.49 t/ha (19). Similarly, in wheat cultivation, the BARI GOM-26 variety demonstrated optimal nitrogen utilization efficiency (NUE) when treated with 50 mg/L NAA and 50 % N-fertilizer during blooming (20). Furthermore, peak NUE was achieved during the grain-filling phase by employing 25 mg/L NAA and 75 % nitrogen fertilizer. The BARI GOM-25 cultivar also exhibited improved NUE when a blend of N-fertilizer and NAA was used (21). For baby corn growth, dividing N-fertilizer application into split doses, alongside 40 mg/L of NAA, notably enhanced growth parameters (22).

The simultaneous application of nitrogen fertilizer and NAA has been shown to have a significant influence on the growth, production and nutritional qualities of *Stevia* plants. Understanding the synergistic effects of this combination is critical to improving the crop's overall performance and quality. *Stevia* leaves typically contain approximately 10.0 to 18.0 % protein and 52 to 64.06 % carbohydrates on a dry weight basis (23, 24). The stevioside content varies depending on the leaf's dry weight and growing conditions (25, 26). Therefore, there is a need to enhance agricultural practices to boost both biomass yield and stevioside production in *Stevia*. Previous studies have highlighted the impact of nitrogen and NAA on *Stevia* growth, development, yield and nutrient uptake (3, 27). However, there remains a gap in understanding the combined effects of these elements under local conditions, warranting further investigation. This study aims to close this gap by investigating the combined effects of externally applied nitrogen and NAA on *Stevia* plant growth, productivity and nutritional composition.

Materials and Methods

Location

The experimental site is located at the coordinates of 25^0 38' North latitude and 88^0 41' East longitude, positioned at an elevation of 34.5 m above sea level. Fig. 1 illustrates the field study zone situated within the Bangladesh Sugarcrop Research Institute's regional station in Thakurgaon, Bangladesh.

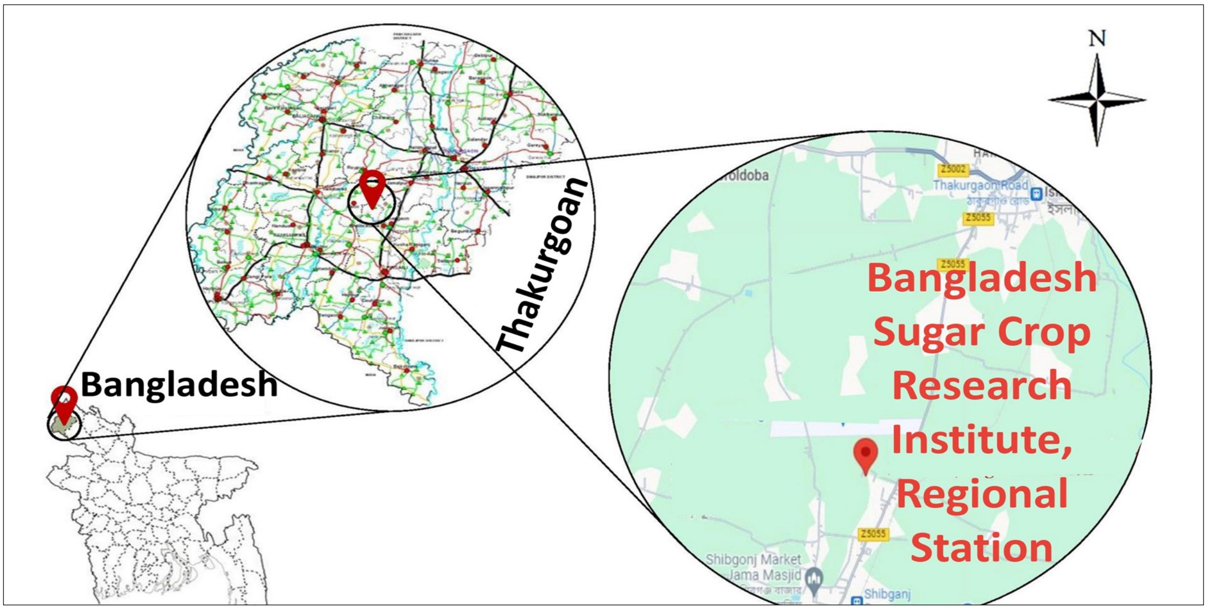

Fig. 1. Study area. Map showing the location of Bangladesh Sugarcrop Research Institute (BSRI), Regional Station, Thakurgoan, Bangladesh. Map created using Google map and Local Government Engineering Department Maps of Bangladesh.

Soil and land

The study was conducted in a farm field with well-drained soil situated in an Agro-Ecological Zone categorized as AEZ No.1, within the Old Himalayan Piedmont plain. The soil type identified is sandy loam, falling under the classification of hyperthermic Aeric Haplaquept according to the Inceptisol order. This soil type typically exhibits a limited number of layers, formed under fluctuating moisture levels and varying temperatures. The characterization of the soil's general and chemical properties was conducted in February 2021, through the collection of an initial composite soil sample from the top 15 cm depth, as outlined in the Fertilizer Recommendation Guide (28).

Climate and weather

Fig. 2 illustrates the highest, lowest and average air temperatures (ºC), relative humidity (%), total precipitation (mm), sunshine duration (hours month^{-1}) and average monthly Pan evaporation (mm) throughout the experiment.

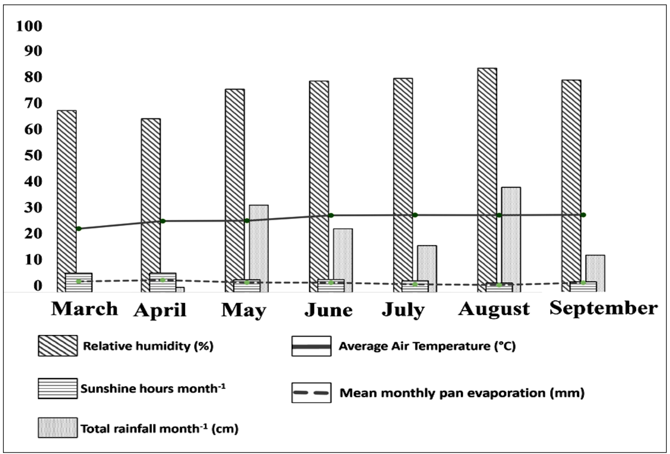

Fig. 2. Climate information collected during the study period (March to September 2021) at BSRI, Regional Station, Thakurgaon, Bangladesh.

Experimental design

The study employed a Randomized Complete Block Design (RCBD), featuring 2 variables: varying levels of nitrogen doses and NAA. The experiment comprised a total of 60 plots, each measuring 2 m × 2 m in size.

Land preparation and application of treatments

Following appropriate soil cultivation, an initial fertilizer application was carried out, incorporating a total of 50 kg ha^{-1} of Triple Super Phosphate (TSP) during the final soil preparation stage. Subsequently, nitrogen (N) in the form of urea was administered in 3 parts: one-third as a side dressing 30 days after transplanting (DAT) and the remaining portion along with Muriate of Potash (MOP) totaling 40 kg ha^{-1}, was divided into 2 equal parts for top dressing at 60 and 90 DAT. Additionally, Naphthaleneacetic acid (NAA) was sprayed at monthly intervals starting from 15 DAT until reaching 90 days. The crop was harvested 6 months after transplantation.

Procedure of data collection

The height of plants was assessed before harvesting, starting from the ground level and extending to the topmost leaf, with measurements expressed in cm. The count of leaves per plant was manually conducted and recorded. The leaf area for each plant was determined using a leaf area meter after separating the leaves. Plant samples from each pot in the plot were collected and subjected to drying at 60 °C for approximately 48 h. Subsequently, the dried samples were ground to pass through a 20-mesh sieve using a grinding mill. These processed samples were then placed into paper bags and stored in desiccators until further analysis.

Chemical analysis

N content estimation utilized the Micro-Kjeldahl method (29). Other nutrient analysis involved extracting 1 g of finely ground leaves using a di-acid mixture (HNO_3:$HClO_4$= 2:1), following Jackson's method (30). Subsequently, P, K, S, Ca and Mg were assessed. Phosphorus levels were determined calorimetrically employing the stannous chloride method and a spectrophotometer (Supertonic® GENESYS TM 5 336001 CAT) at 660 nm (30). Potassium content was measured using a flame photometer at 768 nm wavelengths. Sulphur content was determined turbidimetrically with a spectrophotometer (Supertonic® GENESYS TM 5 336001 CAT) at 420 nm within 2 to 8 min, following Black's procedure (31). Calcium and magnesium contents were measured using an Atomic Absorption Spectrometer (AAS).

Statistical analysis

Analysis of variance was done with the help of MSTATC. The mean differences of the treatments were adjusted by the Least Significant Difference (LSD) test.

Results and Discussion

Effect on plant height, number of leaves plant^{-1}, leaf area plant^{-1} (cm^{-2}), dry leaf yield plant^{-1} (g)

The height of *Stevia* plants showed a gradual increase as they progressed through different growth stages, ranging from 21 days after transplanting (DAT) to 147 DAT. Notably, significant variations in plant height were observed across different levels of nitrogen and NAA application, with the N_3H_3 treatment (nitrogen 140 kg ha^{-1} × NAA 100 mg/L) yielding the tallest plants at all growth stages, measuring 27.15, 38.78, 53.45, 66.54, 75.87, 90.47 and 98.80 cm respectively, compared to the control group (Table 1). This finding aligns with previous research, where also documented positive effect on *Stevia* plant height under various cultivation conditions (32, 33). However, it's worth noting that excessive nitrogen doses beyond N_3H_3 began to negatively impact plant growth. Interestingly, when nitrogen dosage was highest and NAA was lowest (N_5H_1), plant height closely resembled that of the N_1H_1 treatment. Nevertheless, a consistent N_5 dose coupled with increased NAA dosage led to another uptick in plant height.

The findings consistently showed that leaf area per plant and dry leaf yield per plant were notably highest at the N_3H_3 treatment compared to the control, as depicted in Supplementary Tables 1, 2 and Fig. 3. This aligns with previous research (3), which noted comparable leaf dry weights between plants grown in non-calcareous and acidic soils. While a broader range of leaf dry weights per plant, from 15 to 35 g, the highest number of leaves per plant was observed at the N_4H_3 dosage (34), as detailed in Supplementary Table 3. Similarly, a positive correlation between nutrient doses and leaf count in *Stevia* cultivation using hydroponics (33). On the other hand, excessive nitrogen doses did not enhance phytochemical content, potentially explaining the limited growth associated with high nitrogen rates (35). Moreover, it's noted that elevated nitrogen concentrations could adversely affect both aboveground and belowground plant growth (11).

Table 1. Effect of N and NAA on *Stevia* plant height.

Treatments Interaction	Plant height (cm)						
	21DAT	42 DAT	63DAT	84DAT	105DAT	126DAT	147DAT
N_1H_1	20.18c	34.12c	42.80d	55.34e	63.45d	74.38h	91.45b
N_1H_2	22.87a-c	36.76ab	46.65b-d	58.34b-e	68.29b-d	80.23e-h	94.56ab
N_1H_3	26.76 a	38.67ab	51.28ab	64.63a-c	72.38a-c	88.29a-d	96.28ab
N_1H_4	24.65a-c	36.87ab	49.67a-c	62.57a-e	69.52a-d	81.52c-h	94.66ab
N_2H_1	20.43c	34.54bc	43.83cd	57.35c-e	64.32 d	74.40h	91.50b
N_2H_2	23.12a-c	36.87ab	47.78a-d	60.47a-e	69.39a-d	82.46b-g	95.26ab
N_2H_3	26.87a	38.72ab	51.90ab	65.21ab	73.78a-c	88.82a-c	97.84ab
N_2H_4	25.10ab	36.92ab	49.72a-c	62.87a-d	69.88a-d	82.74b-g	95.32ab
N_3H_1	21.12bc	35.43a-c	44.35cd	58.32b-e	66.71cd	77.37f-g	93.28ab
N_3H_2	24.65a-c	37.56ab	48.17a-d	62.37a-e	72.39a-c	85.35a-e	96.47ab
N_3H_3	27.15a	38.78a	53.45a	66.54a	75.87a	90.47a	98.80a
N_3H_4	26.56a	37.60ab	51.35ab	64.31a-c	73.67a-c	87.22a-e	97.24ab
N_4H_1	20.54c	34.56bc	42.85d	57.46c-e	64.37 d	75.55gh	92.16ab
N_4H_2	23.18a-c	36.94ab	47.80a-d	61.95a-e	70.36a-d	83.25a-f	95.34ab
N_4H_3	26.88a	38.74ab	52.10ab	66.03a	74.34ab	89.38ab	98.35ab
N_4H_4	25.34ab	36.96ab	49.76a-c	63.19a-d	70.38a-d	83.30a-f	95.41ab
N_5H_1	20.19c	34.20bc	42.82d	56.23de	64.10 d	74.39h	91.46b
N_5H_2	22.92a-c	36.79ab	46.73b-d	59.29a-e	68.83a-d	80.89d-h	94.70ab
N_5H_3	26.80a	38.70ab	51.95ab	65.14ab	72.80a-c	88.75a-c	97.32ab
N_5H_4	24.70a-c	36.90ab	49.68a-c	62.97a-d	69.65a-d	81.77c-h	94.69ab
LSD (0.05)	4.533	5.163	6.028	7.415	7.579	7.426	6.912

Mean values in a column having the same letter (s) do not differ significantly at the 5 % level of DMRT. **NS** = Non-significant, ** indicates 1 % level of significance. * indicates 5 % level of significance. Here, $\mathbf{N_1}$: Control, $\mathbf{N_2}$: 105 kg ha^{-1}, $\mathbf{N_3}$: 140 kg ha^{-1}, $\mathbf{N_4}$: 175 kg ha^{-1}, $\mathbf{N_5}$: 210 kg ha^{-1}, $\mathbf{H_1}$: control, $\mathbf{H_2}$: 50 mg/L, $\mathbf{H_3}$: 100 mg/L and $\mathbf{H_4}$: 150 mg/L.

Effect on nutrient content in the Stevia leaf

Various concentrations of nitrogen (N) and Naphthaleneacetic acid (NAA) only show notable impacts on nitrogen (N), potassium (K) and magnesium (Mg) levels among the analyzed nutrients (Table 2). The highest nitrogen content (3.47 %) was observed in plots treated with N_3H_3. Conversely, the control plot exhibited the lowest nitrogen content at 2.56 %, significantly lower than all other treatments. This trend is consistent for potassium and magnesium as well. The N_3H_3 treatment displayed the highest potassium (0.19 %) and magnesium (0.15 %) levels, while the lowest levels recorded were 0.15 % for potassium and 0.12 % for magnesium respectively. Additionally, there was a consistent decrease in nutrient content as nitrogen doses increased. Notably, the N_5H_1 dose yielded similar results to N_1H_1. Similarly, the impact of different foliar urea dosages on nitrogen (N) levels and absorption in *Stevia* leaves during harvest. Increased urea levels were found to elevate leaf nitrogen content regardless of soil composition (3). However, nitrogen intake did not fully align with the increase in leaf nitrogen content. Notably, the application of urea to foliage boosted nitrogen uptake, with the highest uptake recorded at 2.0 g. Interestingly, higher urea doses (U2.5 and U3.0) led to a decrease in nitrogen absorption, irrespective of soil type. Furthermore, *Stevia* exhibited greater uptake and nitrogen content in acidic soil compared to non-calcareous soil. Regarding the effect of foliar urea spraying, wheat leaves exhibited enhanced nitrogen absorption following urea treatment (36). Specifically, a 4 % urea foliar spray was identified as the most effective for promoting wheat nitrogen uptake.

Conclusion

The research findings demonstrated that variations in nitrogen (N) levels and NAA (naphthalene acetic acid) doses significantly influenced key morphological aspects of *Stevia* plants, such as their height, leaf area per plant and dry leaf yield per plant, in comparison to untreated plants. The application of nitrogen at 140 kg ha^{-1} combined with 100 mg/L NAA (N_3H_3) resulted in the highest nitrogen content at 3.47 %. Furthermore, essential chemical parameters like potassium (K), magnesium (Mg) and zinc (Zn) showed

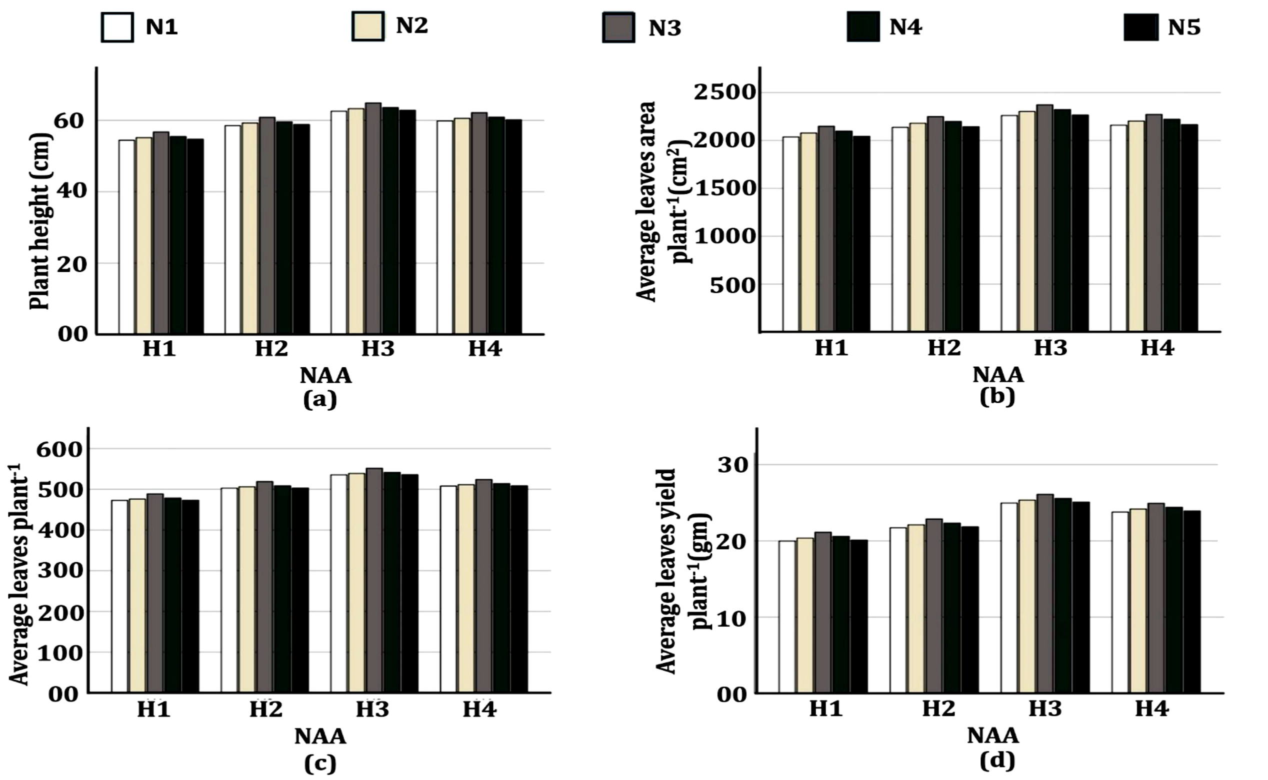

Fig. 3. Effect of N and NAA on plant height (**a**), average leaf area plant^{-1} (**b**), average leaves plant^{-1} (**c**) and average dry leaves yield plant^{-1} (**d**) of *Stevia*. All the measurements were converted to average values for better visualization. Here, **N_1**: Control, **N_2**: 105 kg ha^{-1}, **N_3**: 140 kg ha^{-1}, **N_4**: 175 kg ha^{-1}, **N_5**: 210 kg ha^{-1}, **H_1**: control, **H_2**: 50 mg/L, **H_3**: 100 mg/L and **H_4**: 150 mg/L.

Table 2. Effect of N and NAA on N, P, K, S, Ca, Mg and Zn of *Stevia* leaf.

Treatments	N %	P %	K %	S %	Ca %	Mg %
N_1H_1	2.56c	0.16	0.15g	0.26	1.31	0.12g
N_1H_2	2.69bc	0.16	0.16e-g	0.27	1.45	0.13c-f
N_1H_3	2.98a-c	0.17	0.18a-e	0.29	1.66	0.14a-d
N_1H_4	2.72bc	0.16	0.16c-g	0.28	1.48	0.14b-e
N_2H_1	2.61bc	0.16	0.16fg	0.27	1.35	0.12fg
N_2H_2	2.74bc	0.17	0.16c-g	0.28	1.48	0.14b-d
N_2H_3	3.10a-c	0.18	0.19a-c	0.30	1.69	0.14a-c
N_2H_4	2.78bc	0.17	0.17a-g	0.28	1.52	0.14a-d
N_3H_1	2.75bc	0.17	0.17b-g	0.28	1.42	0.13d-g
N_3H_2	2.93a-c	0.18	0.18a-f	0.28	1.63	0.15a-c
N_3H_3	3.47a	0.18	0.19a	0.30	1.72	0.15a
N_3H_4	3.01a-c	0.18	0.18a-e	0.29	1.66	0.15ab
N_4H_1	2.64bc	0.16	0.16e-g	0.27	1.37	0.12e-g
N_4H_2	2.78bc	0.18	0.16b-g	0.28	1.50	0.14a-d
N_4H_3	3.19ab	0.18	0.19ab	0.30	1.70	0.15ab
N_4H_4	2.80bc	0.18	0.17a-g	0.28	1.55	0.14a-c
N_5H_1	2.58c	0.16	0.16fg	0.27	1.33	0.12g
N_5H_2	2.70bc	0.16	0.16d-g	0.27	1.46	0.14b-e
N_5H_3	3.02a-c	0.17	0.18a-d	0.30	1.67	0.14a-c
N_5H_4	2.74bc	0.17	0.17b-g	0.29	1.51	0.14b-d
LSD (0.05)	0.601	NS	0.024	NS	NS	0.014

Mean values in a column having the same letter (s) do not differ significantly at the 5 % level of DMRT. **NS** = Non-significant, ** indicates 1 % level of significance. ***** indicates 5 % level of significance. Here, **N_1**: Control, **N_2**: 105 kg ha^{-1}, **N_3**: 140 kg ha^{-1}, **N_4**: 175 kg ha^{-1}, **N_5**: 210 kg ha^{-1}, **H_1**: control, **H_2**: 50 mg/L, **H_3**: 100 mg/L and **H_4**: 150 mg/L.

notable enhancements. Hence, the combination of nitrogen at 140 kg ha^{-1} and NAA at 100 mg/L presents a promising approach for optimizing *Stevia* cultivation, particularly in the northern regions of Bangladesh.

Acknowledgements

The authors acknowledge the support of the Bangladesh Sugarcrop Research Institute to allow us to conduct the research during the Corona pandemic period and SAURES (Sher-e-Bangla Agricultural University Research System) to provide financial support in the 2021-22 financial year with grant award number 387.

Authors' contributions

MSI was the day to day guide for the field experiment part, he also help the student in collection and preparation samples. The experiment was conducted during Covid time. RM took the responsibility to draft the paper including the text, graph, table compilation and also took part in chemical analysis. MSH carried out the field experiment, collected and prepared the samples from field with the financial help of SAURES project and physical-technical help of BSRI. MA and MSIK did the chemical analysis part. MAI, arranged the funding, planned the experiment, help to conduct the chemical analysis, write the MS thesis and help to write the manuscript in regular basis.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Supplementary data

Supplementary Table 1. Effect of N and NAA on Leaf area $plant^{-1}$ of *Stevia*

Supplementary Table 2. Effect of N and NAA on dry leaf yield $plant^{-1}$ of *Stevia*

Supplementary Table 3. Effect of N and NAA on the number of leaves $plant^{-1}$ of *Stevia*

References

1. Soejarto DD, Compadre CM, Medon PJ, Kamath SK, Kinghorn AD. Potential sweetening agents of plant origin. II. field search for sweet-tasting *Stevia* species. Economic Botany. 1983;37(1):71-79. https://doi.org/10.1007/BF02859308
2. Uddin MS, Chowdhury MSH, Khan M, Uddin MB, Ahmed R, Baten M. *In vitro* propagation of *Stevia rebaudiana* Bert in Bangladesh. African Journal of Biotechnology. 2006;5(13). https://www.ajol.info/index.php/ajb/article/view/43088
3. Zaman MM, Chowdhury MAH, Chowdhury T, [eds.]. Integrated effects of poultry manure and chemical fertilizer on the growth, leaf yield and stevioside content of *Stevia*. Journal of the Bangladesh Agricultural University. 2015; https://doi.org/10.22004/ag.econ.235278
4. Sardessai YN, Bhosle S. Industrial potential of organic solvent tolerant bacteria. Biotechnology Progress. 2004;20(3):655-60. https://doi.org/10.1021/bp0200595
5. Kinghorn AD, Soaejarto DD. *Stevia* sweeteners. Alternative Sweeteners. 1991;2:157-71.
6. Gregersen S, Jeppesen PB, Holst JJ, Hermansen K. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism. 2004;53(1):73-76. https://doi.org/10.1016/j.metabol.2003.07.013
7. Das K, Dang R, Shivananda TN, Sur P. Interaction between phosphorus and zinc on the biomass yield and yield attributes of the medicinal plant *Stevia* (*Stevia rebaudiana*). The Scientific World Journal. 2005;5:390-95. https://doi.org/10.1100/tsw.2005.49
8. Hasnain M, Chen J, Ahmed N, Memon S, Wang L, Wang Y *et al*. The effects of fertilizer type and application time on soil properties, plant traits, yield and quality of tomato. Sustainability. 2020;12(21):9065. https://doi.org/10.3390/su12219065
9. Inugraha M, Maghfoer D, Widaryanto E. Response of *Stevia* (*Stevia rebaudiana* Bertoni M) to nitrogen and potassium fertilization. Int J Agric Vet Sci. 2014;10(1):47-55. https://doi.org/10.9790/2380-071014755
10. Akbari F, Arminian A, Kahrizi D, Fazeli A, Ghaheri M. Effect of nitrogen sources on gene expression of *Stevia rebaudiana* Bertoni under *in vitro* conditions. Cellular and Molecular Biology. 2018;64(2):11-16. https://doi.org/10.14715/cmb/2018.64.2.3
11. Uçar E, Turgut K, Özyiğit Y, Özek T, Özek G. The effect of different nitrogen levels on yield and quality of *Stevia* (*Stevia rebaudiana* bert.). Journal of Plant Nutrition. 2018;41(9):1130-37. https://doi.org/10.1080/01904167.2018.1431673
12. Youssef MA, Yousef AF, Ali MM, Ahmed AI, Lamlom SF, Strobel WR *et al*. Exogenously applied nitrogenous fertilizers and effective microorganisms improve plant growth of *Stevia* (*Stevia rebaudiana* Bertoni) and soil fertility. AMB Express. 2021;11(1):133. https://doi.org/10.1186/s13568-021-01292-8
13. Nasrin D. Effect of nitrogen on the growth, yield and nutrient uptake by *Stevia* (MS Thesis), Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh; 2008.
14. Michael G, Nickell LG. Plant growth regulators. Agricultural Uses. Springer-verlag berlin-heidelberg-new york. 1982;173 seiten,29 abb.,dm 47, 50. Zeitschrift für Pflanzenernährung und Bodenkunde. 1983;146(1):128-28. https://doi.org/10.1002/jpln.19831460115
15. Sarkar PK, Haque MS, Abdul Karim M. Effects of GA and IAA and their frequency of application on morphology, yield 3. Pakistan Journal of Agronomy. 2002 Jan 1;1(4):119-22. https://doi.org/10.3923/ja.2002.119.122
16. Sarker BC, Roy B, Nashirullah MT, Islam MA, Sarker SC, Rahmatullah NM. Root growth, hydraulic conductance and cell wall properties of rice root under interactive effect of growth regulator and limited water. Journal of Agroforestry and Environment. 2009;3(2):227-30.
17. Bakhsh I, Khan HU, Khan MQ, Javaria S. Effect of naphthalene acetic acid and phosphorus levels on the yield potential of transplanted coarse rice. Sarhad J Agric. 2011;27(2):161-65.
18. Lee JI, Kang KH, Lee EU. Studies on new sweetening resource plant *Stevia* (*Stevia rebaudiana* Bertoni) in Korea. I. Effects of transplanting-date shifting by cutting and seeding dates on agronomic characteristics and dry leaf yields of *Stevia*. The Research Reports of the Office of Rural Development. Crops. 1979.
19. Siddik A, Shirazy BJ, Islam MM, Hoque A, Haque M. Combined effect of nitrogen and NAA on the yield of sesame (*Sesamum indicum* L.). Scientia. 2016;13(1):1-9. https://doi.org/10.15192/PSCP.SA.2016.13.1.19

20. Islam S, Jahan N. Effects of NAA and different nitrogen levels on nutrient uptake by BARI Gom-26 (*Triticum aestivum* L.). Journal of the Asiatic Society of Bangladesh, Science. 2016 Jun 30;42 (1):69-76. https://doi.org/10.3329/jasbs.v42i1.31751

21. Adam AG, Jahan N, Rashid P. Effects of NAA and nitrogen fertilizer on NPK uptake and nitrogen use efficiency of BARI gom-25 (*Triticum aestivum* L.). Bangladesh Journal of Botany. 2020;49 (3):643-51. https://doi.org/10.3329/bjb.v49i3.50004

22. Muthukumar VB, Velayudham K, Thavaprakaash N. Growth and yield of baby corn (*Zea mays* L.) as influenced by plant growth regulators and different time of nitrogen application. Research Journal of Agriculture and Biological Sciences. 2005;1(4):303-07.

23. Srivastava S, Srivastava M. Improving tolerance of *Stevia rebaudiana* to water deficit stress through foliar spray of potassium nitrate. International Journal of Applied and Natural Sciences. 2016;5(4):1-8.

24. Segura-Campos M, Barbosa-Martín E, Matus-Basto Á, Cabrera-Amaro D, Murguía-Olmedo M, Moguel-Ordoñez Y *et al*. Comparison of chemical and functional properties of *Stevia rebaudiana* (Bertoni) varieties cultivated in mexican Southeast. American Journal of Plant Sciences. 2014;5(3):286-93. https://doi.org/10.4236/ajps.2014.53039

25. Nepovim A, Drahosova H, Valicek P, Vanek T. The effect of cultivation conditions on the content of stevioside in *Stevia rebaudiana* Bertoni plants cultivated in the Czech Republic. Pharmaceutical and Pharmacological Letters. 1998;8(1):19-21.

26. Geuns JMC. Stevioside. Phytochemistry. 2003;64(5):913-21. https://doi.org/10.1016/S0031-9422(03)00426-6

27. Chalapathi MV, Shivaraj B, Ramakrishana VR. Nutrient uptake and yield of *Stevia (Stevia rebaudiana* Bertoni) as influenced by methods of planting and fertilizer levels. Crop Research-Hisar. 1997;14:205-08.

28. BARC. Fertilizer recommendation guide. 2018. https://moa.portal.gov.bd/sites/default/files/files/moa.portal.gov.bd/page/9d1b92d4_1793_43af_9425_0ed49f27b8d0/FRG-2018%20%28English%29.pdf

29. Bremner JM, Mulvaney CS. Nitrogen-total. In: Page AL (ed.) Agronomy monographs. Madison, WI, USA: American Society of Agronomy, Soil Science Society of America. 2015;p. 595-624. https://doi.org/10.2134/agronmonogr9.2.2ed.c31

30. Jackson WA, Flesher D, Hageman RH. Nitrate uptake by dark-grown corn seedlings: Some characteristics of apparent induction. Plant Physiology. 1973;51(1):120-27. https://doi.org/10.1104/pp.51.1.120

31. Black JW, Duncan WAM, Shanks RG. Comparison of some properties of pronethalol and propranolol. British Journal of Pharmacology and Chemotherapy. 1965;25(3):577-91. https://doi.org/10.1111/j.1476-5381.1965.tb01782.x

32. Noor-E-Ferdous RA, Roy B, Sarker BC, Islam MS. Effects of different cultivation methods on growth, yield and nutrient content of *Stevia*. International Journal of Agriculture and Medicinal Plants. 2021;2(4):10-18.

33. Tavakoli Hasanaklou H, Ghadim AEK, Moradi F, Mirzaei HH, Hasanaklou NT. Morphological and physiological characteristics of *Stevia rebaudiana* cultivated under different nitrogen supplements and growth regulators. Russian Journal of Plant Physiology. 2022;69(3):49. https://doi.org/10.1134/S1021443722030141

34. Zewdinesh D, Beemnet M, Weldemariam G. *Stevia* production guideline.

35. Tavarini S, Sgherri C, Ranieri AM, Angelini LG. Effect of nitrogen fertilization and harvest time on steviol glycosides, flavonoid composition and antioxidant properties in *Stevia rebaudiana* Bertoni. Journal of Agricultural and Food Chemistry. 2015;63 (31):7041-50. https://doi.org/10.1021/acs.jafc.5b02147

36. Khan P, Memon MY, Imtiaz M, Aslam M. Response of wheat to foliar and soil application of urea at different growth stages. Pakistan Journal of Botany. 2009;41(3):1197-204.