



RESEARCH ARTICLE

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

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## 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<sub>1</sub>: untreated, N<sub>2</sub>: 105 kg ha<sup>-1</sup>, N<sub>3</sub>: 140 kg ha<sup>-1</sup>, N<sub>4</sub>: 175 kg ha<sup>-1</sup>, N<sub>5</sub>: 210 kg ha<sup>-1</sup>) and NAA applications (H<sub>1</sub>: untreated, H<sub>2</sub>: 50 mg/L, H<sub>3</sub>: 100 mg/L, H<sub>4</sub>: 150 mg/L) were administered employing a randomized complete block design (RCBD). Results showed that N<sub>3</sub> and H<sub>3</sub> 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<sub>3</sub>H<sub>3</sub> treatment. The study suggests that for *Stevia* cultivation in northern Bangladesh, an optimal nitrogen application rate of 140 kg ha<sup>-1</sup> 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 anti-hyperglycemic 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

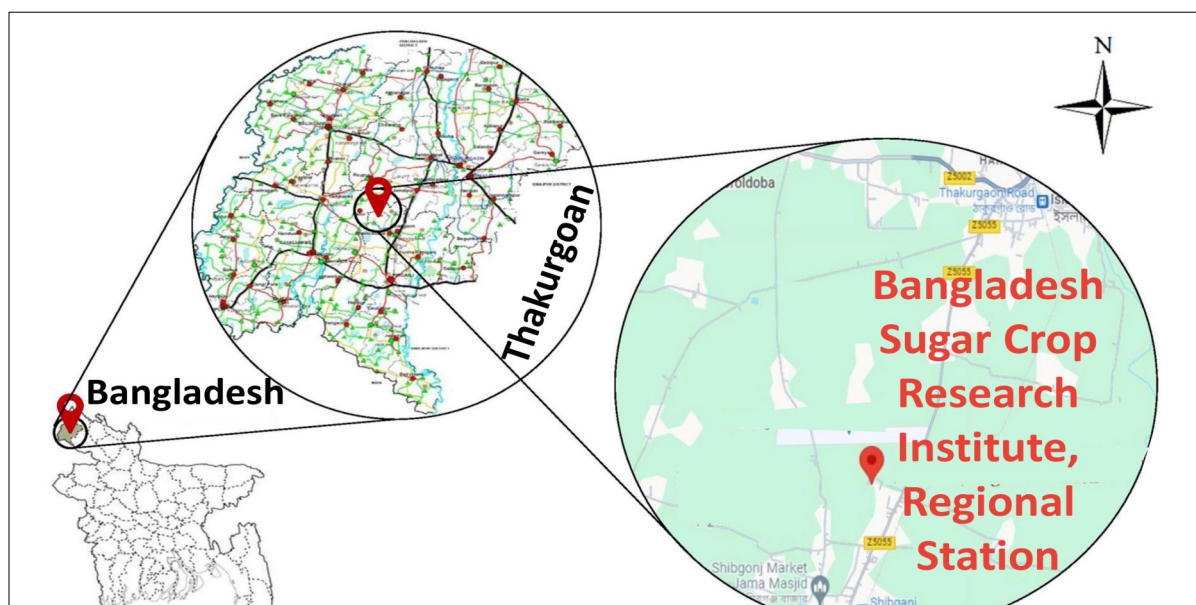
employing 25 mg/L NAA and 75 % nitrogen fertilizer. The BARI GOM-25 cultivar also exhibited improved NUE when a blend of N-fertilizer and NAA was used (21). For baby corn growth, dividing N-fertilizer application into split doses, alongside 40 mg/L of NAA, notably enhanced growth parameters (22).

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° 38' North latitude and 88° 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, Thakurgaon, 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 Aeris 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 (°C), relative humidity (%), total precipitation (mm), sunshine duration (hours month<sup>-1</sup>) and average monthly Pan evaporation (mm) throughout the experiment.

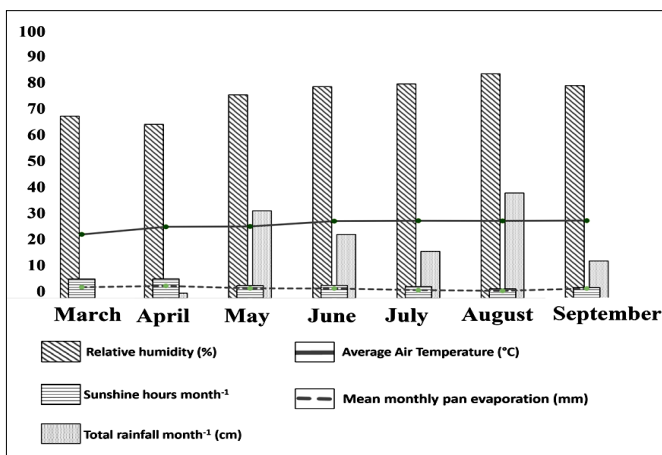


Fig. 2. Climate information collected during the study period (March to September 2021) at BSRI, Regional Station, Thakurgaon, Bangladesh.

## 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<sup>-1</sup> 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<sup>-1</sup>, 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<sub>3</sub>:HClO<sub>4</sub>= 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<sup>-1</sup>, leaf area plant<sup>-1</sup> (cm<sup>2</sup>), dry leaf yield plant<sup>-1</sup> (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<sub>3</sub>H<sub>3</sub> treatment (nitrogen 140 kg ha<sup>-1</sup> × 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<sub>3</sub>H<sub>3</sub> began to negatively impact plant growth. Interestingly, when nitrogen dosage was highest and NAA was lowest (N<sub>5</sub>H<sub>1</sub>), plant height closely resembled that of the N<sub>1</sub>H<sub>1</sub> treatment. Nevertheless, a consistent N<sub>5</sub> 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<sub>3</sub>H<sub>3</sub> 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<sub>4</sub>H<sub>3</sub> 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).

es 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 com-

**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 <sub>1</sub> H <sub>1</sub>	20.18c	34.12c	42.80d	55.34e	63.45d	74.38h	91.45b
N <sub>1</sub> H <sub>2</sub>	22.87a-c	36.76ab	46.65b-d	58.34b-e	68.29b-d	80.23e-h	94.56ab
N <sub>1</sub> H <sub>3</sub>	26.76 a	38.67ab	51.28ab	64.63a-c	72.38a-c	88.29a-d	96.28ab
N <sub>1</sub> H <sub>4</sub>	24.65a-c	36.87ab	49.67a-c	62.57a-e	69.52a-d	81.52c-h	94.66ab
N <sub>2</sub> H <sub>1</sub>	20.43c	34.54bc	43.83cd	57.35c-e	64.32 d	74.40h	91.50b
N <sub>2</sub> H <sub>2</sub>	23.12a-c	36.87ab	47.78a-d	60.47a-e	69.39a-d	82.46b-g	95.26ab
N <sub>2</sub> H <sub>3</sub>	26.87a	38.72ab	51.90ab	65.21ab	73.78a-c	88.82a-c	97.84ab
N <sub>2</sub> H <sub>4</sub>	25.10ab	36.92ab	49.72a-c	62.87a-d	69.88a-d	82.74b-g	95.32ab
N <sub>3</sub> H <sub>1</sub>	21.12bc	35.43a-c	44.35cd	58.32b-e	66.71cd	77.37f-g	93.28ab
N <sub>3</sub> H <sub>2</sub>	24.65a-c	37.56ab	48.17a-d	62.37a-e	72.39a-c	85.35a-e	96.47ab
N <sub>3</sub> H <sub>3</sub>	27.15a	38.78a	53.45a	66.54a	75.87a	90.47a	98.80a
N <sub>3</sub> H <sub>4</sub>	26.56a	37.60ab	51.35ab	64.31a-c	73.67a-c	87.22a-e	97.24ab
N <sub>4</sub> H <sub>1</sub>	20.54c	34.56bc	42.85d	57.46c-e	64.37 d	75.55gh	92.16ab
N <sub>4</sub> H <sub>2</sub>	23.18a-c	36.94ab	47.80a-d	61.95a-e	70.36a-d	83.25a-f	95.34ab
N <sub>4</sub> H <sub>3</sub>	26.88a	38.74ab	52.10ab	66.03a	74.34ab	89.38ab	98.35ab
N <sub>4</sub> H <sub>4</sub>	25.34ab	36.96ab	49.76a-c	63.19a-d	70.38a-d	83.30a-f	95.41ab
N <sub>5</sub> H <sub>1</sub>	20.19c	34.20bc	42.82d	56.23de	64.10 d	74.39h	91.46b
N <sub>5</sub> H <sub>2</sub>	22.92a-c	36.79ab	46.73b-d	59.29a-e	68.83a-d	80.89d-h	94.70ab
N <sub>5</sub> H <sub>3</sub>	26.80a	38.70ab	51.95ab	65.14ab	72.80a-c	88.75a-c	97.32ab
N <sub>5</sub> H <sub>4</sub>	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, **N<sub>1</sub>**: Control, **N<sub>2</sub>**: 105 kg ha<sup>-1</sup>, **N<sub>3</sub>**: 140 kg ha<sup>-1</sup>, **N<sub>4</sub>**: 175 kg ha<sup>-1</sup>, **N<sub>5</sub>**: 210 kg ha<sup>-1</sup>, **H<sub>1</sub>**: control, **H<sub>2</sub>**: 50 mg/L, **H<sub>3</sub>**: 100 mg/L and **H<sub>4</sub>**: 150 mg/L.

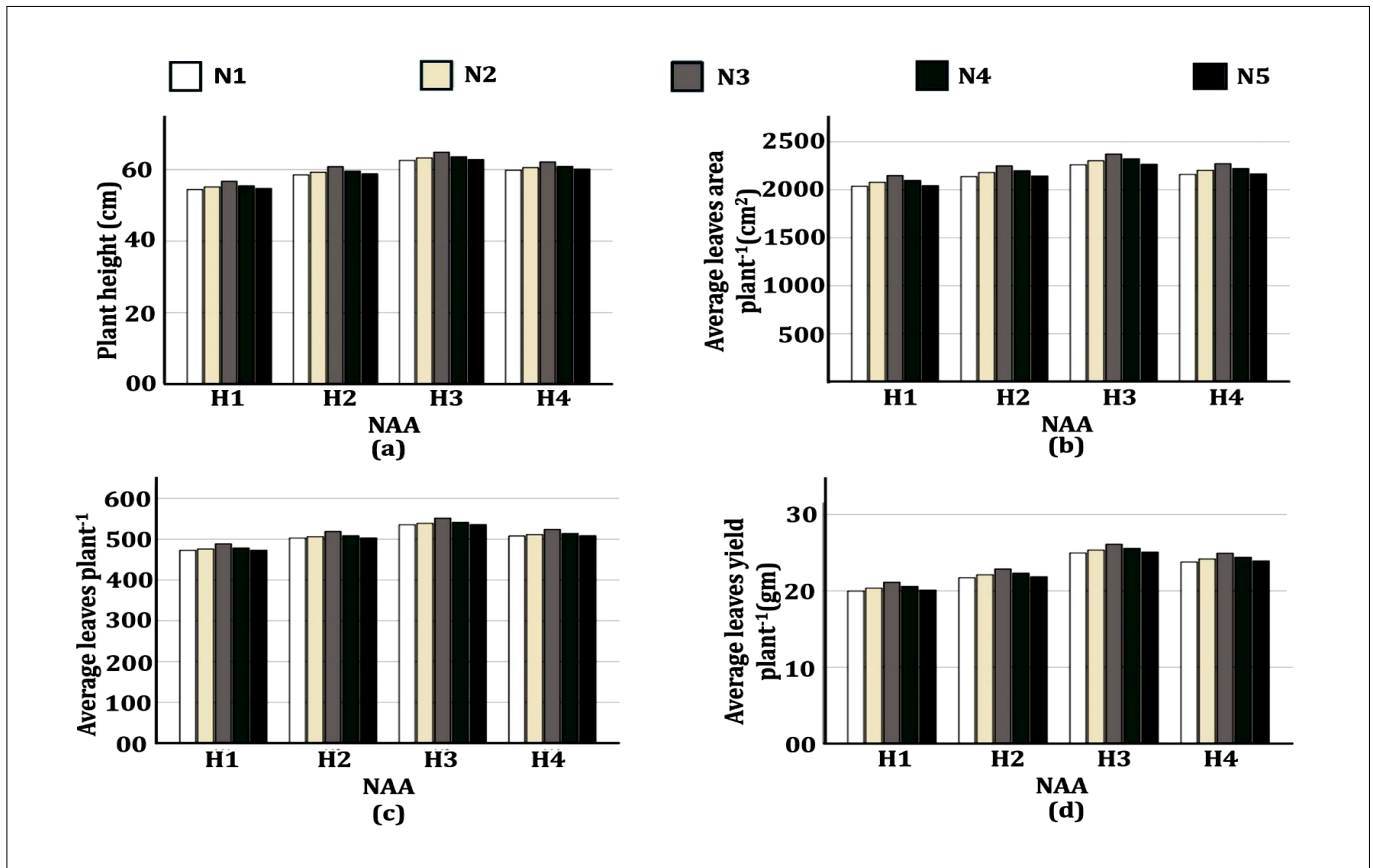
### Effect on nutrient content in the *Stevia* leaf

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<sub>3</sub>H<sub>3</sub>. 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<sub>3</sub>H<sub>3</sub> 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<sub>5</sub>H<sub>1</sub> dose yielded similar results to N<sub>1</sub>H<sub>1</sub>. Similarly, the impact of different foliar urea dosag-

pared 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<sup>-1</sup> combined with 100 mg/L NAA (N<sub>3</sub>H<sub>3</sub>) 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<sup>-1</sup> (b), average leaves plant<sup>-1</sup> (c) and average dry leaves yield plant<sup>-1</sup> (d) of *Stevia*. All the measurements were converted to average values for better visualization. Here, **N<sub>1</sub>**: Control, **N<sub>2</sub>**: 105 kg ha<sup>-1</sup>, **N<sub>3</sub>**: 140 kg ha<sup>-1</sup>, **N<sub>4</sub>**: 175 kg ha<sup>-1</sup>, **N<sub>5</sub>**: 210 kg ha<sup>-1</sup>, **H<sub>1</sub>**: control, **H<sub>2</sub>**: 50 mg/L, **H<sub>3</sub>**: 100 mg/L and **H<sub>4</sub>**: 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 <sub>1</sub> H <sub>1</sub>	2.56c	0.16	0.15g	0.26	1.31	0.12g
N <sub>1</sub> H <sub>2</sub>	2.69bc	0.16	0.16e-g	0.27	1.45	0.13c-f
N <sub>1</sub> H <sub>3</sub>	2.98a-c	0.17	0.18a-e	0.29	1.66	0.14a-d
N <sub>1</sub> H <sub>4</sub>	2.72bc	0.16	0.16c-g	0.28	1.48	0.14b-e
N <sub>2</sub> H <sub>1</sub>	2.61bc	0.16	0.16fg	0.27	1.35	0.12fg
N <sub>2</sub> H <sub>2</sub>	2.74bc	0.17	0.16c-g	0.28	1.48	0.14b-d
N <sub>2</sub> H <sub>3</sub>	3.10a-c	0.18	0.19a-c	0.30	1.69	0.14a-c
N <sub>2</sub> H <sub>4</sub>	2.78bc	0.17	0.17a-g	0.28	1.52	0.14a-d
N <sub>3</sub> H <sub>1</sub>	2.75bc	0.17	0.17b-g	0.28	1.42	0.13d-g
N <sub>3</sub> H <sub>2</sub>	2.93a-c	0.18	0.18a-f	0.28	1.63	0.15a-c
N <sub>3</sub> H <sub>3</sub>	3.47a	0.18	0.19a	0.30	1.72	0.15a
N <sub>3</sub> H <sub>4</sub>	3.01a-c	0.18	0.18a-e	0.29	1.66	0.15ab
N <sub>4</sub> H <sub>1</sub>	2.64bc	0.16	0.16e-g	0.27	1.37	0.12e-g
N <sub>4</sub> H <sub>2</sub>	2.78bc	0.18	0.16b-g	0.28	1.50	0.14a-d
N <sub>4</sub> H <sub>3</sub>	3.19ab	0.18	0.19ab	0.30	1.70	0.15ab
N <sub>4</sub> H <sub>4</sub>	2.80bc	0.18	0.17a-g	0.28	1.55	0.14a-c
N <sub>5</sub> H <sub>1</sub>	2.58c	0.16	0.16fg	0.27	1.33	0.12g
N <sub>5</sub> H <sub>2</sub>	2.70bc	0.16	0.16d-g	0.27	1.46	0.14b-e
N <sub>5</sub> H <sub>3</sub>	3.02a-c	0.17	0.18a-d	0.30	1.67	0.14a-c
N <sub>5</sub> H <sub>4</sub>	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<sub>1</sub>**: Control, **N<sub>2</sub>**: 105 kg ha<sup>-1</sup>, **N<sub>3</sub>**: 140 kg ha<sup>-1</sup>, **N<sub>4</sub>**: 175 kg ha<sup>-1</sup>, **N<sub>5</sub>**: 210 kg ha<sup>-1</sup>, **H<sub>1</sub>**: control, **H<sub>2</sub>**: 50 mg/L, **H<sub>3</sub>**: 100 mg/L and **H<sub>4</sub>**: 150 mg/L.

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

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## 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<sup>-1</sup> of *Stevia*

Supplementary Table 2. Effect of N and NAA on dry leaf yield plant<sup>-1</sup> of *Stevia*

Supplementary Table 3. Effect of N and NAA on the number of leaves plant<sup>-1</sup> of *Stevia*

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