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OPTIMIZING UREA APPLICATION FOR ENHANCED GROWTH AND STEVIOL GLYCOSIDE BUILDUP IN STEVIA

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Abstract

Stevia rebaudiana is a high-value natural sweetener crop known for its steviol glycosides, primarily stevioside and rebaudioside A. Nitrogen is a precarious nutrient influencing both vegetative growth and secondary metabolite production in stevia. This study aimed to optimize urea (a widely used nitrogen source) application levels for maximizing stevia biomass and glycoside accumulation. A field experiment was piloted using a randomized complete block design (RCBD) with five nitrogen levels: 0-160 kg N ha^{-1} with difference of 40 kg, designated as T1 to T5. Results exhibited that all growth parameters i.e., plant height, branches, leaf area and dry biomass, improved significantly with enchanting nitrogen rates up to 120 kg N ha^{-1}. The same trend was observed for leaf yield per plant and per hectare, as well as dry leaf yield. Stevioside and rebaudioside A contents, along with total glycoside yield, also peaked at 120 kg N ha^{-1}. Although the highest nitrogen dose (160 kg N ha^{-1}) produced similar results, it did not significantly outperform the 120 kg level, indicating a point of diminishing returns. Soil analysis confirmed improved organic matter and nitrogen availability without adverse effects on pH or electrical conductivity. These findings suggest that 120 kg N ha^{-1} is the optimum dose for achieving maximum productivity and steviol glycoside yield under current field conditions. The study provides evidence-based nitrogen management strategies to enhance the economic and functional value of stevia cultivation. Further multi-location trials are recommended to validate these findings across diverse agro-ecological zones.

Keywords: “Stevia, “Urea”, “Stevioside”, “Rebaudioside”, “Steviol”, “Leaf Yield”.

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INTRODUCTION

Stevia (Stevia rebaudiana Bertoni), a perennial herb of the Asteraceae family, has extended attention globally as a natural, zero-calorie sweetener. Native to Paraguay and Brazil, stevia has become a viable commercial crop in many parts of the world, including Asia, due to the increasing demand for plant-based alternatives to artificial sweeteners 1. The principal compounds liable for its intense sweetness are steviol glycosides—mainly stevioside and rebaudioside A—which are testified to be 200–300 times sweeter than sucrose but without caloric content or glycemic effects 2. The growing market for stevia-based products in food, pharmaceuticals, and nutraceutical industries has necessitated agronomic strategies to maximize both biomass and glycoside yield.

Among the various factors affecting stevia productivity, nutrient management—particularly nitrogen—plays a critical role. Nitrogen is essential for chlorophyll synthesis, protein formation, and vegetative growth in plants 3. However, the nitrogen requirement of stevia is unique, as excessive application may promote vegetative growth at the cost of steviol glycoside accumulation, while insufficient nitrogen can reduce overall biomass and leaf development 4. Urea, a widely used nitrogenous fertilizer, is a cost-effective and easily available source of nitrogen. Yet, its optimization in terms of dose and timing remains a critical area for maximizing stevia's agronomic and biochemical output.

Several studies have demonstrated the positive impact of nitrogen application on stevia growth, yet there is inconsistency regarding the optimal rate. For example, AlJafer et al. 5 reported that moderate levels of urea application significantly improved leaf area, biomass accumulation, and glycoside content, while higher doses had a diminishing return or even toxic effects. Similarly, Silverman6 emphasized that nitrogen application up to 120 kg ha^{-1} enhanced plant height, number of branches, and stevioside yield, but levels beyond this threshold resulted in lodging and poor leaf quality. These observations underline the need for careful calibration of nitrogen levels to avoid imbalance between vegetative growth and secondary metabolite synthesis.

In addition to quantity, the form and timing of nitrogen application influence stevia's physiological performance. Split applications or slow-release formulations can enhance nitrogen-use efficiency and limit leaching losses, particularly in light-textured soils where stevia is often cultivated 7. Moreover, stevia's nitrogen metabolism is closely linked with photosynthetic efficiency and carbon-nitrogen balance, both of which have direct implications for steviol glycoside biosynthesis 8.

Despite the mounting body of literature, there vestiges a gap in considerate the specific nitrogen dynamics under different agroecological conditions and their effects on both agronomic traits and biochemical quality of stevia. In particular, studies focusing exclusively on urea as a nitrogen source, and its dose optimization for enhancing steviol glycoside content alongside biomass, are limited in South Asian and semi-arid contexts. Addressing this gap is essential to develop site-specific nutrient management practices that ensure high-quality stevia production without compromising environmental sustainability.

Therefore, the present study aims to estimate the consequence of different urea application levels on the growth, leaf yield, and steviol glycoside buildup in Stevia rebaudiana. The findings of this study will contribute to formulating efficient nitrogen management strategies that can support sustainable and economically viable stevia cultivation.

RESEARCH METHODS

The field research was piloted at research farm of Agriculture Research Institute Tarnab, Peshawar, all through the spring season of 2023. The region comes under a semi-arid agro-climatic zone with a middling annual rainfall of 300-350 mm, mean extreme temperature of 42 °C, and sandy loam soil with good drainage properties. Soil samples from the experimental field were analyzed before sowing to determine physicochemical properties, pH (7.1), electrical conductivity (EC) (0.24 dSm-1), organic matter (0.50%) and available nitrogen (43 ppm), phosphorus (9.0 ppm) and potassium (138 ppm).

Experimental Design and Treatments

The experiment was laid out in a Randomized Complete Block Design (RCBD) with five urea application levels and three replications. The treatment structure was Urea (kg ha-1) as follows:

- T1: Control (No urea application)
- T2: 40
- T3: 80
- T4: 120
- T5: 160

Each plot measured 3 m × 2 m with plant spacing of 30 cm × 30 cm, accommodating 5 rows per plot. Buffer zones of 0.5 m amid plots and 1.0 m amid replications were maintained to minimize nutrient overlap.

Planting and Crop Management

Stevia rebaudiana seedlings (30 days old) were transplanted into the field during the second week of February. Uniform and healthy seedlings were selected from the nursery. All plots received a basal dose of phosphorus and potassium at the rates of 60 kg P_2O_5 ha^{-1} and 40 kg K_2O ha^{-1}, respectively, at the time of transplanting. Urea was applied in two identical splits—half at 15 days after transplanting (DAT) and the remaining half at 45 DAT.

Standard agronomic practices, including irrigation, weeding, and pest control, were followed uniformly across all treatments. Irrigation was applied weekly using furrow irrigation based on crop water requirements and climatic conditions.

Data Collection

a. Growth Parameters

At 90 days after transplanting, five arbitrarily selected plants from apiece plot were evaluated for the following:

- Plant height
- Number of branches per plant
- Leaf area
- Total dry biomass

b. Yield Attributes

At harvest (around 120 DAT), leaf yield was recorded. Leaves were separated, shade-dried, and weighed to determine dry leaf yield.

c. Steviol Glycoside Estimation

Dried leaf samples were ground and subjected to steviol glycoside extraction following the method of Brandle and Rosa 9 with trivial modifications. Roughly 0.5 g of powdered leaf material was mined with 80% methanol, sifted, and evaluated using High-Performance Liquid Chromatography (HPLC) to quantify stevioside and rebaudioside A content. Glycoside yield was calculated as:

Glycoside yield (kg ha^{-1}) = Dry leaf yield (kg ha^{-1}) × Glycoside concentration (%) / 100

Soil Analysis

Pre- and post-harvest soil samples (0–15 cm depth) were poised from each plot. Samples were air-dried, sieved, and analyzed for pH, EC, organic matter, and available NPK using standard methods.

The collected data were statistically analyzed using Statistix 8.1 software for means comparisons.

RESULTS

Plant Height (cm)

Plant height was pointedly influenced by urea application (Figure 1). A consistent escalation was observed from T1 (control) to T4, with the highest height (85 cm) recorded at 120 kg N ha^{-1} (T4), followed closely by T5 (83 cm). The increase in height with nitrogen application is attributed to enhanced cell elongation and division due to better nitrogen availability, which supports protein synthesis and vegetative growth. However, the non-significant difference between T4 and T5 suggests a threshold beyond which additional nitrogen does not result in further height gains. This trend is consistent with outcomes by Kumar et al. 10, Sharma et al. 11 who reported that excessive nitrogen beyond optimal levels fails to expressively impact plant height due to nutrient saturation.

Number of Branches per Plant

The number of branches per plant also showed a marked increase with urea levels (Figure 1). Maximum branching (13 branches per plant) occurred in T4, whereas T1 had the least (6 branches). Nitrogen promotes axillary bud development and shoot proliferation, leading to increased branching. The reduction at T5 suggests possible hormonal imbalance or excessive vegetative growth hindering lateral development. Similar observations were reported by Saleem et al. 12, Huang et al. 13 who noted an optimum nitrogen range beyond which structural development is compromised.

Leaf Area per Plant (cm^2)

Urea application significantly enhanced leaf area (Figure 1). The maximum leaf area (250 cm^2) was recorded at T4, while T1 had the lowest (100 cm^2). Increased nitrogen availability facilitates higher chlorophyll content and cell expansion, promoting leaf growth. According to Li et al. 14, nitrogen boosts photosynthetic apparatus development, resulting in larger leaves and improved light interception efficiency, especially under optimum nitrogen supply.

Total Dry Biomass (g per Plant)

Total dry biomass was significantly higher in urea-treated plots, peaking at T4 (38 g) and slightly decreasing at T5 (37 g), as shown in Figure 1. The rise in dry matter with urea application reflects increased metabolic activity and assimilate translocation. However, biomass stagnation at higher doses indicates diminishing returns, which may be due to luxury consumption or reduced nutrient-use efficiency 15,16,17.

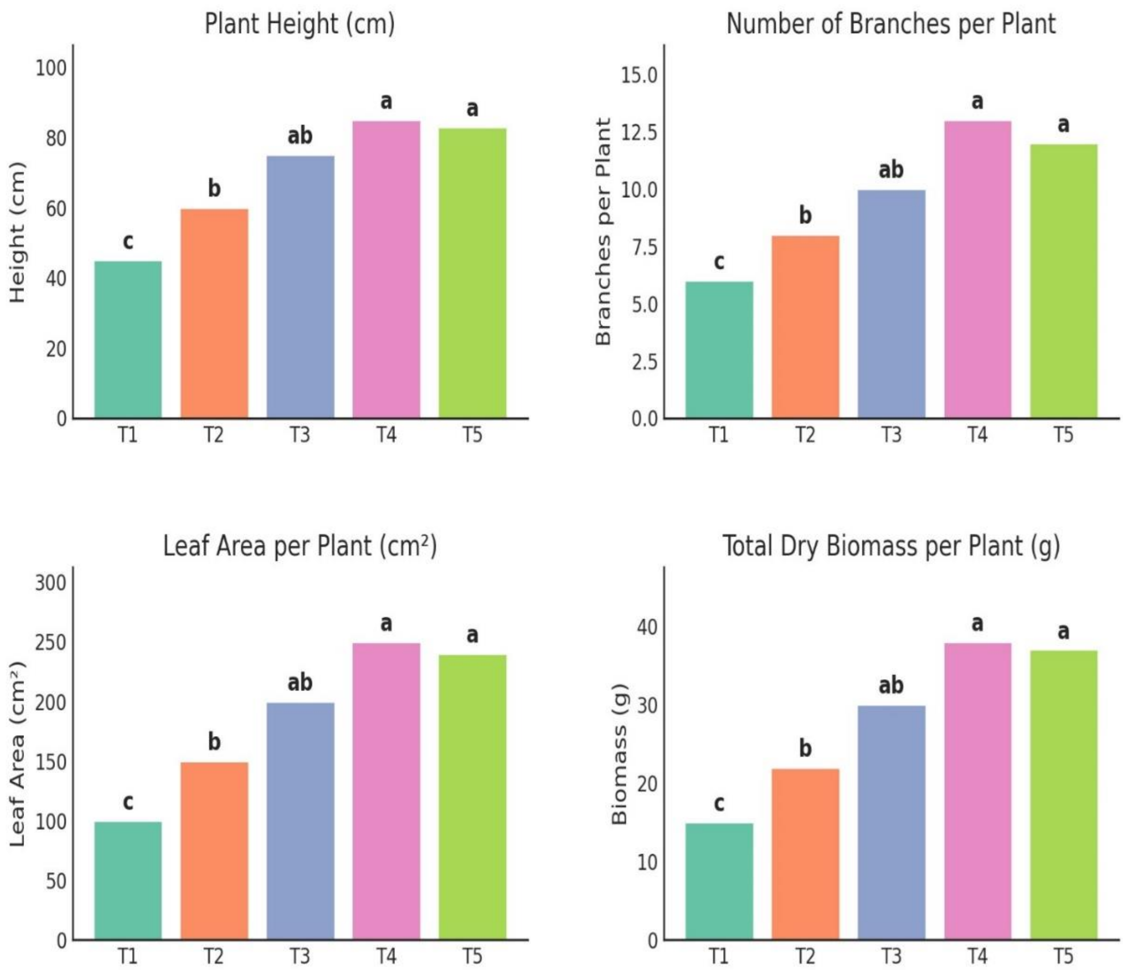

Figure 1. Effect of Urea Levels on plant height, branches plant-1, leaf area and biomass in Stevia rebaudiana

Leaf Yield per Plant and per Hectare

Leaf yield responded significantly to increasing urea levels (Figure 2). The highest yield was recorded at T4 (33 g per plant and 3300 kg ha^{-1}), followed by T5. The control plot yielded the lowest. Nitrogen enhances leaf formation and expansion, leading to greater total biomass accumulation. Rana et al. 18 reported that intermediate nitrogen doses favor biomass allocation to leaves, while excess nitrogen may lead to vegetative overgrowth with reduced economic yield.

Dry Leaf Yield (g per Plant)

Dry leaf yield followed a similar trend as fresh leaf yield, with a maximum at T4 (24 g) and a slight decline at T5 (23 g) (Figure 2). The increase is attributed to enhanced dry matter accumulation in leaf tissues due to nitrogen-induced photosynthetic efficiency. Tanveer et al. 19 noted that appropriate nitrogen doses improve structural leaf tissue development, but excessive application may lead to nutrient imbalance and lower dry matter partitioning.

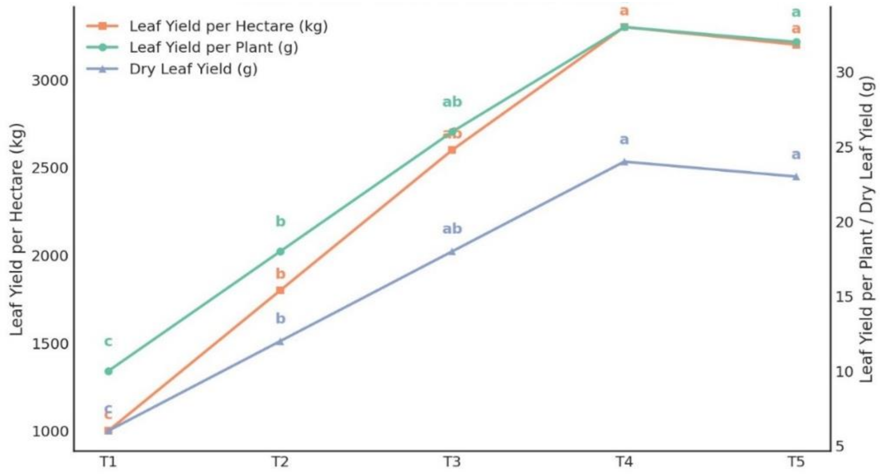

Figure 2. Effect of Urea Levels on leaf yield (per hectare/per plant) and dry leaf yield in Stevia rebaudiana

Stevioside and Rebaudioside A Content (%)

Both stevioside and rebaudioside A content increased with urea application, peaking at T4 (6.9% and 3.2%, respectively) (Table 1). These values were significantly higher than those recorded in T1 (4.5% and 2.1%). Increased nitrogen availability likely facilitated secondary metabolite biosynthesis by improving plant vigor and metabolic activity. However, T5 values slightly decreased, suggesting that excessive nitrogen may dilute metabolite concentrations. These results are consistent with Zhang et al. 20, who demonstrated that glycoside accumulation is optimal at moderate nitrogen levels.

Total Glycosides and Glycoside Yield

Total glycoside content and yield followed the trend of primary glycosides, with the highest values at T4 (10.1% and 333 kg ha^{-1}) and a slight reduction at T5 (Table 1). Glycoside yield is a product of both leaf biomass and concentration; hence, the reduction at T5 is attributed to lower efficiency of nitrogen utilization. Mehmood et al. 21 highlighted that over-fertilization may promote vegetative growth at the expense of secondary metabolite concentration, reducing net glycoside output.

Table 1. Effect of Urea Levels on Steviol Glycoside Content and Yield in Stevia rebaudiana

Treatment	Stevioside (%)	Rebaudioside A (%)	Total Glycosides (%)	Glycoside Yield (kg ha^{-1})
T_1	4.5 c	2.1 c	6.6 c	66 c
T_2	5.8 b	2.6 b	8.4 b	151 b
T_3	6.5 ab	3.0 ab	9.5 ab	247 ab
T_4	6.9 a	3.2 a	10.1 a	333 a
T_5	6.8 a	3.1 a	9.9 a	317 a

Soil Properties (pH, EC, Organic Matter, and Available NPK)

Post-harvest soil analysis showed a moderate increase in available nitrogen and organic matter in plots treated with urea (Table 2). The highest values were recorded in T4 and T5, while soil pH and EC remained statistically unchanged across treatments, indicating that short-term urea application did not acidify or salinize the soil. Available phosphorus and potassium remained stable, suggesting that nitrogen application did not significantly affect their dynamics. These results agree with Nawaz et al. 22, who reported improved nitrogen availability and soil organic carbon without significant changes in pH and EC in short-term fertilization trials.

Table 2. Soil Properties Before and After Urea Application in Stevia rebaudiana Field

Treatment	pH	EC (dS m^{-1})	Organic Matter (%)	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
T_1	7.1 a	0.25 a	0.52 c	45 c	9.2 b	140 a
T_2	7.0 a	0.28 a	0.60 bc	68 b	11.1 ab	142 a
T_3	6.9 a	0.30 a	0.68 ab	91 a	12.0 a	143 a
T_4	6.9 a	0.32 a	0.72 a	95 a	12.3 a	145 a
T_5	6.8 a	0.34 a	0.70 a	92 a	12.1 a	146 a

CONCLUSIONS

The present study clearly demonstrates that urea application significantly influences the growth, biomass, leaf yield, and steviol glycoside content in Stevia rebaudiana. Among the five nitrogen treatments evaluated, the application of 120 kg N ha^{-1} (T4) resulted in the highest plant height, branching, leaf area, and total dry biomass. This treatment also led to the maximum fresh and dry leaf yields, along with enhanced stevioside, rebaudioside A concentrations, and glycoside yield. Although 160 kg N ha^{-1} (T5) showed comparable performance, it did not yield statistically significant improvements over T4, indicating a threshold beyond which additional nitrogen offers diminishing returns. Moreover, soil health indicators such as pH and EC remained stable, while organic matter and available nitrogen levels increased post-application, suggesting no immediate adverse effects on soil quality. These results suggest that 120 kg N ha^{-1} is the optimum urea dose for maximizing both biomass and steviol glycoside production under the given agro-climatic conditions. This level ensures a balanced approach to improving both agronomic and phytochemical traits in stevia without compromising soil sustainability. Future research should evaluate long-term impacts and economic feasibility under different ecological zones and irrigation regimes to develop region-specific nitrogen recommendations.

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