



RESEARCH ARTICLE

Elite *Stevia rebaudiana* clones for sustainable tropical highland cultivation: Agronomic traits and glycoside profiles

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Abstract

Stevia rebaudiana Bertoni (Bertoni) is a high-value source of non-caloric sweeteners, yet its genotype-specific performance under tropical highland conditions remains insufficiently documented. Eleven elite stevia clones grown in the tropical highlands of Indonesia were evaluated, integrating agronomic performance with the steviol glycoside profile. A random block design with four replicates was implemented across three harvests. Majalengka exhibited the greatest plant height (45.06 cm) and total biomass (21.32 g plant⁻¹), while Karang Anyar and Garut also exceeded 20 g plant⁻¹, indicating robust vegetative growth. Dry leaf yield ranged from 4.98 to 14.00 g plant⁻¹, with Majalengka, Karang Anyar, and TIA-001A among the highest-yielding genotypes. High-performance liquid chromatography (HPLC) identified Garut as the leading clone in total glycoside content (346.78 mg g⁻¹), with the highest concentrations of stevioside (75.91 mg g⁻¹, 7.59%) and rebaudioside A (26.40 mg g⁻¹, 2.64%). However, its Reb A/stevioside ratio (0.35) was lower than that of TIA-006 (0.74), TIA-003 (0.54), and Karang Anyar (0.52), which exhibited more favorable sweetness profiles. TIA-003 combined high glycoside content (286.58 mg g⁻¹, 28.66%) with environmental stability, despite moderate leaf yield. Pujon and TIA-002A showed consistent glycoside profiles suitable for herbal and blended extract applications, while Karang Anyar demonstrated dual potential for leaf yield and sweetener quality. These findings identify Garut, TIA-003, and Karang Anyar as priority genotypes for glycoside extraction and agro-industrial scaling, while Majalengka and TIA-001A offer biomass advantages for whole-plant utilization. These findings offer genotype-specific insights to guide breeding programs and facilitate agro-industrial deployment in tropical highland stevia systems.

Keywords: *Stevia rebaudiana*; tropical highland; sustainability; genotype evaluation; steviol glycosides; yield stability.

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1. Introduction

Stevia rebaudiana (Bertoni) Bertoni has gained global recognition as a natural sweetener, valued for its intense sweetness and non-caloric nature. Its primary bioactive compounds, steviol glycosides, particularly rebaudioside A (Reb A), offer a clean, sugarlike taste and have been associated with antibacterial, antifungal, and antiviral activities, extending stevia's applicability beyond the food industry (Arumugam et al., 2020; Amarakoon, 2021). These pharmacological properties further support their use in functional foods and herbal formulations (Bhasker et al., 2015; Myint et al., 2020).

Recent international studies have highlighted advances in stevia cultivation and glycoside enhancement. Hydroponic systems under optimized light regimes significantly improved leaf biomass and glycoside accumulation in Taiwan (Chou et al., 2025a), while nitrogen source and cytokinin application enhanced rebaudioside A content beyond 7% in Iran (Ahmadirad et al., 2024). Genotypic responses to salt stress in Egypt revealed "High Sugar" lines with superior glycoside biosynthesis (Hashem et al., 2024), underscoring the importance of stress tolerance in breeding programs. On the processing side, hot water extraction methods achieved

glycoside recovery efficiencies above 85% in Pakistan (Joshi et al., 2024), and tissue culture approaches reported in nature plants accelerated regeneration and increased glycoside yield, supporting both industrial production and genetic conservation (Abouelela et al., 2025).

Environmental factors such as altitude, temperature, photoperiod, and planting density continue to influence yield and glycoside content, while agronomic practices such as fertility management and pinching can further improve productivity (Pal et al., 2015; Benhmimou et al., 2017; Uçar et al., 2018; Xu et al., 2024). Despite these advances, most genotypic evaluations have been conducted under temperate or subtropical environments, leaving tropical highlands underrepresented in literature. Soil type has also been shown to significantly influence vegetative growth and biomass yield (Zaman, 2015). Global efforts to develop elite stevia cultivars with enhanced yield and glycoside profiles are ongoing (Huber & Wehner, 2023; Castro et al., 2025), yet few studies offer detailed morpho-biochemical characterization of genotypes in equatorial uplands.

To support global stevia breeding and cultivation efforts, Indonesia has initiated improvement programs through conventional hybridization, polyploidization, induced mutations, and local selection (Adabiyah et al., 2019; Amien et al., 2021; Heliyanto et al., 2024). Several elite lines have emerged from these initiatives, yet their field performance under diverse agroecological conditions remains poorly understood. Thus, this study aims to evaluate the growth traits, yield components, and steviol glycoside profiles of selected *S. rebaudiana* clones cultivated under tropical highland conditions in Batu, East Java. The findings are expected to inform breeding strategies, guide genotype selection, and contribute to sustainable stevia value chains in similar environments worldwide.

2. Methodology

2.1. Genetic material used

The eleven elite *S. rebaudiana* (Bertoni) Bertoni clones assessed in this study were derived through diverse improvement pathways (Table 1). This diverse genetic background reflects a strategic integration of regional adaptation and institutional innovation, forming the basis for multi-trait selection under highland cultivation.

2.2. Seedling propagation

Seedling propagation was carried out in Karangploso, Malang, from 12 December 2023 to March 2024. Seedlings were vegetatively propagated using the method recommended by Sinta & Sumaryono (2019). This method is widely adopted to ensure genetic uniformity in elite stevia lines (Martini et al., 2015).

2.3. Research location and conditions

The field experiment was conducted in the designated stevia cultivation area situated in Junggo, Tulungrejo Village, Batu District, East Java, Indonesia. This site lies at an elevation of 1,300 meters above sea level, with geographic coordinates of 59341.77821 E and 6348269.02589 N. The region receives an average annual rainfall of approximately 1,346 mm and maintains a mean temperature of 25.7 °C throughout the year. The experimental soil was classified as sandy loam (63% sand, 30% silt, 7% clay) with moderate organic matter (3.92% C-organic, equivalent to ~6.7% organic matter), slightly acidic reaction (pH H₂O 6.5), and a cation exchange capacity of 29.29 cmol(+) kg⁻¹, indicating good nutrient and water retention. Nutrient status was adequate, with total N 0.57%, available P₂O₅ 289 ppm, and exchangeable K 0.43 cmol(+) kg⁻¹, while micronutrients (Fe, Mn, Zn, Cu) were present at sufficient levels. These characteristics, combined with organic amendment, provided a favorable environment for stevia establishment and growth.

Table 1

Name and origin of Stevia clones evaluated in the field trial

No	Clone name	Origin	Reference
1	TIA-001B	Local clone, North Sumatera	
2	P-9	Hybridisation Program	
3	M-16	Mutation Program	
4	TIA-003	Local clone	
5	Pujon	Local clone	
6	Karang Anyar	Local clone	Amien et al. (2021); Heliyanto et al. 2024)
7	TIA-001A	Local clone, North Sumatera	
8	TIA-002	Local clone	
9	TIA-006	Local clone	
10	Majalengka	Local clone, West Java	
11	Garut	Local clone, West Java	

2.4. Transplanting of seedlings

Stevia seedlings were transplanted following a structured sequence of field operations. Raised beds were prepared with dimensions of 1 m in width, 4 m in length, and 15 cm in height. Prior to transplanting, the soil was amended with chicken manure at a rate of 20,000 kg ha⁻¹ to improve fertility and organic matter content. Chicken manure applied in the experiment was characterized by high organic matter and essential macronutrients, making it a suitable biofertilizer for stevia cultivation. Previous studies conducted in Malang and Surabaya reported nutrient ranges of nitrogen (N) between 1.5%–3%, phosphorus (P₂O₅) 1%–2%, and potassium (K₂O) 1%–2%. The manure also contained approximately 30%–40% organic carbon and 60%–70% moisture, reflecting its capacity to improve soil fertility and structure. This composition underscores the role of chicken manure in enhancing nutrient availability and sustaining soil organic matter during the establishment phase of stevia seedlings. Each bed was then covered with perforated plastic mulch to regulate soil temperature, conserve moisture, and suppress weed growth. Circular perforations of 3 cm diameter were made to facilitate aeration and water infiltration.

Transplanting was conducted on 7 March 2024. Planting holes were aligned with the mulch perforations to ensure uniform spacing and ease of establishment. A total of 24 seedlings were arranged per bed, with a spacing of 25 cm × 25 cm to provide adequate room for root development and canopy expansion. Immediately after transplanting, seedlings were irrigated to secure root–soil contact and reduce transplanting shock. Subsequent monitoring was performed to maintain optimal soil moisture and ensure uniform establishment.

2.5. Experimental set up and pruning strategy

To assess the performance of eleven elite stevia clones, a randomized block design (RBD) was employed with four replications. This layout ensured statistical reliability and minimized environmental variation across plots. To enhance leaf biomass and promote uniform plant architecture, a two-stage pruning protocol was implemented. The initial pruning occurred roughly one month after transplanting, where the main stem was trimmed to 15 cm above ground level to stimulate primary branch formation. A second pruning was conducted once 5%–10% of the plant population began flowering, encouraging secondary branching and denser canopy development. This approach followed best-practice guidelines for

stevia cultivation and supported consistent vegetative growth across genotypes.

2.6. Observations

Agronomic and biochemical traits were assessed across three consecutive harvests, following the multi-harvest observation protocol established by **Sinta & Sumaryono (2019)** for stevia field trials in Indonesia. This approach enables robust evaluation of genotype performance over time, capturing both productivity and environmental responsiveness. The first harvest was conducted 2.5 months after transplanting, when approximately 10% of the plant population had initiated flowering. The second and third harvests were performed at two-month intervals, allowing for consistent temporal spacing and comparative analysis across growth stages.

Stevia plant traits measured included height, stem diameter, and canopy width, along with fresh and dry weights of leaves and stems, and total biomass. Six plants per plot were chosen for destructive sampling. Pruning was done at about 5 cm above ground using sterilized scissors to keep cuts uniform and reduce damage. This method supports analysis of genotype × environment interaction and helps models yield stability, following best practices for stevia agronomic research.

2.7. Glucoside extraction and quantification

The determination of steviol glycoside content in stevia leaves followed a modified protocol based on **Martono et al. (2016)**, optimized for precision and reproducibility. The procedure comprised three main stages: (i) Reagent Preparation High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile (Merck, Germany), trifluoroacetic acid, and analytical-grade ethanol (E-Merck) were used alongside doubly distilled water produced in-house. Nylon membrane filters (0.45 µm, Whatman, UK) ensured sample purity. Stevioside and Rebaudioside A standards (≥99.0% purity, Wako, Japan) were dissolved in the mobile phase to prepare calibration solutions.

(ii) Sample Extraction. For each analysis, 0.50 g of dried stevia leaf powder was subjected to ultrasonic-assisted extraction using 25 mL of 60% ethanol at 40 °C for 15 minutes, repeated twice. The combined filtrates were adjusted to a final volume of 100 mL and filtered through membrane filters prior to chromatographic analysis (iii) Chromatographic Conditions. HPLC was performed using a Knauer system (Germany) equipped with a Eurosphere C-18 column (250 × 4.6 mm, 5 µm) and UV detection at 210 nm. The mobile phase consisted of a water–methanol mixture (90:10, pH

3.0 with phosphoric acid), acetonitrile, and trifluoroacetic acid in a 65:35:0.01 v/v/v ratio. The mixture was sonicated for 30 min to ensure homogeneity. Chromatographic runs were conducted at a flow rate of 0.6 mL/min, with the column maintained at 30 °C and an injection volume of 20 µL using a Rheodyne 7726i injector.

2.8. Statistical analysis and stability assessment

All agronomic data, excluding glycoside concentrations, were subjected to analysis of variance (ANOVA) to determine significant differences among the eleven stevia clones. When significant variation was detected, Duncan's Multiple Range Test (DMRT) was applied at the 5% level to identify statistically distinct performance groups and facilitate genotype ranking.

To evaluate the stability of clone performance across three harvests, the Eberhart and Russell model was employed. This method estimates two key parameters: the regression coefficient (b_i), which reflects a genotype's responsiveness to environmental variation, and the deviation from regression (S_{2d}), which indicates the consistency of performance. Genotypes with b_i values close to 1 and non-significant S_{2d} were considered stable and broadly adapted to highland conditions. This approach has been widely validated in genotype-

by-environment interaction studies, including in maize (Sowmya et al., 2018) and stevia (Amien et al., 2022), and is particularly effective for identifying clones with both high productivity and environmental resilience. Stability classification was based on the combined interpretation of b_i and S_{2d} values, with emphasis on clones that maintained consistent agronomic traits across harvests.

3. Results and discussion

3.1. Growth performance

Genotypic variation significantly influenced plant height, stem diameter, and canopy width (Tables 2–4), confirming the role of genetic architecture in shaping stevia morphology. These findings align with Huber & Wehner (2023), who reported high heritability for vegetative traits in elite stevia populations. Similar genotype-driven morphological differentiation has been observed in sugarcane (Heliyanto et al., 2020), and rice (Alves et al., 2020). Majalengka recorded the tallest plants (45.06 cm), followed by TIA-002 and TIA-001B, indicating superior vertical growth. TIA-006 had the shortest stature (28.26 cm), suggesting limited vigor under highland conditions. Stem diameter was thickest in TIA-001B (3.52 mm), supporting its mechanical strength and assimilate transport capacity.

Table 2

Plant height (cm) across three consecutive harvests in eleven stevia elite clones cultivated across three harvests during the 2024 season in Batu District, Malang Regency, East Java

Clone name	1 st harvest	2 nd harvest	3 rd harvest	Average
TIA-001B	34.69 d-l	52.00 a	37.08 d-j	41.26 AB
P-9	32.96 f-l	44.75 a-d	25.00 l	34.24 C
M-16	35.19 d-k	49.71 a-c	29.42 j-l	38.10 BC
TIA-003	32.25 g-l	41.38 b-h	30.96 i-l	34.86 C
Pujon	29.67 j-l	40.42 c-i	34.88 d-l	34.99 C
Karang Anyar	31.54 h-l	40.17 c-i	31.50 h-l	34.40 C
TIA-001A	35.46 d-k	42.21 a-g	35.96 d-k	37.88 BC
TIA-002	38.75 d-j	40.38 c-i	29.67 j-l	36.26 C
TIA-006	25.98 kl	33.71 e-l	25.08 l	28.26 D
Majalengka	42.71 a-f	51.25 ab	41.21 b-h	45.06 AB
Garut	31.56 h-l	43.71 a-e	30.96 i-l	35.41 C

Values sharing identical letters within a column indicate no significant difference according to the 5% level of Duncan's Multiple Range Test.

Table 3

Stem diameter (mm) in eleven stevia elite clones cultivated across three harvests during the 2024 season in Batu District, Malang Regency, East Java

Clone name	1 st harvest	2 nd harvest	3 rd harvest	Average
TIA-001B	4.53 a	3.38 b-e	2.63 d-h	3.52 A
P-9	3.48 b-d	3.19 b-f	3.15 b-g	3.27 A
M-16	3.53 bc	3.00 c-g	3.54 bc	3.36 A
TIA-003	3.37 b-e	3.82 a-c	3.50 b-d	3.56 A
Pujon	3.23 b-f	2.54 e-h	2.28 gh	2.68 B
Karang Anyar	3.06 b-g	2.38 f-h	2.02 h	2.49 B
TIA-001A	3.66 a-c	3.88 ab	2.98 c-g	3.51 A
TIA-002	3.35 b-e	3.12 b-g	3.05 b-g	3.17 A
TIA-006	3.06 b-g	3.83 a-c	3.55 bc	3.48 A
Majalengka	3.89 ab	3.02 b-g	2.55 e-h	3.15 A
Garut	3.17 b-f	2.63 d-h	1.96 h	2.59 B

Values sharing identical letters within a column indicate no significant difference according to the 5% level of Duncan's Multiple Range Test.

Table 4

Width of canopy (mm) in eleven stevia elite clones cultivated across three harvests during the 2024 season in Batu District, Malang Regency, East Java

Clone name	1 st harvest	2 nd harvest	3 rd harvest	Average
TIA-001B	27.46 f-l	37.92 a	37.75 a	34.38 A
P-9	24.67 j-l	32.96 a-e	26.29 g-l	27.97 E
M-16	22.08 lm	31.67 b-g	27.88 e-k	27.21 EF
TIA-003	18.88 mn	30.38 d-i	25.34 i-l	24.86 F
Pujon	24.88 j-l	35.21 a-d	32.17 b-f	30.75 D
Karang Anyar	27.79 e-k	34.96 a-d	32.29 b-f	31.68 B-D
TIA-001A	25.35 i-l	36.92 ab	38.29 a	33.52 A-C
TIA-002	23.42 k-m	28.08 e-k	24.96 j-l	25.49 EF
TIA-006	14.58 n	25.63 h-l	23.00 k-m	21.07 G
Majalengka	28.92 e-j	36.13 a-c	36.67 ab	33.90 AB
Garut	27.42 f-l	34.67 a-d	30.79 c-h	30.96 CD

Values sharing identical letters within a column indicate no significant difference according to the 5% level of Duncan's Multiple Range Test.

Canopy width was widest in TIA-001B, Majalengka, and TIA-001A (>33 cm), enhancing light interception and photosynthetic efficiency. These results are consistent with [Burgess et al. \(2017\)](#), who demonstrated that wider canopy structures improve light distribution in rice. [Libik-Konieczny et al. \(2018\)](#) and [Duan et al. \(2024\)](#) further support the role of canopy architecture in optimizing radiation use efficiency. [Li et al. \(2021\)](#) also found that narrow spacing increased canopy expansion in stevia, reinforcing the influence of planting density.

3.2. Yield traits and stability

Dry leaf yield varied significantly across genotypes and harvests ([Table 5](#)). Majalengka, Karang Anyar, and TIA-001A exceeded 13 g plant⁻¹, with Majalengka reaching 14.00 g. However, TIA-001A and Pujon showed instability ($S_{2d} >$ threshold), indicating sensitivity to microclimatic variation. Stable high-yielding clones included Karang Anyar, Majalengka, and TIA-001B ($b_i \approx 1.2$), consistent with [Amien et al. \(2022\)](#), who emphasized genotype-by-environment interactions in stevia.

TIA-003, though moderate in yield (8.97 g), demonstrated strong environmental stability ($b_i = 0.976$), aligning with [Andrade et al. \(2016\)](#) and

supporting its suitability for consistent production systems. Dry stem biomass ([Table 6](#)) was highest in Majalengka, Karang Anyar, and Garut (>7 g plant⁻¹), with Garut showing excellent stability ($S_{2d} = 0.240$). Total biomass ([Table 7](#)) followed a similar trend, with Majalengka (21.32 g), Karang Anyar (20.50 g), and Garut (18.01 g) leading. These results reinforce the need to integrate yield quantity and stability in genotype selection, especially under variable highland climates ([Chou et al., 2025b](#); [Ribeiro et al., 2021](#)).

3.3. Steviol glycoside profile

Glycoside composition varied markedly among clones ([Figure 1](#)). Garut led in total glycoside content (346.78 mg g⁻¹), stevioside (75.91 mg g⁻¹), and rebaudioside A (26.40 mg g⁻¹), surpassing the average concentrations reported by [Gardana et al. \(2010\)](#) in southern Italian *Stevia rebaudiana* accessions, where stevioside and Reb A were 5.8% and 1.8%, respectively. This highlights the superior productivity of tropical highland clones. However, Garut's Reb A/stevioside ratio (0.35) was lower than that of TIA-006 (0.74), TIA-003 (0.54), and Karang Anyar (0.52), indicating a less favorable sweetness profile despite its high total glycoside yield.

Table 5

Dry leaf weight (g plant⁻¹) and stability parameters across three consecutive harvest in eleven elite stevia clones cultivated during the 2024 season in Batu District, Malang Regency, East Java

Clone name	1 st harvest	2 nd harvest	3 rd harvest	Average	b_i	S_{2d}	Stability
TIA-001B	4.83 l-n	14.55 e-g	16.31 cd	11.90 A	1.2 ns	2.316 ns	Stable
P-9	3.14 o-q	8.47 i	5.80 j-m	5.81 C	0.4 ns	3.090 ns	Stable
M-16	2.04 pq	7.31 ij	7.35 ij	5.57 C	0.6 ns	1.985 ns	Stable
TIA-003	2.88 o-q	10.85 h	13.19 g	8.97 B	1.0 ns	1.964 ns	Stable
Pujon	4.79 mn	14.10 fg	20.56 a	13.15 A	1.5 ns	7.254 *	Not stable
Karang Anyar	6.16 j-m	15.18 d-f	19.83 a	13.72 A	1.3 ns	1.522 ns	Stable
TIA-001A	3.79 no	14.81 d-f	21.38 a	13.33 A	1.7 *	6.492 *	Not stable
TIA-002	3.27 n-p	7.97 i	5.59 k-m	5.61 C	0.4 ns	1.968 ns	Stable
TIA-006	1.57 q	7.00 i-k	6.37 j-l	4.98 C	0.6 ns	1.274 ns	Stable
Majalengka	7.00 i-k	16.91 bc	18.08 b	14.00 A	1.2ns	2.175 ns	Stable
Garut	5.48 k-m	15.92 c-e	14.24 fg	11.88 A	1.5 ns	2.851 ns	Stable

Values sharing identical letters within a column indicate no significant difference according to the 5% level of Duncan's Multiple Range Test; * = significantly different at 5% level; ns = not significantly different.

Table 6

Dry stem weight (g plant⁻¹) and stability parameters across three consecutive harvest in eleven elite stevia clones cultivated during the 2024 season in Batu District, Malang Regency, East Java

Clone name	1 st harvest	2 nd harvest	3 rd harvest	Average	b _i	S _{2d}	Stability
TIA-001B	1.73 m-p	9.79 b-e	10.09b-d	7.20 A	1.5*	0.557 ns	Not stable
P-9	1.48 m-p	5.58 g-i	2.87 k-o	3.31 C	0.5ns	3.618 **	Not stable
M-16	1.12 n-p	5.23 h-j	4.01 h-l	3.45 C	0.6ns	0.484 ns	Stable
TIA-003	0.93 o-p	5.28 h-j	6.08 f-h	4.10 C	0.9ns	0.520 ns	Stable
Pujon	1.21 n-p	7.61 e-g	12.39a	7.07 AB	1.6ns	7.781 **	Not stable
Karang Anyar	2.02 l-p	8.08 d-f	10.24a-d	6.78 AB	1.3ns	0.676 ns	Stable
TIA-001A	1.09 n-p	8.07 d-f	11.78a-b	6.98 AB	1.6ns	3.932 **	Not stable
TIA-002	1.77 m-p	4.75 h-k	3.55 i-m	3.36 C	0.4ns	0.343 ns	Stable
TIA-006	0.39 p	2.65 k-o	3.25 j-n	2.10 D	0.5*	0.530 ns	Not stable
Majalengka	2.93 k-o	10.35a-c	8.71 c-n	7.33 A	1.1ns	1.684 **	Not stable
Garut	1.83 l-p	8.69 c-e	7.88 e-f	6.13 B	1.1ns	0.240 ns	Stable

Values sharing identical letters within a column indicate no significant difference according to the 5% level of Duncan's Multiple Range Test; * = significantly different at 5% level; ns= not significantly different.

Table 7

Dry total biomass (g plant⁻¹) and stability parameters in eleven elite *Stevia rebaudiana* clones cultivated in Batu, East Java

Clone name	1 st harvest	2 nd harvest	3 rd harvest	Average	b _i	S _{2d}	Stability
TIA-001B	6.56 i-n	24.34 b-d	26.40 a-d	19.10 A	1.3*	4.3 ns	Not Stable
P-9	4.63 l-n	14.05 f-i	8.68 h-n	9.12 C	0.4ns	14.9*	Not stable
M-16	3.15 m-n	12.54 g-k	11.36 h-l	9.02 C	0.6ns	1.8 ns	Stable
TIA-003	3.81 l-n	16.13 e-h	19.27 d-g	13.07 B	0.9ns	3.5 ns	Stable
Pujon	5.99 j-n	21.71 c-f	32.95 a	20.22 A	1.5ns	31.2**	Not stable
Karang Anyar	8.18 i-n	23.26 b-e	30.06 ab	20.50 A	1.3ns	5.2 ns	Stable
TIA-001A	4.88 k-n	22.88 b-e	33.16 a	20.31 A	1.7ns	21.8*	Not stable
TIA-002	5.04 j-n	12.72 g-j	9.14 h-n	8.97 C	0.4ns	4.9 ns	Stable
TIA-006	1.97 n	9.65 h-n	9.63 h-n	7.08 C	0.5ns	3.9 ns	Stable
Majalengka	9.93 h-m	27.25a-c	26.79 a-d	21.32 A	1.2ns	0.9 ns	Stable
Garut	7.30 i-n	24.61b-d	22.12 c-e	18.01 A	1.0ns	5.9	Stable

Values sharing identical letters within a column indicate no significant difference according to the 5% level of Duncan's Multiple Range Test; * = significantly different at 5% level; ns= not significantly different.

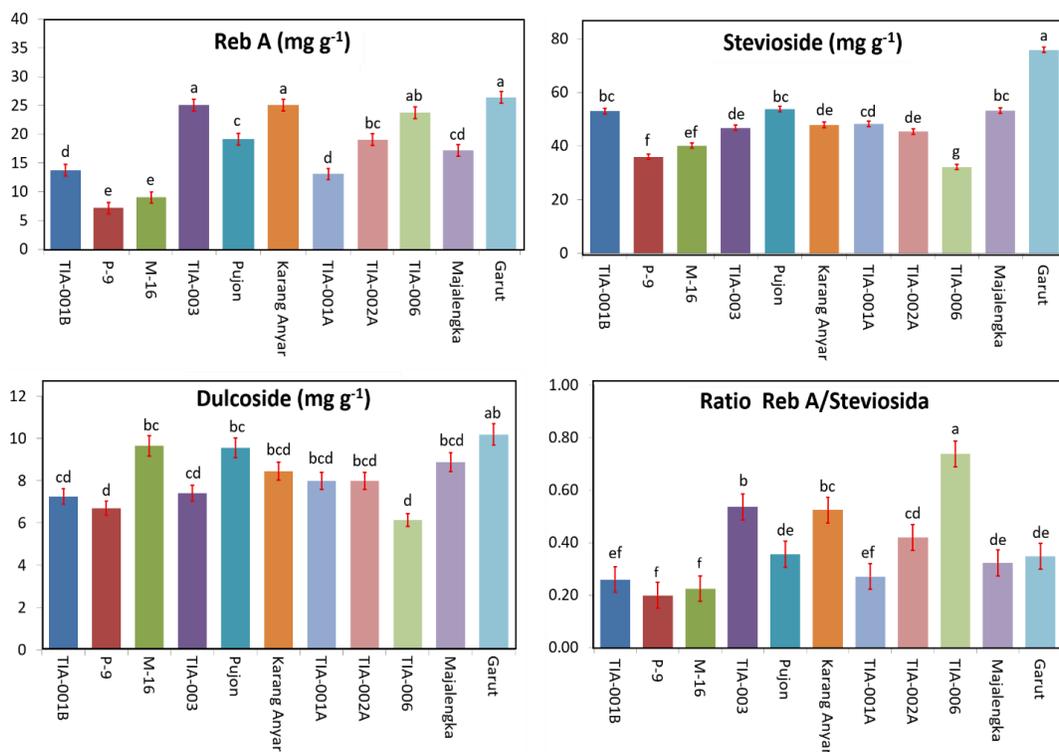


Figure 1. Glucoside contents of eleven elite *Stevia rebaudiana* Bertoni clones cultivated in Batu, East Java.

These ratios are critical for sensory quality and bitterness reduction, as demonstrated by **Tian et al. (2022)**, who correlated glycoside structure with taste dynamics, and by **Parris et al. (2016)**, who emphasized the importance of Reb A dominance in improving leaf-based sweetener quality.

Majalengka and Karang Anyar also showed high Reb A concentrations ($>170 \text{ mg g}^{-1}$), reinforcing their potential for premium sweetener markets. TIA-003 combined high Reb A (250.88 mg g^{-1}) with consistent production, making it ideal for flavor-focused applications (**Andrade et al., 2016**).

Clones like Pujon and TIA-001A had balanced profiles suitable for mid-grade extracts, while P-9 and M-16 showed lower Reb A ($<100 \text{ mg g}^{-1}$), likely contributing to bitterness. These findings support a dual-pathway strategy: high-yield clones for extraction and high-ratio clones for direct consumption (**Pal et al., 2015; Cho et al., 2025**).

Functional food potential was evident in TIA-003 and Garut, given their elevated dulcoside A and Reb A levels. **Bundgaard Anker et al. (2019)** highlighted steviol glycosides' benefits for diabetic and hypertensive populations. These results align with global health trends and regulatory shifts favoring natural sweeteners (**Tao & Cho, 2020; Zhou et al., 2021**).

4. Conclusions

The present study demonstrates significant genotype-dependent variation in agronomic traits and steviol glycoside composition among elite stevia clones cultivated in Indonesia's tropical highlands. Clones such as Garut, TIA-003, and Karang Anyar exhibit promising profiles for glycoside extraction and agro-industrial scaling, while Majalengka and TIA-001A offer biomass advantages for whole-plant utilization. These findings provide a strategic basis for targeted breeding, cultivar deployment, and value-added stevia production under highland tropical conditions. Future studies should explore genotype \times environment interactions and soil-nutrient management strategies to optimize stevia yield and glycoside stability across diverse agroecological conditions.

Author contribution

Conceptualization was performed by **B. Heliyanto**; methodology by **B. Heliyanto** and **R. D. Purwati**; investigation by **M. Murianingrum**, **S. Hartati**, **R. D. Purwati**, **B. W. Hapsari**, and **A. Fadillah**; data curation by **M. Murianingrum**, **A. Fadillah**, and **R. L. Hendrati**; formal analysis by **B. Heliyanto** and **Marjani**; writing—original draft by **B. Heliyanto** and **M. Murianingrum**; writing—review and editing by **S. Hartati**, **C. Suhara**, and **R. L. Hendrati**; visualization by **E. A. Hafidz** and **C. Suhara**; supervision by **S. Amien** and **M. Murianingrum**; resources by **Parnidi** and **B.W. Hapsari**; project administration by **B. Heliyanto** and **M.**

Murianingrum; and funding acquisition by **B. Heliyanto**; all authors having reviewed and approved the final version of the manuscript prior to submission.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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