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Physiological Performance and Growth Responses of Ratoon Sugarcane to Hydrogel, Nano-Silica Particles, and K-Humate Applications

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ABSTRACT

Ratoon sugarcane cultivation systems experience declining productivity due to soil degradation, which adversely affects plant physiological capacity. This study evaluated physiological and growth responses of the Bulu Lawang ratoon sugarcane variety to hydrogel, silica nanoparticles (SiNPs), and K-humate, applied individually and in combination to improve soil physical, chemical, and biological properties. A field experiment used a randomized complete block design with seven treatments under uniform NPKS fertilization. Parameters observed included nitrogen metabolism, chlorophyll content, gas exchange, photosynthetic rate, vegetative growth, and biomass. The combination of SiNPs and K-humate, with or without hydrogel, produced the most favorable responses, increasing nitrate reductase activity by 147%, total chlorophyll by 75%, stomatal conductance by 194%, and photosynthetic rate by 89% relative to the control, with corresponding gains in leaf area, root area, plant population, and dry biomass. Hydrogel provided no additional benefit beyond that of SiNPs and K-humate under the high-rainfall conditions of this study. Correlation analysis showed strong positive relationships between nitrogen metabolism and chlorophyll synthesis, photosynthetic rate and leaf and root area, and photosynthesis and biomass, indicating an integrated physiological response. These findings indicate that SiNPs and K-humate, rather than the hydrogel, primarily drive improvements in ratoon sugarcane productivity on degraded soils.



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1. Introduction

Ratoon sugarcane (RC) refers to sugarcane plants that regrow after harvest through the emergence of lateral shoots from the old sugarcane root system (Wang *et al.* 2024). Ratoon sugarcane cultivation systems are commonly adopted by Indonesian farmers due to several advantages, including faster plant growth and lower production costs compared to plant cane (PC), with cost savings of approximately 20-25% (Xu *et al.* 2021). However, intensive and continuous ratoon sugarcane

cultivation practices lead to declining productivity as ratoon cycles increase (Marin *et al.* 2019; De Oliveira *et al.* 2022).

The decline in ratoon sugarcane productivity is inherently linked to soil quality deterioration resulting from intensive cultivation practices that degrade the physical, chemical, and biological properties of the rhizosphere (Rossato *et al.* 2017; Wang *et al.* 2024). Degradation of soil physical properties includes decreased porosity, increased bulk density, and soil compaction due to heavy machinery use during harvest, which impedes root penetration and reduces soil aeration (Esteban *et al.* 2019; De Oliveira *et al.* 2022; Dlamini and Zhou 2022). Deterioration of soil chemical properties is characterized

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by depletion of essential nutrients, decreased soil pH, reduced cation exchange capacity (CEC), and diminished soil organic matter content, which serves as a buffer for soil fertility (Rossato *et al.* 2017; Pang *et al.* 2021). Meanwhile, degradation of soil biological properties is reflected in decreased soil microbial activity and altered composition of rhizosphere microbial communities that support nutrient cycling and plant health (Pang *et al.* 2021; Wang *et al.* 2024).

These soil degradation conditions directly affect the ratoon sugarcane root system, which originates from old ratoons and gradually experiences declining metabolic vitality (Chumphu *et al.* 2019; Esteban *et al.* 2019; Rahardiansyah *et al.* 2025). Soil quality deterioration inhibits root growth and the ability to absorb water and nutrients, ultimately reducing plant physiological capacity, including photosynthetic rate, water use efficiency, nitrogen metabolism, and biomass accumulation (Pissolato *et al.* 2021; Garcia *et al.* 2021). Limited nutrient uptake, particularly nitrogen and phosphorus, inhibits chlorophyll and photosynthetic enzyme synthesis, thereby significantly reducing ratoon sugarcane productivity compared to plant cane (Zambrosi *et al.* 2017; Shukla *et al.* 2019). Therefore, the decline in millable cane yield and sugar content in ratoon sugarcane systems poses a major challenge to achieving national sugar self-sufficiency and improving sugarcane farmers' incomes.

Addressing soil degradation issues in ratoon sugarcane systems requires innovative strategies to improve soil conditions in an integrated manner of enhancing soil physical, chemical, and biological properties. Several potential approaches for improving soil quality include the application of polymer-based soil amendments (hydrogel), silica nanoparticles (SiNPs) synthesized from geothermal mud (Petrus *et al.* 2025), and K-humate extracted from low-calorie coal (Timotius *et al.* 2025), each serving specific functions in improving different components of soil properties.

Hydrogels specifically improve soil physical properties, particularly water retention capacity, soil structure, porosity, and infiltration rate (Singh *et al.* 2018). Hydrogel can absorb and retain water up to hundreds of times its weight, then release it gradually according to plant needs, thereby creating a stable rhizosphere zone with improved water availability. Application of hydrogel at a dose of 5 kg ha⁻¹ has been reported to increase soil moisture content, reduce bulk density, and improve soil structure in sugarcane grown on sandy alluvial soils (Singh *et al.* 2018). This

improvement in soil physical properties is crucial for supporting the growth of ratoon sugarcane root systems experiencing declining vitality.

Potassium humate (K-humate) improves soil chemical and biological properties simultaneously. From a soil chemistry perspective, K-humate increases cation exchange capacity (CEC), improves soil aggregates, and enhances the availability and release of nitrogen, phosphorus, and potassium through the formation of humic acid-mineral complexes (Liu *et al.* 2019; Zhang *et al.* 2019a, 2019b). Arjumend *et al.* (2015) reported that K-humate application increased soil organic matter by 9%, total nitrogen by 30%, available phosphorus by 100%, and available potassium by 50%. From a soil biology perspective, K-humate increases soil microbial activity and stimulates root growth through hormone-like effects that improve cell respiration, protein synthesis, and water and nutrient uptake (Gholamhoseini *et al.* 2018; Ampong *et al.* 2022). Thus, K-humate serves as a bridge between improving soil chemical fertility and enhancing rhizosphere biological activity.

Silica nanoparticles (SiNPs) have a unique role in enhancing plant resistance to abiotic stress while improving plant physiological efficiency at the cellular level (Goswami *et al.* 2022; Misra *et al.* 2023). SiNPs enhance plant resistance to drought stress through improved water use efficiency, maintenance of cell membrane integrity, and increased antioxidant enzyme activity (Ghorbanpour *et al.* 2020; Malik *et al.* 2021). Additionally, SiNPs increase photosynthetic capacity by enhancing chlorophyll content and net photosynthetic rate. Li *et al.* (2023) reported that the application increased chlorophyll a content by up to 17%, chlorophyll b by up to 50%, and net photosynthetic rate by up to 30%. Silicon accumulation in plant tissues also enhances the structural integrity of stems and leaves, thereby supporting plant growth and productivity under suboptimal environmental conditions.

Considering that each soil amendment material has specific functions in improving different components of soil properties—hydrogel for soil physical properties, K-humate for soil chemical and biological properties, and SiNPs for plant physiological resilience—an integrated application is expected to have greater potential to address soil degradation problems than single applications comprehensively. The integrated application of these three materials potentially generates synergistic effects through: (1) improvement of water retention and soil structure by hydrogel, creating optimal conditions for K-humate activity in enhancing nutrient

availability; (2) enhanced nutrient uptake facilitated by K-humate, subsequently optimized by SiNPs in improving photosynthetic efficiency; and (3) increased plant stress resistance by SiNPs, maintaining stability of physiological processes under improved water availability conditions provided by hydrogel.

This study aimed to evaluate the physiological and growth responses of Bulu Lawang ratoon sugarcane variety to the application of hydrogel, silica nanoparticles (SiNPs), and K-humate, both individually and in combination. The research hypothesis posits that the synergistic combination of these three components will enhance water and nutrient retention in the root zone, improve nutrient uptake by plants, ameliorate soil conditions, and sustainably increase the physiological capacity and productivity of ratoon sugarcane.

2. Materials and Methods

2.1. Time and Place

This study was conducted from August to December 2025. The field experiment was carried out in Bedilan Hamlet, Margokaton Village, Seyegan District, Sleman Regency, Special Region of Yogyakarta (GPS coordinates: -7.724220°S, 110.288557°E). The research site is an agricultural land specifically dedicated to sugarcane cultivation. The soil type at the research location is classified as Inceptisol. Laboratory analyses were performed at the Laboratory of the Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta.

2.2. Study Design and Setting

The field experiment was conducted using a Randomized Complete Block Design (RCBD) with a single factor consisting of seven treatments and four replications. The total experimental area covered 1,400 m², divided into 28 experimental plots, each measuring 50 m². The treatments tested were hydrogel (Agrigel MSG 3, CV. Maulana Says Green 3), silica nanoparticles (geothermal-derived silica), and K-humate (*Gamahumat*, Universitas Gadjah Mada, Indonesia). The standard doses applied were 5 kg ha⁻¹ hydrogel, 5 kg ha⁻¹ SiNPs, and 15 kg ha⁻¹ K-humate. There were six soil amendment treatment units with various dose levels and one control unit without soil amendment treatment (Table 1).

This study utilized the Bululawang sugarcane variety in the first ratoon cycle. The sugarcane had undergone one harvest following initial planting in 2024, and the experiment was therefore conducted during the first ratoon growth stage. All experimental units received

Table 1. Soil amendment treatment doses

Treatment code	Hydrogel	SiNPs	K-Humate
T0 (kontrol)	-	-	-
T1	(5) kg/ha ⁻¹	-	-
T2	-	(5) kg/ha ⁻¹	-
T3	-	-	(15) kg/ha ⁻¹
T4	(5) kg/ha ⁻¹	(5) kg/ha ⁻¹	-
T5	-	(5) kg/ha ⁻¹	(15) kg/ha ⁻¹
T6	(5) kg/ha ⁻¹	(5) kg/ha ⁻¹	(15) kg/ha ⁻¹

the same basal fertilization treatment, consisting of NPKS fertilizer (14-9-18-4) at a dose of 1,000 kg ha⁻¹. Soil amendment materials, including hydrogel, silica nanoparticles, and K-humate, were applied after sugarcane harvest and ratooning. The application was carried out by creating furrows around the sugarcane rows, into which the treatment materials were placed. To ensure uniform distribution, given the relatively small quantities involved, each treatment material was first dissolved in 16 liters of water and then applied by the drenching method to each plot at the designated treatment dose. Upon completion of the application, the furrows were covered with soil.

Regarding land management, drainage ditches were constructed between treatment and replication blocks to channel rainwater and prevent waterlogging in the experimental area. No supplemental irrigation was applied, as rainfall at the research site was sufficient to meet crop water requirements during the early growth phase. Weed control was carried out through selective herbicide spraying after harvest. Pest control measures were not implemented during the study period because no significant pest incidence was observed in the sugarcane crop.

2.3. Observation Parameters

Observation parameters in this study included physiological, growth, and biomass parameters of ratoon sugarcane at the vegetative stage. All observations were conducted 4 months after treatment application, coinciding with the late vegetative phase, when photosynthetic activity and plant metabolism are relatively high, allowing optimal measurement of physiological parameters.

2.3.1. Leaf Sampling for Physiological and Biochemical Parameters

Leaf samples for physiological and biochemical analyses were collected from plants in the observation rows of each treatment plot. The leaf used as the sample

was the third leaf from the apex, or the youngest fully expanded leaf, known as the Kuijper +1 leaf, characterized by a clearly visible dewlap, the tissue located between the leaf blade and leaf sheath, which serves as a standard sampling marker in sugarcane (McCray *et al.* 2021). One leaf sample was collected per treatment plot, and measurements were performed with four replications for each parameter.

2.3.2. Physiological Parameters

Physiological parameters observed included net photosynthetic rate (PN), stomatal conductance (gs), and transpiration rate (Em), measured using a Portable Photosynthesis and Fluorescence System (LI-COR 6800 Education Model, LI-COR Biosciences, Lincoln, NE, USA). The gas exchange measurement approach was previously applied in sugarcane (Guo *et al.* 2023). Data were recorded every few seconds until photosynthesis values stabilized. For each measurement, eight data points were recorded within approximately 1 minute and subsequently averaged to obtain a representative value for each leaf observation.

2.3.3. Nitrate Reductase Activity (NRA)

Nitrate reductase activity (NRA) was measured four months after treatment using the same Kuijper +1 leaves used for chlorophyll analysis (McCray *et al.* 2021). Leaf pieces (~1 mm, 0.2 g) excised from the midrib were incubated in 5 mL of 0.1 M phosphate buffer (pH 7) for 24 h, after which the buffer was replaced with fresh buffer containing 0.1 mL of 5 M NaNO₃ and incubated for 2 h. A 0.1 mL aliquot of the incubation solution was then mixed with a color reagent (0.2 mL 1% sulphanilamide in 3 N HCl and 0.2 mL 0.02% N-naphthylethylenediamine) and left for 15 min to develop a pink color, indicating nitrite formation. After adding 2.5 mL of distilled water, absorbance was measured at 540 nm using a UV-Vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific) (Ende *et al.* 2022). NRA was calculated as: $NRA = (Abs_{\sim sample} / Abs_{\sim std}) \times (1000 / FW) \times (1 / W1) \times (500 / 1000)$, expressed in $\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$.

2.3.4. Chlorophyll Content (Chl a, Chl b, Chl t)

Chlorophyll content analysis was carried out four months after treatment using the method of Arnon (1949), employing 80% acetone as solvent and measuring absorbance at wavelengths of 663 nm and 645 nm with a UV-Vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific). Fresh leaf samples of 1 g were ground using a mortar until a paste was formed, then mixed with

20 mL of 80% acetone solution. The mixture was filtered through Whatman No. 2 filter paper, and the filtrate was collected into test tubes. The chlorophyll extract was then placed into a cuvette and measured for absorbance. Chlorophyll a and b contents were calculated using the following formulas (Arnon, 1949): $\text{Chl a} = (0.0127 \times A_{663} - 0.00269 \times A_{645}) \times 20 \text{ mL}$, $\text{Chl b} = (0.0229 \times A_{645} - 0.00468 \times A_{663}) \times 20 \text{ mL}$, $\text{Chl t} = \text{Chl a} + \text{Chl b}$.

2.3.5. Growth Parameters

Growth parameters observed included plant population (PP), stalk diameter (SD), stalk length (SL), plant height (PH), leaf number (LN), leaf area (LA), and root area (RA). The experiment was arranged in a randomized block design (RBD) with seven treatments and four replications. Destructive sampling was conducted 4 months after treatment application, with 3 sample plants per treatment per replication, yielding a total of 84 sample plants. PP was determined by counting the number of main stalks and tillers along the observation row using a hand tally counter, following procedures commonly applied in sugarcane agronomic studies (Chanda *et al.* 2018).

LA was measured destructively using a leaf image analysis system (WinDIAS, Delta-T Devices Ltd. (2009), Cambridge, UK), following previously reported image-based procedures (Bodor *et al.* 2012; Brazaitytė *et al.* 2019). Leaf samples were arranged individually on a high-contrast background without overlapping, photographed under controlled lighting, and analyzed using WinDIAS software to obtain total leaf surface area (cm² per sample) based on pixel-color segmentation. RA was measured using the same imaging principle adapted for root samples: roots were cleaned of soil debris, spread evenly on the background, photographed, and analyzed to obtain total root surface area (cm² per sample).

2.3.6. Biomass Parameters

Biomass parameters were evaluated through fresh weight (FW) and dry weight (DW) measurements. Destructive sampling was conducted 4 months after treatment application, with 3 sample plants per treatment, yielding a total of 84 sample plants across 4 replications. Each plant sample was separated into stalk, root, and leaf fractions, weighed fresh, and the fractions were summed to obtain total FW per plant. Samples were then air-dried and oven-dried at 70°C for 72 hours until a constant weight was reached, after which each fraction was weighed again and summed to obtain total DW per plant.

2.4. Data Analysis

Data analysis was performed using Analysis of Variance (ANOVA) at a 95% confidence level ($\alpha = 5\%$) to determine treatment effects on all observation parameters. When ANOVA results indicated significant differences, mean comparisons among treatments were conducted using Tukey's Honestly Significant Difference (HSD) test at $\alpha = 5\%$. Furthermore, relationships among observation variables were analyzed using Pearson correlation analysis to identify associations between physiological, growth, and biomass parameters. All data analyses were performed using RStudio software.

3. Results

3.1. Physiological and Biochemical Responses of Ratoon Sugarcane

The application of hydrogel, silica nanoparticles (SiNPs), and K-humate, individually and in combination, significantly influenced the physiological and biochemical performance of ratoon sugarcane. Nitrate reductase activity (NRA) differed significantly among treatments (Table 2), increasing progressively from T0 to T6. T6 and T5 produced the highest NRA values, T2, T3, and T4 formed a statistically comparable intermediate group, and T1 showed the smallest increase among the amended treatments. This pattern indicates that combined hydrogel, silica nanoparticles, and K-humate application enhanced nitrogen assimilation capacity, with the response scaling with treatment intensity.

Leaf chlorophyll content followed a trend consistent with NRA. Chlorophyll a, b, and total chlorophyll all increased significantly from T0 to T6, with T5 and T2 also showing marked gains over the control. Chlorophyll b showed the largest relative increase among the three

fractions. Low coefficients of variation for Chl a and Chl t confirmed reliable treatment differentiation. Leaf gas exchange parameters also varied significantly among treatments, with T0 lowest and T6 highest for stomatal conductance (gs), transpiration rate (Em), and net photosynthetic rate (PN). However, gs did not differ significantly among the amended treatments (T1–T5). PN showed a clearer separation, with T3–T5 forming an intermediate group above T1 and T2. Em followed the same increasing trend but with relatively high variability across treatments (CV>28%).

3.2. Growth Response and Biomass Yield of Ratoon Sugarcane

Growth parameters and biomass of ratoon sugarcane were significantly affected by the soil amendment treatments (Table 3). Leaf number, leaf area, and root area all increased significantly from T0 to T6, with root area showing the largest relative gain among the three growth parameters observed. T6 and T5 consistently formed the top-performing group for these parameters, T2, T3, and T4 formed an intermediate group, and T1 showed the smallest response among the amended treatments. Coefficients of variation were low for leaf number and root area (1.13% and 7.44%) and moderate for leaf area (12.95%), indicating high measurement precision across replications.

Plant population, stem diameter, stem length, and plant height also differed significantly among treatments and increased progressively from T0 to T6. T3, T4, and T5 were statistically equivalent to T6 for plant population, whereas T1 and T2 did not differ significantly from the control. Plant height increased steadily across treatments with low variability (CV = 0.64%), and visual differences in plant architecture among treatments are shown in Figure 1. Fresh and

Table 2. Effects of hydrogel, silica nanoparticles, and K-humate application on physiological parameters and chlorophyll content related to nitrogen metabolism, leaf chlorophyll synthesis, leaf gas exchange behavior, and photosynthetic rate of ratoon sugarcane

Treatment	NRA $\mu\text{mol NO}_2^- \text{g}^{-1} \text{FW h}^{-1}$	Chl a $\text{mg g}^{-1} \text{FW}$	Chl b $\text{mg g}^{-1} \text{FW}$	Chl t $\text{mg g}^{-1} \text{FW}$	gs $\text{mol m}^{-2} \text{s}^{-1}$	Em $\text{mol m}^{-2} \text{s}^{-1}$	PN $\text{mol m}^{-2} \text{s}^{-1}$
T0	0.92±0.07 ^c	2.08±0.40 ^d	0.55±0.18 ^d	2.63±0.58 ^d	0.18±0.08 ^b	3.47±0.46 ^b	19.53±2.34 ^c
T1	1.43±0.11 ^{bc}	2.20±0.05 ^a	0.64±0.02 ^{cd}	2.84±0.07 ^{cd}	0.37±0.11 ^{ab}	6.45±1.87 ^a	25.98±4.21 ^{bc}
T2	1.54±0.11 ^b	2.96±0.07 ^{ab}	0.98±0.03 ^{ab}	3.95±0.11 ^{ab}	0.41±0.09 ^{ab}	7.91±0.67 ^a	30.51±3.76 ^{ab}
T3	1.44±0.09 ^b	2.35±0.05 ^{cd}	0.70±0.02 ^{cd}	3.05±0.07 ^{cd}	0.43±0.15 ^{ab}	7.08±2.45 ^a	32.21±2.74 ^{ab}
T4	1.53±0.41 ^b	2.76±0.34 ^{ab}	0.90±0.16 ^{bc}	3.67±0.50 ^{bc}	0.39±0.06 ^{ab}	7.16±2.27 ^a	31.91±0.79 ^{ab}
T5	1.86±0.25 ^{ab}	3.10±0.10 ^{ab}	1.05±0.05 ^{ab}	4.15±0.15 ^{ab}	0.38±0.06 ^{ab}	5.71±0.46 ^a	32.28±1.80 ^{ab}
T6	2.27±0.30 ^a	3.40±0.10 ^a	1.20±0.05 ^a	4.60±0.15 ^a	0.53±0.13 ^a	8.68±2.38 ^a	36.96±1.27 ^a
CV (%)	11.36	7.80	11.12	8.57	28.21	28.23	8.72

Values followed by the same letter within the same column are not significantly different according to Tukey's test at the 5% level. T0 = control; T1 = Hydrogel; T2 = K-humate; T3 = SiNPs; T4 = Hydrogel + SiNPs; T5 = K-humate + SiNPs; T6 = Hydrogel + K-humate + SiNPs. NRA = nitrate reductase activity; Chl a = chlorophyll a; Chl b = chlorophyll b; Chl t = total chlorophyll; gs = stomatal conductance; Em = transpiration rate; PN = net photosynthetic rate

Table 3. Effects of hydrogel, silica nanoparticles (SiNPs), and K-humate application on growth parameters of ratoon sugarcane

Treatment	LN (no. plant ⁻¹)	LA (cm ² plant ⁻¹)	RA (cm ² plant ⁻¹)	FW (g plant ⁻¹)	DW (g plant ⁻¹)	PP (‘000 ha ⁻¹)	SD (mm)	SL (cm)	PH (cm)
T0	6.73±0.05 ^c	1571.79±220.94 ^b	121.77±9.40 ^d	838.66±0.57 ^d	167.73±0.11 ^d	106±7.6 ^b	24.93±2.13 ^c	154.00±1.41 ^c	299.93±0.75 ^c
T1	7.33±0.15 ^d	2074.37±300.83 ^{ab}	154.62±5.82 ^d	973.66±9.45 ^c	194.73±1.89 ^c	119±6.65 ^b	28.13±0.45 ^b	163.13±3.29 ^{bc}	303.50±1.17 ^c
T2	7.86±0.11 ^{bc}	2313.13±184.40 ^{ab}	258.39±8.25 ^b	1160.00±21.93 ^b	232.00±4.38 ^b	107±3.60 ^b	28.73±1.26 ^{ab}	167.56±5.18 ^{cd}	325.12±4.93 ^c
T3	7.73±0.05 ^c	2331.64±144.03 ^{ab}	168.37±26.86 ^{cd}	979.66±15.50 ^c	195.93±3.10 ^c	143±14.01 ^a	29.10±1.03 ^{ab}	167.73±4.98 ^{cd}	318.10±1.63 ^d
T4	7.80±0.00 ^{bc}	2579.08±490.83 ^a	205.98±11.48 ^c	1140.00±17.34 ^b	228.00±3.46 ^b	142±5.50 ^a	29.60±0.26 ^{ab}	175.10±1.01 ^{bc}	344.50±1.41 ^b
T5	8.00±0.00 ^{ab}	2917.70±560.09 ^a	349.05±12.08 ^a	1191.33±38.68 ^{ab}	238.26±7.73 ^{ab}	150±6.55 ^a	29.50±0.79 ^{ab}	180.83±2.27 ^{ab}	346.86±0.81 ^{ab}
T6	8.23±0.05 ^a	2938.07±224.71 ^a	370.92±24.78 ^a	1232.33±21.57 ^a	246.46±4.31 ^a	151±2.64 ^a	31.40±0.52 ^a	186.40±2.20 ^a	350.77±0.64 ^a
CV (%)	1.13	12.95	7.44	1.96	1.96	5.62	3.59	1.98	0.64

Values followed by the same letter within the same column are not significantly different according to Tukey's test at the 5% level. T0 = control; T1 = Hydrogel; T2 = K-humate; T3 = SiNPs; T4 = Hydrogel + SiNPs; T5 = K-humate + SiNPs; T6 = Hydrogel + K-humate + SiNPs. PP = plant population; SD = stem diameter; SL = stem length; PH = plant height; LN = leaf number; LA = leaf area; RA = root area; FW = fresh weight; DW = dry weight

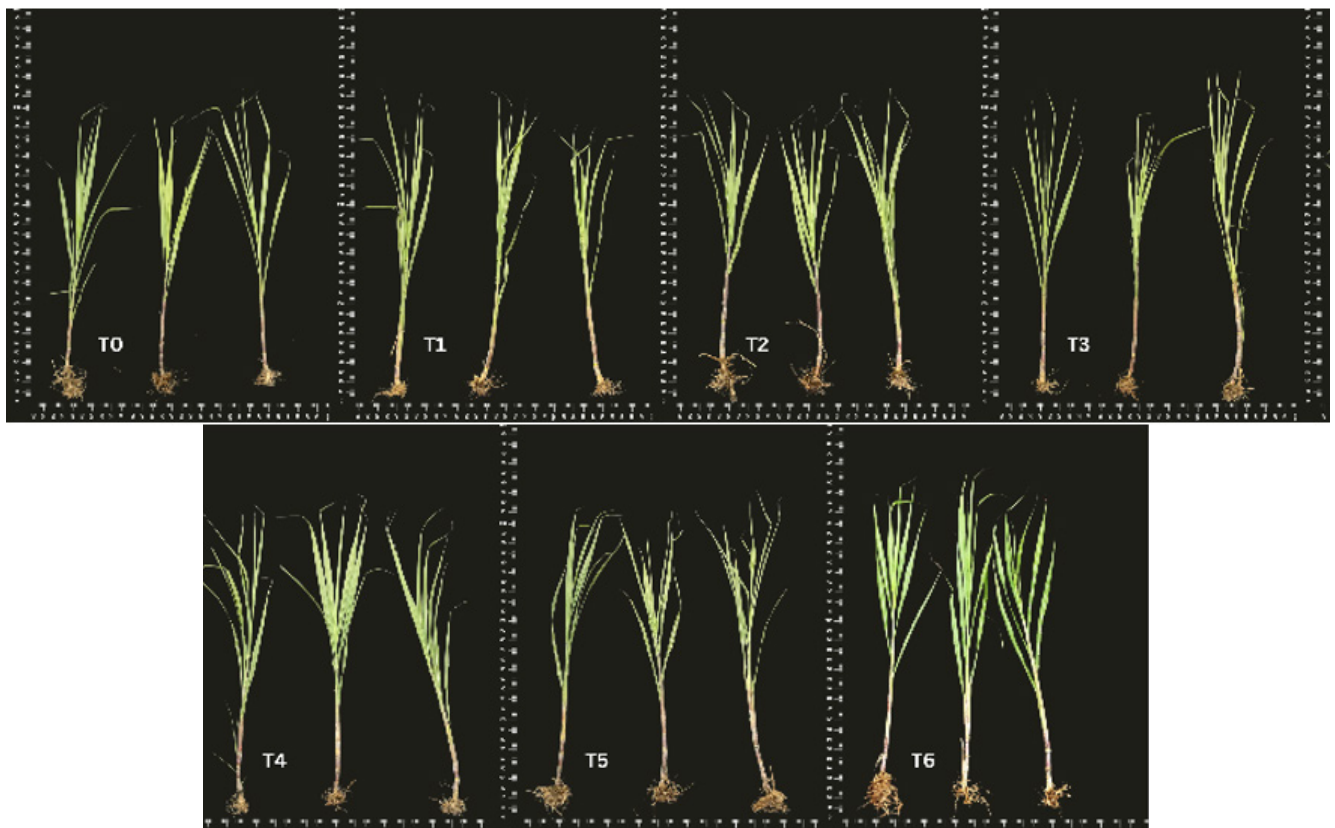


Figure 1. Bululawang ratoon sugarcane variety under various treatments. The photograph shows visual differences in plant height, leaf number, and root development among treatments: T0 (control), T1 (hydrogel), T2 (SiNPs), T3 (K-humate), T4 (hydrogel + SiNPs), T5 (K-humate + SiNPs), and T6 (hydrogel + K-humate + SiNPs). The combination treatment T6 exhibited optimal vegetative growth, with higher plant height, leaf number, and root area than the other treatments

dry weights showed the same increasing trend, with T6, T5, T4, and T2 producing the highest biomass accumulation and significantly higher values than the control; variability in both biomass parameters was low (CV = 1.96%).

3.3. Correlation among Physiological, Biochemical, Growth, and Biomass Parameters

Pearson correlation analysis revealed extensive positive relationships among physiological, biochemical, growth, and biomass parameters (Figure 2). Photosynthetic rate

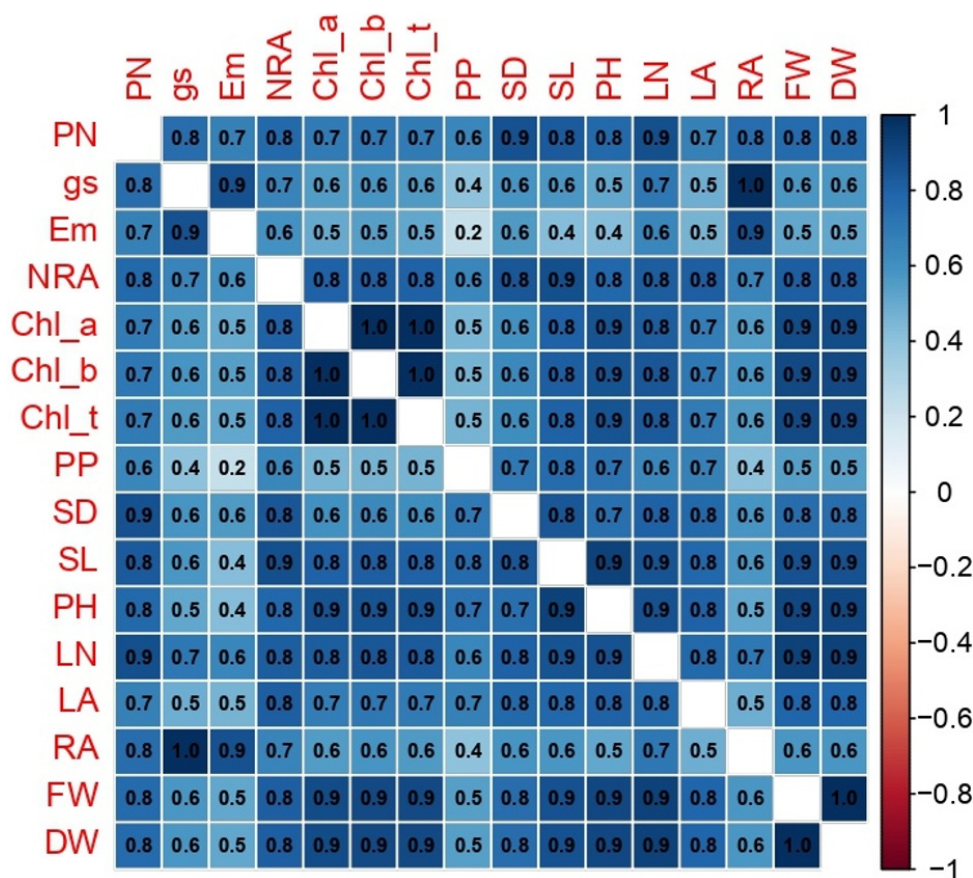


Figure 2. Pearson correlation heatmap showing relationships among physiological, growth, and biomass parameters of sugarcane plants. Physiological parameters observed included photosynthetic rate (PN), stomatal conductance (gs), transpiration rate (Em), and nitrate reductase activity (NRA). Biochemical parameters were represented by chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl t) content. Growth parameters consisted of plant population (PP), stem diameter (SD), stem length (SL), plant height (PH), leaf number (LN), leaf area (LA), and root area (RA), while biomass parameters included fresh weight (FW) and dry weight (DW). The blue color scale indicates positive correlations, whereas the red color scale indicates negative correlations, with color intensity reflecting the strength of relationships ($r = -1$ to $+1$).

and NRA showed their strongest associations with stem diameter and leaf number. Both parameters also correlated strongly with the remaining growth and biomass traits. The three chlorophyll fractions were perfectly intercorrelated with one another. They showed their strongest associations with plant height and biomass. Stem length, plant height, and leaf number were strongly intercorrelated with each other and with fresh and dry weight. Stomatal conductance showed a near-perfect correlation with root area. Plant population consistently showed the weakest correlations among all parameters. Overall, these patterns indicate a tightly coordinated physiological and morphological response across treatments.

4. Discussion

The application of hydrogel, silica nanoparticles (SiNPs), and K-humate, alone and in combination,

improved the physiological performance, growth, and biomass of Bululawang ratoon sugarcane. The triple-combination treatment (T6) consistently outperformed the single and double-combination treatments across nearly all parameters, indicating a synergistic interaction among the three amendments rather than a simple additive effect (Mindari *et al.* 2025). Notably, the hydrogel-only treatment (T1) showed no significant advantage over the untreated control (T0) in either physiological or growth parameters. This outcome is attributable to the prevailing high-rainfall conditions at the experimental site (monthly precipitation 268.27 to 392.10 mm, mean 328.53 mm month⁻¹ (NASA Power 2025), under which soil water availability was inherently sufficient and the primary water-retention function of hydrogel became redundant (Wang *et al.* 2025). This is further supported by the comparable performance of T5 (SiNPs + K-humate) and T6 (hydrogel + SiNPs + K-humate) across nearly all

measured growth parameters, indicating that the addition of hydrogel to this combination provided no significant additional benefit. This is consistent with previous reports indicating that hydrogel benefits are most pronounced under water-deficit or drought conditions rather than in high-rainfall environments (Gholamhoseini *et al.* 2018; Abdelghafar *et al.* 2024).

The increase in nitrate reductase activity (NRA) under the amended treatments reflects improved nitrogen assimilation capacity, the entry point of the physiological response observed in this study. K-humate enhances NRA by increasing soil cation exchange capacity through dissociation of carboxyl and hydroxyl functional groups, forming humate-nitrogen complexes that reduce leaching and stimulate the activity of beneficial rhizosphere microorganisms involved in the nitrogen cycle (Arjumend *et al.* 2015; Ampong *et al.* 2022; Ma *et al.* 2024). This mechanism is consistent with findings in foxtail millet, where potassium humate increased NRA by 34.4%, with the enhancement directly linked to greater soluble protein synthesis and yield (Shen *et al.* 2024), and in sugarcane, where humic substances elevated nitrate reductase and glutamine synthetase activities, increasing total protein concentration (Leite *et al.* 2020).

SiNPs contribute to NRA enhancement through improved cell membrane integrity and structural preservation of leaf mesophyll cells, including intact cell walls and normal nuclei essential for efficient nitrogen transport and enzyme function (Yuvaraj *et al.* 2023; Faisal *et al.* 2024), with a 63.7% increase in NR activity reported following SiO₂-NPs application, an effect mechanistically linked to coordinated regulation of nitrogen and carbon metabolism (Ashraf *et al.* 2024). The strong positive correlation between NRA and the other physiological parameters (Figure 2) confirms the central and upstream role of nitrogen metabolism in orchestrating the downstream physiological responses observed across treatments, consistent with the view that humic-acid-driven nitrogen assimilation directly drives chlorophyll production, enzyme synthesis, and growth hormone activity (Ampong *et al.* 2022).

The higher chlorophyll content recorded under T6 and T5 is consistent with the strong correlation between NRA and total chlorophyll ($r > 0.8$), since nitrogen constitutes the porphyrin ring of chlorophyll and is an essential component of key photosynthetic enzymes (Akhtar *et al.* 2024). This relationship is consistent with Bhat *et al.* (2024), who reported notable positive correlations between NR activity and total chlorophyll across treatments. The higher chlorophyll content under T6 and T5 reflects a combination of amplified nitrogen supply and direct

pigment-protective effects of SiNPs and K-humate. SiNPs are reported to protect chlorophyll from photodegradation through enhanced expression of chlorophyll biosynthesis genes and direct binding with chloroplasts (Li *et al.* 2023; Yang *et al.* 2025), with increases of up to 42.3% in total chlorophyll reported following SiO₂-NPs application in rice and *Ocimum tenuiflorum* (Ashraf *et al.* 2024; Bhat *et al.* 2024). K-humate improves uptake of macro and micronutrients, particularly iron, an essential cofactor in chlorophyll biosynthesis, and promotes chlorophyll synthesis through hormone-mediated signaling that upregulates photosynthetic pigment production (Zhang *et al.* 2019b; Nassar *et al.* 2023; Shen *et al.* 2024).

Notably, T5 and T6 did not differ significantly in any chlorophyll fraction (Table 2), again indicating that the addition of hydrogel to the SiNPs and K-humate combination provided no further pigment-related benefit. The close agreement among Chl a, Chl b, and Chl t ($r > 0.8$) (Figure 2) indicates a coordinated pigment response to nitrogen enrichment, with Chl a remaining the dominant light-harvesting fraction (Akhtar *et al.* 2024). The elevated chlorophyll pool in T6 and T5 represents the functional prerequisite for the subsequent enhancement in gas exchange and photosynthetic performance.

The rise in stomatal conductance, transpiration, and net photosynthetic rate under T6 and T5 corresponds with the increase in chlorophyll content, since greater pigment concentration raises the demand for CO₂ uptake. This is consistent with the strong positive correlations between Chl t and *g_s* ($r > 0.6$) and *E_m* ($r > 0.5$) (Figure 2), and with Akhtar *et al.* (2024), who established that higher leaf nitrogen content directly sustains elevated stomatal sensitivity and photosynthetic capacity. The increase in *g_s* under T6 reflects the combined contributions of K-humate, which enhances stomatal opening through hormone-like signaling and improved turgor regulation (Ampong *et al.* 2022; Shen *et al.* 2024), and SiNPs, which improve cell wall flexibility and plasticity to facilitate stomatal mechanics and CO₂-O₂ exchange (Faisal *et al.* 2024). The parallel increase in *E_m* is consistent with reports of a 52% increase in transpiration rate alongside enhanced *g_s* following the application of SiO₂-NPs (Shen *et al.* 2024; Faisal *et al.* 2024).

Notably, T5 and T6 did not differ significantly in *g_s*, *E_m*, or PN (Table 2), consistent with the pattern observed for NRA and chlorophyll content, and further indicating that the hydrogel provided no additional benefit to gas exchange or photosynthetic performance beyond that conferred by SiNPs and K-humate. The resulting increase in net photosynthetic rate is consistent with previous

reports in sugarcane, in which humic substances improved PN and instantaneous water-use efficiency by increasing mesophyll conductance and carboxylation efficiency (Leite *et al.* 2020). A similar effect was reported in potato, where humic acid improved PN and gs across varied water-deficit conditions (Man-hong *et al.* 2020). A 35% increase in PN has similarly been reported in rice following SiO₂-NPs treatment (Ashraf *et al.* 2024). The strong intercorrelation among NRA, chlorophyll, gas exchange, and photosynthetic parameters ($r = 0.6-0.8$) (Figure 2) indicates that these physiological responses operate as a coordinated system rather than independent traits. This supports a sequential physiological chain in which enhanced nitrogen assimilation drives chlorophyll synthesis, which in turn promotes wider stomatal aperture and ultimately maximizes CO₂ fixation (Shen *et al.* 2024; Bhat *et al.* 2024; Akhtar *et al.* 2024).

This physiological improvement translated into measurable growth gains. The expansion in leaf area under T6 and T5 represents a direct expansion of the photosynthetically active surface, consistent with its strong correlation with biomass parameters and with previous reports linking leaf area index to yield potential in root and stem crops, where a one-unit increase in LAI induced a 65% increase in root yield in sugar beet (Nassar *et al.* 2023), and where a decline in LAI was identified as a primary yield-limiting factor through reduced photosynthesis (Khodadadi *et al.* 2020). K-humate stimulates leaf expansion through hormone-mediated cell division governed by auxin and cytokinin (Ampong *et al.* 2022; Shen *et al.* 2024), while SiNPs improve the efficiency of light energy utilization to support leaf area development (Yang *et al.* 2025).

Root area followed the same pattern, with correlations to nitrogen metabolism ($r > 0.7$) and chlorophyll content ($r > 0.6$), indicating that root development supported, rather than merely followed, the upstream physiological response. K-humate stimulates lateral root proliferation through auxin and cytokinin-like mechanisms and by enhancing root hair density and cortical cell expansion (Ampong *et al.* 2022; Ma *et al.* 2024), with reported increases of 22.80-28.38% in root production under K-humate (Nassar *et al.* 2023) and humic substances-driven enhancement of lateral root emergence and root hair initiation in sugarcane (Leite *et al.* 2020). SiNPs contributed to RA expansion through reported increases in primary root length of 33.93% and in lateral root number of 25.00% (Ashraf *et al.* 2024; Yang *et al.* 2025). Notably, T5 and T6 did not differ significantly in LA or RA (Table 3), again indicating that the hydrogel provided no additional

benefit to leaf or root development beyond that already conferred by SiNPs and K-humate.

The increases in plant population, stem diameter, stem length, and plant height under T6 and T5 represent the structural outcome of this improved physiological and root performance. Plant population is linked to hormone-mediated tiller initiation under K-humate and SiNPs application, consistent with reports that combined humic acid and Si application improves nitrogen availability and tiller count (Mindari *et al.* 2025), and that SiO₂ nanoparticles elevate cytokinin, gibberellin, and indole acetic acid levels, regulating gene transcription related to shoot and tiller formation (Faisal *et al.* 2024). Unlike the other growth parameters, plant population was the one trait in which T6 differed significantly from T5 (Table 3), suggesting that hydrogel may still contribute marginally to tiller emergence even under otherwise sufficient soil moisture. Stem diameter and plant height responded to K-humate in a dose-dependent manner (Ampong *et al.* 2022; Shen *et al.* 2024), whereas SiNPs increased stem-related biomass by enhancing protective enzyme activity, chlorophyll synthesis, and photosynthetic efficiency (Yang *et al.* 2025). Stem elongation closely tracked plant height ($r > 0.9$), consistent with height serving as an integrative indicator of overall plant performance (Shen *et al.* 2024; Basha *et al.* 2020), and T5 and T6 again did not differ significantly in SD, SL, or PH (Table 3), reinforcing the limited additional contribution of hydrogel to stem development.

The resulting biomass gain in T5 and T6 therefore reflects the cumulative effect of improvements occurring simultaneously at the molecular, cellular, and whole-plant levels, rather than a single dominant mechanism. This is consistent with reports of HA-induced improvements of 14.12-36.63% in above-ground dry biomass (Man-hong *et al.* 2020), and nSiO₂-mediated increases of 29.18% in leaf dry weight alongside a 13.84% gain in photosynthetic rate (Yang *et al.* 2025). T5 and T6 did not differ significantly in FW or DW (Table 3), consistent with the pattern observed across nearly all physiological and growth parameters in this study, and further supporting the conclusion that hydrogel contributed no measurable additional benefit once SiNPs and K-humate were already present.

The combined application of SiNPs and K-humate (T5 and T6) produced the most favorable physiological and growth responses in Bululawang ratoon sugarcane. Nitrate reductase activity, total chlorophyll, net photosynthetic rate, leaf area, root area, and dry biomass all reached their highest values relative to the untreated control. The strong positive correlations among physiological

and growth parameters indicate a coordinated system-level response. K-humate primarily supported nitrogen availability and root development, while SiNPs reinforced cellular physiological resilience. Hydrogel contributed no measurable additional benefit under the high-rainfall conditions of this study. This is evidenced by the consistent statistical equivalence between T5 and T6 across nearly all parameters measured.

These findings demonstrate that the combined application of SiNPs and K-humate offers a sustainable strategy to enhance ratoon sugarcane productivity. This strategy works by optimizing the integrated physiological process from nitrogen metabolism to biomass accumulation, particularly on degraded soils. Hydrogel may be unnecessary where soil moisture is not limiting. Future research should extend this evaluation to stalk yield, sucrose content, and soil biological properties across a full production cycle. Such studies should ideally examine these amendments under contrasting rainfall or water-deficit conditions, to clarify whether hydrogel provides a measurable benefit under more moisture-limited circumstances.

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