



Acclimatization of micropropagated sugarcane plantlets: Growth, physiology, and nutrition under contrasting light environments

Aclimatização de plântulas micropropagadas de cana-de-açúcar: Crescimento, Fisiologia, nutrição sob ambientes contrastantes de luz

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ABSTRACT

In the final stage of micropropagation, sugarcane plantlets (*Saccharum officinarum* L.) undergo *ex vitro* acclimatization, a period in which important light-influenced morphophysiological adjustments occur. This study aimed to establish acclimatization protocols for micropropagated sugarcane plantlets under different light conditions. The experiment was conducted from May to July 2022 at the Northeast Center for Strategic Technologies and the Sugarcane Experimental Station in Carpina, Pernambuco, Brazil, in a randomized block design with four replications. Acclimatization occurred under shaded, unshaded greenhouse, and full sun field conditions for 51 days, comprising 10 treatments (T1–T10). Survival, biometrics, leaf area, SPAD index, biomass, root/shoot ratio, and nutritional parameters were evaluated. Treatment T8 (shaded for 51 days) promoted greater plant height, leaf length, leaf area, and leaf number, while T9 (unshaded in a greenhouse for 51 days) showed a higher survival rate, culm diameter, shoot dry mass, and SPAD index. In contrast, T10 (full sun) showed the lowest overall development. These results demonstrate that protected environments, whether shaded or unshaded, favor the initial growth and acclimatization of micropropagated plantlets of the RB-041443 variety.

Keywords: Plant tissue culture; *Ex vitro* establishment; Light management; Morphophysiology; *Saccharum* spp.

RESUMO

Na fase final da micropropagação, mudas de cana-de-açúcar (*Saccharum officinarum* L.) passam pela aclimatização *ex vitro*, período em que ocorrem importantes ajustes morfofisiológicos influenciados pela luz. Este estudo objetivou estabelecer protocolos de aclimatização para mudas micropropagadas de cana-de-açúcar sob diferentes condições de luz. O experimento foi conduzido de maio a julho de 2022 no Centro de Tecnologias Estratégicas do Nordeste e na Estação Experimental de Cana-de-Açúcar de Carpina, Pernambuco, Brasil, em delineamento de blocos casualizados, com quatro repetições. A aclimatização ocorreu sob sombreamento, sem sombreamento em casa de vegetação e a pleno sol em campo, durante 51 dias, compreendendo 10 tratamentos (T1–T10). Avaliaram-se a sobrevivência, biometria, área foliar, índice SPAD, biomassa, razão raiz/parte aérea e parâmetros nutricionais. O tratamento T8 (51 dias sob sombreamento) promoveu maior altura de plantas, comprimento foliar, área foliar e número de folhas, enquanto o T9 (51 dias sem sombreamento em casa de vegetação) apresentou maior taxa de sobrevivência, diâmetro do colmo, massa seca da parte aérea e índice SPAD. Em contraste, o T10 (pleno sol) apresentou o menor desenvolvimento geral. Esses resultados demonstram que ambientes protegidos, com ou sem sombreamento, favorecem o crescimento inicial e a aclimatização de mudas micropropagadas da variedade RB-041443.

Palavras-chave: Cultura de tecidos vegetais; Estabelecimento *ex vitro*; Manejo da luz; Morfofisiologia; *Saccharum* spp.

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Recebido em: 27/06/2025. Aceito em: 24/06/2026.

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1 INTRODUÇÃO

Sugarcane (*Saccharum* spp.) is one of the most important crops in tropical and subtropical regions, accounting for approximately 60% of global sugar production. Modern cultivars originate from interspecific crosses between the “noble cane” *Saccharum officinarum* L. and the wild species *Saccharum spontaneum* L., combining high sucrose content with greater adaptability and resistance to biotic and abiotic stresses (Silva *et al.*, 2020). In addition to sugar production, sugarcane is a strategic crop for bioenergy due to its high biomass yield and C4 photosynthetic metabolism, which confers high efficiency in carbon assimilation. Its industrial processing also generates valuable by-products, such as molasses for alcohol production and bagasse for paper manufacturing, animal feed, and energy cogeneration (Redae and Ambaye, 2018; Dias *et al.*, 2021).

According to recent estimates for the 2025/2026 harvest, Brazil remains the world's leading sugarcane producer, with production supported by increases in harvested area and productivity, contributing directly to the expansion of sugar, ethanol, and bioenergy production (Conab, 2026). Sustaining the competitiveness of this sector requires continuous technological advances and the rapid dissemination of superior cultivars adapted to different production environments.

Genetic improvement has played a fundamental role in increasing sugarcane productivity, adaptability, and resistance to pests and diseases (Santos *et al.*, 2023; Silva *et al.*, 2025). However, conventional propagation through stem cuttings is limited by low multiplication rates and the risk of pathogen dissemination. Micropropagation via plant tissue culture overcomes these limitations by enabling the rapid, large-scale production of genetically uniform and disease-free seedlings (Redae and Ambaye, 2018; Salokhe, 2021).

Acclimatization represents the final stage of micropropagation and is considered one of the most critical phases for seedling establishment. During the transition from *in vitro* to *ex vitro* conditions, plants undergo important morphophysiological adjustments, including modifications in cuticle development, stomatal functionality, vascular tissues, and leaf anatomy, which are essential for adaptation to environmental fluctuations and autonomous growth under natural conditions (Caetano *et al.*, 2021; Pinheiro *et al.*, 2022).

Among the environmental factors driving acclimatization, light availability plays a central role in regulating plant growth and development. Variations in irradiance directly affect photosynthetic performance, biomass allocation, leaf expansion, and anatomical organization. Under shaded conditions, plants typically increase leaf area to maximize light interception, whereas higher irradiance promotes structural reinforcement and physiological adjustments that enhance photosynthetic

capacity. These responses reflect the high phenotypic plasticity of sugarcane, a C4 species capable of dynamically adjusting its morphology and physiology to environmental variations (Yogendra *et al.*, 2024). Aligning with these adaptive traits, the sugarcane variety RB-041443 is widely cultivated in Northeastern Brazil, outstanding for its high tillering, rapid growth, elevated sucrose content, disease resistance (Oliveira *et al.*, 2021), and superior agronomic performance under restrictive environmental conditions (Ridesa, 2025).

Despite its regional expansion, its acclimatization requirements under different light regimes remain poorly understood, and institutional practices vary from shaded or unshaded greenhouses to full sun. We hypothesized that gradual exposure to increasing irradiance during acclimatization promotes higher survival and vegetative development in micropropagated plantlets than continuous full sun exposure. Thus, this study aimed to establish acclimatization protocols for micropropagated RB-041443 plantlets under shaded, unshaded, and full sun environments, seeking management strategies to improve growth, survival, and subsequent field establishment.

2 MATERIAL AND METHODS

The experiment was conducted between May and July 2022 using micropropagated sugarcane plantlets. While the broader project evaluated acclimatization periods of both 51 and 65 days (from May 18, 2022, to July 7, 2022, and July 21, 2022, respectively), the present study focuses exclusively on the 51-day acclimatization phase. The research was carried out at the greenhouse of the Governor Miguel Arraes Biofactory, located at the Center for Strategic Technologies of the Northeast (CETENE), Recife, Pernambuco, Brazil (08° 02' 56" S; 34° 56' 51" W, altitude: 10 m), and at the experimental field area of the Carpina Sugarcane Experimental Station (EECAC/UFRPE), Carpina, Pernambuco, Brazil (07° 51' 04" S; 35° 14' 27" W, altitude: 178 m). According to the Köppen (1974) classification, the regional climate is tropical rainy with a dry summer (As).

The experimental period coincided with one of the most severe rainfall events recorded in Northeastern Brazil in recent decades. Intense easterly wave disturbances, enhanced by the passage of a cold front at the end of May 2022, produced exceptional precipitation anomalies throughout the Metropolitan Region of Recife (MARENGO *et al.*, 2023). According to records from the National Institute of Meteorology (INMET) and the Pernambuco Water and Climate Agency (APAC), cumulative rainfall in Recife reached approximately 551 mm between May 25 and 30, exceeding the historical climatological average for the entire month of May (INMET, 2022; APAC, 2022; MARENGO *et al.*, 2023). During this period, daily rainfall events surpassed 100 mm, with peaks approaching 150 mm in a single day, causing severe flooding, landslides, transportation interruptions, and the declaration of a state of emergency in several municipalities of the Recife metropolitan area. These extreme weather conditions substantially restricted access to the experimental areas and limited the execution of

field activities. Consequently, the evaluations originally scheduled for 45 days of acclimatization could not be performed, and the experiment was concluded at 51 days, when access and operational conditions were re-established.

Micropropagated plantlets of the RB-041443 variety were established from apical meristems and multiplied *in vitro* using a Temporary Immersion Bioreactor (TIB) system. This system was configured in a twin-container model utilizing 5-liter polyethylene terephthalate (PET) vessels with periodic nutrient solution renewals, operating under the temporary immersion principles adapted from Escalona *et al.* (1999) up to the sixth subculture.

Released commercially in 2021, this variety stands out for its high adaptability and yield stability. According to the recent national varietal census by Ridesa, RB-041443 has rapidly expanded its commercial footprint, ranking among the top three most cultivated varieties in the North-Northeast region of Brazil, where it accounts for approximately 7.7% of the total sugarcane area. This high adoption rate is driven by its outstanding agronomic traits, including vigorous tillering in both plant and ratoon cane cycles, rapid canopy closure, tall stature, low flowering incidence, medium-early maturation, and high sucrose content (Oliveira *et al.*, 2021).

Prior to planting, a representative sample of forty micropropagated plantlets from the sixth subculture was evaluated to characterize the initial plant material. The biometric values reflect the mean measurements obtained from this lot, which exhibited a plant height of 10.52 cm, leaf length of 7.59 cm, culm diameter of 2.08 mm, leaf width of 3.30 mm, and an average of 9.55 leaves per plantlet. The mean root length was 4.25 cm, with an average rooting rate of 8.84 roots per plantlet. Additionally, the estimated mean leaf area was 1.96 cm², calculated according to Oliveira *et al.* (2007) and Guiselini *et al.* (2013). All linear and diametrical measurements were performed with the aid of a graduated ruler and a digital caliper.

The ten acclimatization treatments were established to simulate different light-transition strategies commonly adopted during the ex-vitro establishment of sugarcane plantlets. The combinations were designed based on acclimatization practices routinely employed at CETENE and EECAC/UFRPE, which differ in the duration and sequence of exposure to shaded, unshaded greenhouse, and full sun conditions. The treatments represented a gradient of light exposure, ranging from continuous cultivation under a single environment (shaded, unshaded, or full sun) to gradual transitions between environments. This approach allowed the evaluation of whether progressive increases in irradiance favor the acclimatization process and improve plantlets survival and growth when compared with abrupt exposure to higher radiation levels. Furthermore, the treatments were designed to identify the most suitable acclimatization strategy for micropropagated sugarcane plantlets intended for subsequent field establishment.

The experimental design was randomized blocks, with four replications for the 10 treatments, with the 10 treatments associated with 51 days of acclimatization (DA): T1 – plantlets with 15 days of shading + 36 days without shading; T2 – 20 days with shading + 15 without shading + 16 in full sun; T3 – 15 days with shading + 15 without shading + 21 in full sun; T4 – 10 days with shading + 10 without shading + 31 in full

sun; T5 – 5 days with shading + 15 without shading + 31 in full sun; T6 – 30 days without shading + 21 in full sun; T7 – 15 days without shading + 36 in full sun; T8 – 51 days with shading; T9 – 51 days without shading; T10 – 51 days in full sun.

Each treatment consisted of 30 plantlets per block, totaling 1,200 plantlets across the entire experiment (10 treatments $\times$ 30 plantlets per treatment $\times$ 4 blocks). For survival evaluation, 30 plantlets per treatment were evaluated, and for biometric evaluations, five plantlets from each treatment were randomly selected and marked with red numbers to ensure the same individuals were measured throughout the monitoring period, resulting in 200 monitored plantlets. The trays were moved according to the respective treatments associated with shading and no shading, while respecting the randomized block design. The application of pesticides and fertilizers followed the CETENE Biofactory protocol. The sugarcane plantlets were irrigated by an automatic sprinkler system, with four daily irrigation events lasting four minutes each.

For plant measurements, the following plant variables were considered: the survival rate (SR), was recorded at 15 days of planting (DAP) for each treatment per block. The following variables were measured weekly: plant height (PH), representing the maximum height considering the plant's architecture in the tube; leaf length (LL), measured from the leaf insertion to the leaf tip; culm diameter (CD), measured at the midpoint of the stem using a digital caliper; and leaf width (LW), measured at the midpoint of leaf +1 using a digital caliper. The values were converted to centimeters to calculate the leaf area.

Morphological variables, including the number of leaves (NL, determined by counting all expanded leaves on the main plantlets, excluding leaves from newly emerged tillers), estimated leaf area (ELA), and estimated leaf area per plant (ELAP), were also evaluated weekly according to the non-destructive mathematical models proposed by Oliveira *et al.* (2007) and Guiselini *et al.* (2013). Concurrently, relative chlorophyll content was assessed weekly using a portable SPAD-502 chlorophyll meter (Soil Plant Analysis Development; Minolta Camera Co., Osaka, Japan). Measurements were taken non-destructively on the middle third of the first fully expanded leaf with a visible dewlap (leaf +1), between 09:00 and 11:00 h. The device estimates relative chlorophyll content by measuring leaf transmittance at wavelengths of 650 nm (red light) and 940 nm (infrared radiation), generating values expressed as SPAD units (Minolta, 1989). The SPAD index serves as a reliable, non-destructive proxy for estimating total chlorophyll levels and nitrogen status, a relationship robustly validated in the literature for both annual crops (Vishwakarma *et al.*, 2023) and specifically for sugarcane cultivation systems (Oliveros *et al.*, 2021; Ilkaee *et al.*, 2016).

At 51 days of acclimatization, the dry mass of the aerial part (DMAP), the dry mass of the root (DMR), and the ratio between the dry mass of the root and the aerial part (DMR/DMAP) were measured. The five plantlets previously selected and marked for biometric and morphological evaluations in each treatment and block were harvested and separated into shoot and root fractions. The samples were placed in pre-labeled paper bags, identified according to treatment and replication, and

weighed. Subsequently, the material was oven-dried at 70 °C for 72 h and reweighed to determine dry mass, discounting the tare weight of each corresponding bag (Bezerra Neto and Barreto, 2011). For root dry mass determination, roots were previously washed under running water to remove substrate residues. Only these five previously monitored plantlets were used for the biomass evaluations, ensuring a direct correspondence between morphological, biometric, and biomass data obtained from the same individuals.

The remaining plantlets from each treatment and block were processed similarly and combined with the five monitored plantlets to form composite samples representative of each treatment. Thus, the dry aerial biomass from all 30 plantlets (or all surviving individuals) was pooled to provide sufficient material for the biochemical and nutritional analyses. The samples were ground in a Fritsch Pulverisette 14 rotor mill and stored in properly identified airtight metallized bags until analysis. Triplicate subsamples were subsequently used to determine total nitrogen content by the Kjeldahl method (digestion, distillation, and titration), following Bezerra Neto and Barreto (2011). Crude protein content was estimated from total nitrogen concentration. The same composite samples were also used for elemental analyses of macro- and micronutrients.

Macro and micronutrient concentrations were determined from the dried shoot biomass of micropropagated sugarcane plantlets to provide a descriptive nutritional characterization of the treatments. For each treatment, the dry aerial biomass obtained from all 30 plantlets (or all surviving individuals) was pooled to form a single composite sample representative of that treatment. The composite samples were ground in a Fritsch Pulverisette 14 rotor mill and subsequently pressed into pellets (5–6 g) using an AMEF hydraulic press. One pellet was prepared for each treatment, resulting in ten composite samples corresponding to the ten acclimatization treatments evaluated at 51 days. Additionally, three independent samples of the cultivation substrate were prepared following the same procedure. Elemental analyses were performed at the Laboratory of Phytochemicals and Processes (LAFIP/CETENE) using an Energy Dispersive X-ray Fluorescence Spectrometer (EDXRF), model S2 PUMA Series 2 (Bruker).

The analytical routine included the reading of reference matrix standards (AB1 and AB2) and the substrate samples, followed by a single non-destructive instrumental reading for each plant treatment pellet. The results were expressed in percentage. Because the nutritional analyses were performed using single composite samples per treatment, no inferential statistical analyses were applied to the elemental data. Therefore, the nutritional results are presented descriptively as complementary information to compare the plantlets' mineral composition with their cultivation substrate and to support the interpretation of growth, biomass, total nitrogen, and crude protein responses.

Survival, biometric, and morphological variables, biomass, total nitrogen content, and crude protein estimation were subjected to analysis of variance (ANOVA) using the F-test ($p \leq 0.05$). When significant, treatment means were compared using the Tukey test ($p \leq 0.05$) via SISVAR software (Ferreira, 2011). For biometric

measurements carried out on the same plantlets over time, a repeated-measures analysis was performed. Crucially, these inferential statistical analyses were applied exclusively to the aforementioned growth and biochemical variables, distinct from the descriptive approach used for the elemental nutritional data.

3 RESULTS AND DISCUSSION

Shading is widely implemented in protected environments for sugarcane plantlet production to mitigate high temperatures and excessive solar radiation (Guiselini *et al.*, 2013; Cordeiro Júnior *et al.*, 2020). In the present study, variations in shading duration among the ten treatments did not result in significant differences ($P > 0.05$) in plantlets survival rate (SR) (Table 1). This statistical uniformity suggests that the sugarcane variety RB-041443 exhibits good adaptability during the acclimatization phase, maintaining satisfactory establishment under different light-management strategies.

Table 1. Survival rate (SR, %) of micropropagated sugarcane plantlets (*Saccharum* spp.) variety RB-041443 subjected to different light environments (combinations of shaded, unshaded greenhouse, and full sun conditions) evaluated at 15 days after planting (DAP)

Treatments	Survival rate (%) ns*
T1	60.00 a
T2	69.17 a
T3	62.50 a
T4	70.00 a
T5	66.67 a
T6	86.67 a
T7	80.00 a
T8	66.67 a
T9	88.34 a
T10	75.00 a
CV	19.18%
GA	72.50

Note: Treatments: T1 – plantlets with 15 days of shading + 36 days unshaded; T2 – 20 days shaded + 15 days unshaded + 16 days full sun; T3 – 15 days shaded + 15 days unshaded + 21 days full sun; T4 – 10 days shaded + 10 days unshaded + 31 days full sun; T5 – 5 days shaded + 15 days unshaded + 31 days full sun; T6 – 30 days unshaded + 21 days full sun; T7 – 15 days unshaded + 36 days full sun; T8 – 51 days continuous shading; T9 – 51 days continuous unshaded greenhouse; T10 – 51 days continuous full sun. Means followed by the same lowercase letter do not differ statistically by Tukey's test ($P > 0.05$). ns: not significant; CV: coefficient of variation; GA: general average.

Source: the authors (2025)

Although the treatments were statistically equivalent, plantlets maintained continuously under unshaded greenhouse conditions (T9) showed the highest numerical survival rate (88.34%), whereas T1 (15 days shaded + 36 days unshaded) presented the lowest value (60.00%) (Table 1). These results indicate that acclimatization under protected environments, whether shaded or unshaded, was sufficient to ensure high survival rates of micropropagated plantlets during ex vitro establishment.

Vegetative growth parameters were strongly governed by light availability. The highest plant height (PH) values were clustered among plantlets subjected to continuous shading (T8; 37.98 cm), continuous unshaded greenhouse conditions (T9; 36.60 cm), and the conventional protocol consisting of 15 days of shading followed by 36 days unshaded (T1; 37.50 cm), showing no statistically significant variations among them ($P > 0.05$; Table 2). These findings indicate that protected greenhouse environments, irrespective of artificial shade-cloth placement, provided a microclimate that favored vertical stem elongation.

Table 2. Morphological traits of micropropagated sugarcane plantlets (*Saccharum* spp.) variety RB-041443 subjected to different light environments (combinations of shaded, unshaded greenhouse, and full sun conditions) evaluated during 51 days of acclimatization (DA): plant height (PH, cm), leaf length (LL, cm), culm diameter (CD, mm), leaf width (LW, mm), number of leaves (NL), estimated leaf area (ELA, cm²), and estimated leaf area per plant (ELAP, cm²)

Treat.	PH	LL	CD	LW	NL	ELA	ELAPT
T1	37.50 ab	31.34 a	3.26 a	7.24 a	4.62 ab	19.29 ab	89.64 ab
T2	29.17 cd	25.15 bc	2.60 ab	6.64 a	4.17 abc	14.00 bc	54.71 cd
T3	30.21 cd	27.72 abc	2.81 ab	7.51 a	3.75 bc	16.57 abc	59.21 cd
T4	28.37 cd	25.08 bc	2.90 ab	6.95 a	3.83 bc	14.10 bc	51.79 cd
T5	32.11 bc	28.42 abc	3.02 ab	7.37 a	3.64 c	16.72 abc	58.97 cd
T6	30.46 cd	28.48 abc	2.71 ab	7.90 a	3.71 bc	16.94 abc	59.60 cd
T7	26.24 d	22.96 c	2.65 ab	6.73 a	3.66 c	12.54 c	44.34 de
T8	37.98 a	32.55 a	2.77 ab	7.39 a	4.81 a	20.59 a	98.77 a
T9	36.60 ab	30.13 ab	3.24 a	6.70 a	4.29 abc	17.01 abc	72.99 bc
T10	19.58 e	15.47 d	2.48 b	4.97 b	4.14 abc	6.26 d	24.87 e
CV	7.37%	8.72%	9.83%	9.00%	9.40%	16.42%	17.23%
GA	30.82	26.73	2.84	6.88	4.06	15.40	61.49

Note: Treat. – Treatments: T1 – plantlets with 15 days of shading + 36 days unshaded; T2 – 20 days shaded + 15 days unshaded + 16 days full sun; T3 – 15 days shaded + 15 days unshaded + 21 days full sun; T4 – 10 days shaded + 10 days unshaded + 31 days full sun; T5 – 5 days shaded + 15 days unshaded + 31 days full sun; T6 – 30 days unshaded + 21 days full sun; T7 – 15 days unshaded + 36 days full sun; T8 – 51 days continuous shading; T9 – 51 days continuous unshaded greenhouse; T10 – 51 days continuous full sun. Means followed by the same lowercase letter do not differ statistically by Tukey's test ($P > 0.05$). CV = coefficient of variation; GA = general average.

Source: the authors (2025)

Solar radiation availability strongly influenced the shoot morphometric characteristics of micropropagated RB-041443 plantlets. Continuous full sun exposure (T10) resulted in the lowest mean values for leaf length (LL; 15.47 cm) and culm diameter (CD; 2.48 mm) (Table 2), indicating a more restricted vegetative development under this condition. Conversely, plantlets maintained under continuous shading (T8) and the transitional protocol (T1) exhibited the highest LL values (32.55 cm and 31.34 cm, respectively), showing no significant differences ($P > 0.05$) compared with the unshaded greenhouse treatment (T9; 30.13 cm). This response suggests a high degree of phenotypic plasticity in sugarcane plantlets during acclimatization, with greater leaf elongation occurring under conditions of reduced light availability.

Culm diameter (CD) reached its highest values in the unshaded greenhouse treatment (T9; 3.34 mm) and in the T1 transitional regime (3.26 mm), with no significant differences between them ($P > 0.05$; Table 2). The similar performance of these treatments indicates that acclimatization under protected environments favored culm development. In contrast, the remaining treatments (T2–T8) presented statistically similar CD values ranging from 2.60 to 2.90 mm, demonstrating a relatively stable response of this trait to the different light-management strategies.

Leaf width (LW) ranged from 4.97 to 7.90 mm across treatments, without significant differences ($P > 0.05$; Table 2). This stability suggests that this parameter was less responsive to the environments than leaf length. Similarly, the number of leaves (NL) varied from 3.64 (T5) to 4.81 per plant (T8), indicating only moderate variation among treatments. The responses of estimated leaf area (ELA) and estimated leaf area per plant (ELAP) were more pronounced to light management during acclimatization. Continuous full sun exposure (T10) resulted in the lowest values for both variables ($ELA = 6.26 \text{ cm}^2$; $ELAP = 24.87 \text{ cm}^2$) (Table 2), indicating a substantial reduction in the photosynthetic surface available.

In contrast, the continuous shaded environment (T8) produced the highest metrics ($ELA = 20.59 \text{ cm}^2$; $ELAP = 98.77 \text{ cm}^2$) (Table 2). The greater leaf expansion observed under shaded and protected conditions reinforces the capacity of sugarcane to adjust growth patterns according to radiation availability, a trait associated with the crop's physiological adaptation (Guiselini *et al.*, 2013; Cordeiro Júnior *et al.*, 2020). Furthermore, intermediate results were observed in T1 ($ELA = 19.29 \text{ cm}^2$; $ELAP = 89.64 \text{ cm}^2$) and T9 ($ELA = 17.01 \text{ cm}^2$; $ELAP = 72.99 \text{ cm}^2$), indicating that both strategies provided favorable conditions for leaf development.

The SPAD index is a widely used indicator of relative chlorophyll status and has been associated with photosynthetic performance and nitrogen status in sugarcane (Ilkae *et al.*, 2016; Oliveros *et al.*, 2021; Vishwakarma *et al.*, 2023). In the present study, SPAD readings were monitored at 15, 21, 27, 34, 41, and 50 days after planting (DAP) throughout the 51 days of acclimatization period (Table 3). plantlets maintained under continuous unshaded greenhouse conditions (T9) exhibited the highest SPAD values at both 15 DAP (34.37 SPAD units) and 50 DAP (33.14 SPAD units), indicating that these environmental conditions favored the maintenance of leaf chlorophyll status during acclimatization.

Table 3. Relative chlorophyll index (SPAD index, SPAD units) of micropropagated sugarcane plantlets (*Saccharum* spp.) variety RB-041443 subjected to different light environments (combinations of shaded, unshaded greenhouse, and full sun conditions) evaluated during 51 days of acclimatization (DA)

Treat.	SPAD index (SPAD units)					
	15 DAP	21 DAP	27 DAP	34 DAP	41 DAP	50 DAP
T1	26.27 bAB	22.8 aB	26.05 aAB	31.71 aA	28.98 abAB	29.87 abA
T2	29.68 abA	23.58 aAB	24.71 aAB	29.68 aA	22.63 bB	23.94 bAB
T3	26.11 bA	25.99 aA	24.95 aA	29.41 aA	22.63 bA	28.65 abA
T4	25.04 bB	26.56 aAB	24.39 aB	33.43 aA	25.95 abB	28.97 abAB
T5	27.61 abAB	26.52 aAB	23.15 aB	30.70 aA	24.78 abAB	27.99 abAB
T6	28.02 abA	26.84 aA	25.01 aA	31.62 aA	26.91 abA	27.74 abA
T7	26.26 bA	26.67 aA	25.18 aA	30.65 aA	27.33 abA	29.23 abA
T8	28.91 abA	29.64 aA	27.29 aA	31.78 aA	29.44 abA	30.19 abA
T9	34.37 aA	26.98 aB	27.87 aAB	34.17 aA	29.94 abAB	33.14 aAB
T10	28.81 abAB	28.23 aAB	24.16 aB	34.39 aA	30.83 aAB	26.99 abB
CV	15.26 %					
GA	27.85					

Note: Treat. – Treatments: T1 – plantlets with 15 days of shading + 36 days unshaded; T2 – 20 days shaded + 15 days unshaded + 16 days full sun; T3 – 15 days shaded + 15 days unshaded + 21 days full sun; T4 – 10 days shaded + 10 days unshaded + 31 days full sun; T5 – 5 days shaded + 15 days unshaded + 31 days full sun; T6 – 30 days unshaded + 21 days full sun; T7 – 15 days unshaded + 36 days full sun; T8 – 51 days continuous shading; T9 – 51 days continuous unshaded greenhouse; T10 – 51 days continuous full sun. Means followed by the same lowercase letter within columns (treatments) and uppercase letter within rows (evaluation periods) do not differ statistically by Tukey's test ($P > 0.05$). DAP = days after planting; CV = coefficient of variation; GA = general average.

Source: the authors (2025)

Dry mass accumulation is an important indicator of plantlet growth and vigor because it reflects the efficiency of carbon assimilation and biomass allocation between the aerial and root portions of the plant (Shipley, 2009). During acclimatization, the balance between dry mass of the aerial part (DMAP) and root dry mass (RDM) is particularly relevant, as it influences both the photosynthetic capacity of the aerial portion and the establishment of a functional root system capable of supporting water and nutrient uptake after transplantation (Taiz *et al.*, 2022).

Among the evaluated treatments, plantlets maintained continuously under unshaded greenhouse conditions (T9) presented one of the highest DMAP values (1.06 g) (Table 4). This result was associated with superior vegetative growth, as evidenced by greater plant height (PH; 36.60 cm), leaf length (LL; 30.13 cm), culm diameter (CD; 3.24 mm), and leaf width (LW; 6.70 mm) (Table 2). In contrast, T9 exhibited one of the

lowest RDM values (0.48 g), indicating a greater proportion of biomass allocated to the aerial part during acclimatization.

Table 4. Dry mass of the aerial part (DMAP, g), root dry mass (RDM, g), and dry mass ratio of root and aerial part (RDM/DMAP) of micropropagated sugarcane plantlets (*Saccharum* spp.) variety RB-041443 subjected to different light environments (shaded, unshaded greenhouse, and full sun conditions) during 51 days of acclimatization (DA)

Treatments	DMAP in g	RDM in g	RDM/DMAP Ratio
T1	1.03 a	0.82 abc	0.78 ef
T2	0.70 bc	0.64 abc	0.91 de
T3	0.46 cd	0.77 abc	1.68 a
T4	0.59 bcd	0.89 ab	1.51 ab
T5	0.72 b	0.98 a	1.38 abc
T6	0.66 bc	0.83 abc	1.24 bcd
T7	0.59 bcd	0.59 bc	1.01 cde
T8	0.73 b	0.74 abc	1.01 cde
T9	1.06 a	0.48 c	0.46 f
T10	0.41 d	0.47 c	1.17 bcde
CV	14.24%	20.67%	16.10%
GA	0.70	0.72	1.11

Note: Treat. – Treatments: T1 – plantlets with 15 days of shading + 36 days unshaded; T2 – 20 days shaded + 15 days unshaded + 16 days full sun; T3 – 15 days shaded + 15 days unshaded + 21 days full sun; T4 – 10 days shaded + 10 days unshaded + 31 days full sun; T5 – 5 days shaded + 15 days unshaded + 31 days full sun; T6 – 30 days unshaded + 21 days full sun; T7 – 15 days unshaded + 36 days full sun; T8 – 51 days continuous shading; T9 – 51 days continuous unshaded greenhouse; T10 – 51 days continuous full sun. Means followed by the same lowercase letter do not differ statistically by Tukey's test ($P > 0.05$). DMAP = Dry mass of the aerial part; RDM = root dry mass; RDM/DMAP = dry mass ratio of root and aerial part; CV = coefficient of variation; GA = general average.

Source: the authors (2025)

Continuous full-sun exposure (T10) resulted in the lowest DMAP value (0.41 g) and a low RDM value (0.47 g), both considerably lower than those observed in the best-performing treatments (Table 4). These results are consistent with the reduced vegetative growth recorded under this condition, reinforcing the negative effects of prolonged full-sun exposure during the acclimatization phase. Plantlets subjected to T5 exhibited the highest RDM value (0.98 g) (Table 4). This greater investment in root biomass was accompanied by lower values for PH (32.11 cm), LL (28.42 cm), CD (3.02 mm), NL (3.64 leaves), ELA (16.72 cm²), and ELAPT (58.97 cm²) (Table 2), suggesting a greater proportion of biomass allocated to root development than to shoot expansion under this light-management regime.

A different growth pattern was observed under continuous shading (T8). Although this treatment produced the highest values for PH (37.98 cm), LL (32.55 cm),

NL (4.81 leaves), ELA (20.59 cm²), and ELAPT (98.77 cm²) (Table 2), DMAP remained relatively low (0.73 g), statistically similar to that observed in T5 (0.72 g) (Table 4). These results indicate that greater aerial growth was not necessarily accompanied by proportional increases in dry matter accumulation. The transitional strategy T1 (15 days shaded followed by 36 days unshaded) combined high DMAP (1.03 g) with an intermediate RDM (0.82 g), resulting in a more balanced distribution between the shoot and root systems. Likewise, the RDM/DMAP ratio highlighted differences in allocation among the acclimatization environments, with the highest ratio observed in T3 (1.68), resulting from a low DMAP (0.46 g) associated with a higher RDM (0.77 g) (Table 4).

Overall, light-management strategies during acclimatization directly influenced biomass accumulation and partitioning in micropropagated sugarcane plantlets. While some treatments favored greater dry mass accumulation in the aerial portion, others promoted root development, indicating that radiation availability determines allocation patterns during the transition from *in vitro* to *ex vitro* conditions. A balanced distribution between the aerial and root portions is considered an important attribute of plantlet quality because it contributes to successful establishment after transplantation (Ritchie *et al.*, 2010).

Nitrogen (N) is an essential macronutrient for the growth and productivity of sugarcane, being the second most demanded by the crop. It plays a fundamental role in the synthesis of chlorophyll, essential amino acids, and energy compounds, directly influencing carbohydrate production and plant development (Malavolta *et al.*, 1997; Oliveira *et al.*, 2022). In addition, it is directly related to the crude protein content in plants, impacting their metabolism and growth.

At 51 days of acclimatization, nitrogen concentrations ranged from 0.72 to 1.08 g 100 g⁻¹ DM, whereas crude protein levels varied from 4.51 to 6.72 g 100 g⁻¹ DM among the acclimatization strategies (T1–T10) (Table 5). Treatment T4 exhibited the highest nitrogen concentration (1.08 g 100 g⁻¹ DM) and crude protein level (6.72 g 100 g⁻¹ DM), followed closely by T3, with 1.04 g 100 g⁻¹ DM of nitrogen and 6.50 g 100 g⁻¹ DM of crude protein (Table 5). These results suggest that the gradual transition from shaded to higher-light environments promoted greater nitrogen accumulation and protein synthesis in the micropropagated sugarcane plantlets.

Table 5. Total nitrogen (N, g 100 g⁻¹ DM) and crude protein (CP, g 100 g⁻¹ DM) of micropropagated sugarcane plantlets (*Saccharum* spp.) variety RB-041443 subjected to different light environments (combinations of shaded, unshaded greenhouse, and full sun conditions) evaluated during 51 days of acclimatization (DA)

Treatments	Total Nitrogen (N, g 100 g ⁻¹ DM)	Crude Protein (CP, g 100 g ⁻¹ DM)
T1	0.72 g	4.51 h
T2	0.85 ef	5.33 ef
T3	1.04 ab	6.50 ab
T4	1.08 a	6.72 a
T5	0.97 bc	6.09 bc

Treatments	Total Nitrogen (N, g 100 g ⁻¹ DM)	Crude Protein (CP, g 100 g ⁻¹ DM)
T6	1.00 abc	6.29 abc
T7	0.95 cd	5.95 cd
T8	0.80 fg	5.03 fg
T9	0.75 g	4.74 gh
T10	0.89 de	5.56 de
CV	3.02%	2.95%
GA	0.91	5.67

Note: Treatments: T1 – plantlets with 15 days of shading + 36 days unshaded; T2 – 20 days shaded + 15 days unshaded + 16 days full sun; T3 – 15 days shaded + 15 days unshaded + 21 days full sun; T4 – 10 days shaded + 10 days unshaded + 31 days full sun; T5 – 5 days shaded + 15 days unshaded + 31 days full sun; T6 – 30 days unshaded + 21 days full sun; T7 – 15 days unshaded + 36 days full sun; T8 – 51 days continuous shading; T9 – 51 days continuous unshaded greenhouse; T10 – 51 days continuous full sun. Means followed by the same lowercase letter do not differ statistically by Tukey's test ($P > 0.05$). CV = coefficient of variation; GA = general average.

Source: the authors (2025)

In contrast, a prolonged period under shading (T8) resulted in lower nitrogen (0.80 g 100 g⁻¹ DM) and crude protein (5.03 g 100 g⁻¹ DM) contents (Table 5). Likewise, continuous exposure to sunlight inside the greenhouse, as in treatment T9, led to a reduction in the levels of these parameters (0.75 g 100 g⁻¹ DM and 4.74 g 100 g⁻¹ DM, respectively), possibly due to the greater energy expenditure associated with adaptation to light stress. These results indicate that adequate management of light exposure influences nitrogen absorption and metabolism, directly affecting protein production and, consequently, sugarcane growth and productivity.

The elemental composition analysis of the sugarcane dry matter pool (from 30 plantlets or survivors per treatment pressed into 5–6 g pellets) revealed high concentrations of the macronutrients potassium (K, 0.583% to 0.706%) and calcium (Ca, 0.132% to 0.198%), alongside the micronutrient chlorine (Cl, 0.067% to 0.197%) and the beneficial element silicon (Si, 0.085% to 0.359%) (Table 6). The high accumulation of K across all light-management strategies reflects its mobility within plant tissues, as its absorption and redistribution occur through efficient transport mechanisms, with the cytoplasm and vacuole serving as major storage compartments (Ahmad and Maathuis, 2014). During acclimatization, K plays an important role in osmotic regulation, stomatal functioning, and cell expansion, contributing to plant establishment under varying light environments.

Table 6. Nutrient composition (% of dry matter) of micropropagated sugarcane plantlets (*Saccharum* spp.) variety RB-041443 evaluated at 51 days of acclimatization (DA)

Concentration													
Macronutrients (%)							Micronutrients (%)						
Traet.	P	K	Ca	Mg	S	Cl	Cu	Fe	Mn	Mo	Ni	Zn	Si *

T1	0.037	0.612	0.16	-	0.019	0.163	-	0.006	0.004	0.003	-	0.001	0.196
T2	0.050	0.656	0.132	-	0.015	0.098	-	0.006	0.004	0.003	-	0.001	0.208
T3	0.052	0.604	0.198	-	0.019	0.077	-	0.004	0.005	0.002	-	0.001	0.110
T4	0.059	0.614	0.197	-	0.016	0.073	-	0.006	0.006	0.003	-	0.001	0.182
T5	0.045	0.583	0.184	-	0.014	0.086	-	0.005	0.006	0.003	-	0.001	0.146
T6	0.044	0.638	0.164	-	0.020	0.095	-	0.006	0.003	0.003	-	0.001	0.085
T7	0.04	0.633	0.167	-	0.022	0.076	-	0.006	0.005	0.003	-	0.001	0.200
T8	0.041	0.715	0.178	-	0.028	0.197	-	0.006	0.004	0.003	-	0.001	0.359
T9	0.037	0.577	0.183	-	0.023	0.166	-	0.005	0.003	0.003	-	0.001	0.220
T10	0.032	0.706	0.155	-	0.014	0.067	-	0.006	0.001	0.003	-	0.000	0.265
Subst.	-	0.110	0.218	-	0.002	-	-	0.252	0.004	-	-	-	2.895

Note: Treat. = treatments; Subst. = substrate; Ca = calcium; Cl = chlorine; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; Mo = molybdenum; Ni = nickel; P = phosphorus; S = sulfur; Si = silicon (subtracted amount of silicon derived from the XRF equipment container); Zn = zinc. Data were evaluated descriptively without formal statistical analysis. Discussion is strictly restricted to numerical trends among light-management strategies to avoid data overinterpretation. These nutritional responses must be interpreted alongside growth and biomass production variables to account for potential nutrient dilution effects.

Source: the authors (2025)

The relationship between K and Ca in plant nutrition is influenced by competitive interactions during root uptake. Costa *et al.* (2021) reported antagonistic effects between these nutrients in sugarcane cultivation systems, while Malavolta *et al.* (1997) highlighted that K, as a monovalent cation with a smaller hydrated ionic radius, may be preferentially absorbed compared with divalent cations such as Ca. Therefore, differences observed between K and Ca concentrations among treatments may be associated with these physiological interactions.

Lower concentrations were observed for phosphorus (P; 0.032% to 0.059%), sulfur (S; 0.014% to 0.028%), iron (Fe; 0.004% to 0.006%), manganese (Mn; 0.001% to 0.006%), molybdenum (Mo; 0.002% to 0.003%), and zinc (Zn; up to 0.001%) (Table 6). Although Basaplant substrate was used, direct comparisons with nutrient sufficiency ranges for mature field-grown sugarcane should be interpreted with caution, since micropropagated plantlets exhibit distinct nutritional requirements. Therefore, these concentrations likely reflect the physiological traits of young plantlets transitioning from *in vitro* to *ex vitro* conditions.

Among primary macronutrients, T4 (10 days shaded + 10 days unshaded + 31 days full sun) exhibited the highest P concentration (0.059%), whereas T8 (continuous shading) showed the highest K concentration (0.715%) (Table 6). These results suggest that different light-management strategies may influence nutrient accumulation patterns in micropropagated sugarcane plantlets. However, because no statistical analysis was performed for nutrient concentrations, these differences should be

interpreted only as numerical trends rather than statistically supported treatment effects.

Among the treatments, T8 (51 days under shade) and T9 (without shade, but in a greenhouse during tropical winter) showed the best overall results. T9 had higher survival rates, culm diameter, aerial biomass accumulation, and total chlorophyll content, while T8 showed greater height, leaf length, number of leaves, and leaf area, indicating more vigorous vegetative growth (Tables 1–3). Although T9 was cultivated under diffuse light due to the plastic-covered greenhouse, its performance did not differ significantly from T8 in several variables, despite T8 being under 40% shade. Both demonstrated superior agronomic potential, as illustrated in Figure 1, which highlights the distinct phenotypes. In contrast, T10 showed the lowest development, suggesting that constant sunlight exposure may have limited its growth and physiological performance.

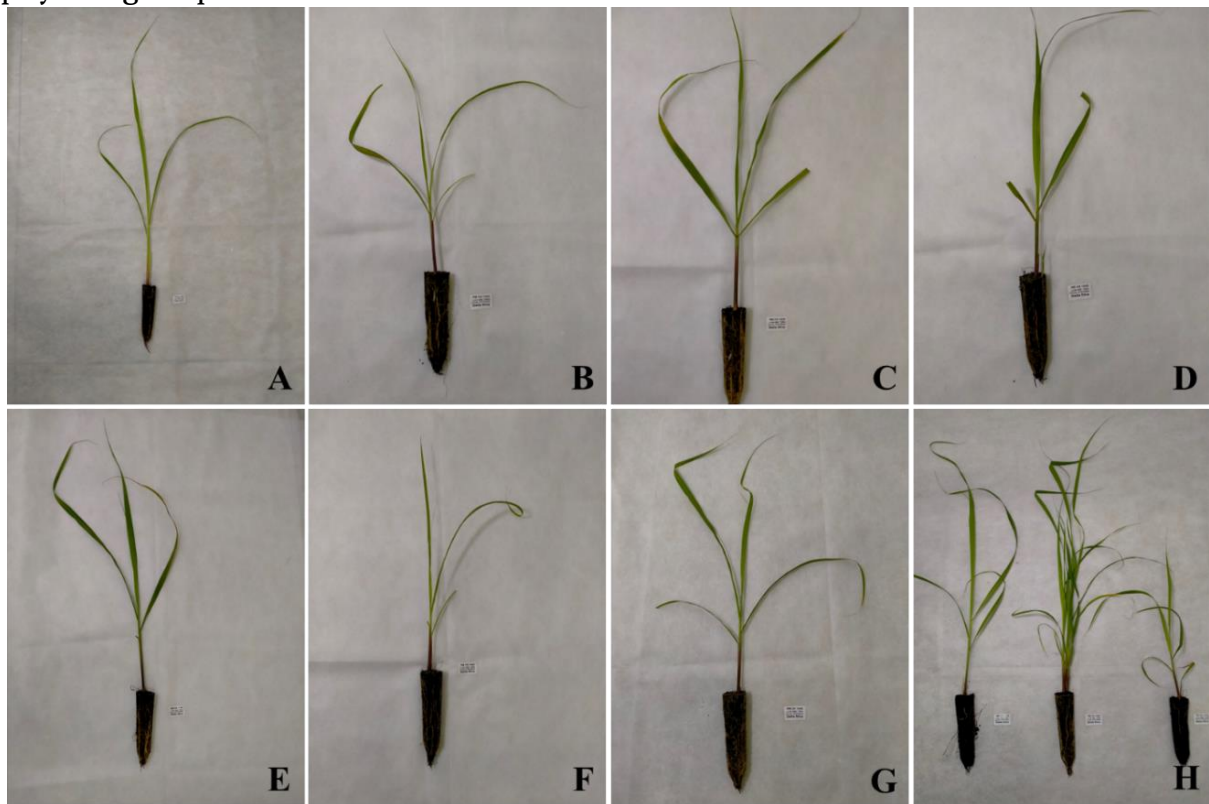


Figure 1. Morphological development of micropropagated sugarcane plantlets (*Saccharum* spp.) variety RB-041443 from treatments T1 (A), T2 (B), T3 (C), T4 (D), T5 (E), T6 (F), and T7 (G), with T8, T9, and T10 grouped in (H), evaluated at 51 days of acclimatization (DA). Treatments: T1 – plantlets with 15 days of shading + 36 days unshaded; T2 – 20 days shaded + 15 days unshaded + 16 days full sun; T3 – 15 days shaded + 15 days unshaded + 21 days full sun; T4 – 10 days shaded + 10 days unshaded + 31 days full sun; T5 – 5 days shaded + 15 days unshaded + 31 days full sun; T6 – 30 days unshaded + 21 days full sun; T7 – 15 days unshaded + 36 days full sun; T8 – 51 days continuous shading; T9 – 51 days continuous unshaded greenhouse; T10 – 51 days continuous full sun.

Source: the authors (2025)

4 CONCLUSIONS

This study demonstrated that light-management strategies during acclimatization influence the morphophysiological development of micropropagated sugarcane plantlets of the RB-041443 variety. Plantlets maintained under continuous shading (T8) showed greater plant height, leaf length, leaf area, and number of leaves, indicating enhanced vegetative growth. In contrast, plantlets maintained continuously in the unshaded greenhouse (T9) exhibited higher numerical values for survival rate, culm diameter, dry mass of the aerial part, and SPAD index, indicating favorable conditions for plantlet establishment and biomass accumulation.

Overall, both greenhouse environments, shaded and unshaded, promoted satisfactory acclimatization and supported the transition from in vitro to ex vitro conditions, whereas continuous full-sun exposure (T10) resulted in the lowest overall growth performance. These findings demonstrate that protected environments, with or without shading, are suitable for the acclimatization of micropropagated sugarcane plantlets and may contribute to improving plantlet quality during the initial stages of development.

5 ACKNOWLEDGMENTS

The team of professionals from the laboratory and greenhouse of the Governador Miguel Arraes Biofactory at the Center for Strategic Technologies of the Northeast - CETENE, the Carpina Sugarcane Experimental Station - EECAC/UFRPE for the space granted for experimentation, and the Council National Institute of Scientific and Technological Development - CNPq for the research grant granted.

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