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RESEARCH ARTICLE
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Gas Exchange and Early Growth Parameters of *Theobroma cacao* under Shading Levels and Substrate Compositions in Tropical Conditions

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ABSTRACT

The study evaluated gas exchange and early growth parameters of *Theobroma cacao* under different shading levels and substrate compositions in tropical conditions. The experiment followed a completely randomized design in a 3 × 5 factorial arrangement (three shading levels × five substrate compositions). The shading treatments consisted of two shade houses covered with black shade nets providing 30% and 50% shading, and an additional treatment under full sun (0% shading). Within each environment, the following substrates were assessed: 100% Carolina® peat substrate (Sub1), 80% Carolina + 20% vermiculite (Sub2), 60% Carolina + 40% vermiculite (Sub3), 40% Carolina + 60% vermiculite (Sub4), and 20% Carolina + 80% vermiculite (Sub5). In tropical environments, growing *Theobroma cacao* seedlings in full sun is not recommended. Changes in light availability and substrate composition affected seedling growth, quality, and gas exchange. Seedling production under 50% shading resulted in taller plants with greater shoot, root, and total dry matter accumulation, as well as improved overall seedling quality. In this environment, seedlings grown in an organic–mineral substrate containing 80% peat exhibited greater stem diameter, while those grown in 100% organomineral substrate showed higher CO_2 assimilation rates and higher instantaneous carboxylation efficiency (A/Ci). In tropical regions, the combination of moderate shading and peat-based substrates promotes better early development of *Theobroma cacao* seedlings.

Keywords: Shading levels; Shade nets; Organomineral substrate; Photosynthetic efficiency.

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INTRODUCTION

Cacao (*Theobroma cacao* L.) is a tree species native to South America, cultivated in humid and sub-humid tropical regions (Almeida & Valle, 2007). It is a major crop in the Amazon, where its production contributes significantly to local income generation. International demand is also increasing, as cacao supplies multiple industries, most notably the food sector, especially chocolate production, as well as pharmaceutical, fertilizer, and animal feed industries (Moda et al., 2019).

Cacao is typically cultivated by planting seedlings or directly sowing viable seeds (Sodré & Gomes, 2019). Seedlings are highly sensitive to water stress and elevated temperatures (Almeida & Valle, 2007). The upper temperature limit for cacao development is around 38°C (Schroth et al., 2016); beyond this threshold, physiological processes are impaired (Lamaoui et al., 2018), leading to leaf dehydration and reductions in leaf production, biomass accumulation, plant height and photosynthetic rate (Almeida & Valle, 2007; Padi et al., 2013; Lahive et al., 2019).

Proper development of cacao seedlings depends on several environmental factors, particularly substrate composition, shading conditions, and the characteristics of the growing region (Hickman, 2011). Growing cacao under shade can mitigate the adverse effects of high temperatures (Richard & Raebild, 2016; Asare et al., 2019) by reducing solar radiation exposure on the plant. Additionally, shaded environments typically exhibit temperatures a few degrees lower due to evaporative cooling, which also enhances the physiological performance and productivity of cacao (Mensah et al., 2022).

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Cocoa is extremely sensitive to high temperatures, from seedling production to development after transplanting into the soil. Therefore, the use of protected environments during seedling formation is essential for plant establishment and development (Visscher et al., 2024). Solar radiation is the primary abiotic factor influencing growth and production, as it serves as the energy source for photosynthetic reactions. However, cocoa cultivation is limited by plant stress when growing seedlings in full sun, resulting from excessive transpiration, which restricts leaf expansion. Intense solar radiation affects the photosynthetic process; therefore, cocoa is considered a partial shade species, adapted to environments with diffuse and moderate light (Lewis et al., 2021). Direct exposure of plants to high light intensity leads to photoinhibition. This process is linked to plant stress caused by light intensity and, consequently, to the inactivation of the photosystem reaction center, particularly photosystem II (PSII) (Mateus-Rodríguez et al., 2023; Zhou et al., 2022).

Even moderate shading reduces the incidence of solar radiation on the leaves, maintains photosystem functioning at adequate levels, acts as a thermal regulator, and promotes better seedling establishment (Lahive et al., 2021). The most suitable microclimate is provided by shading, which can reduce leaf temperature by 2 to 5°C compared to full-sun cultivation (Mensah et al., 2024). This can be related to reduced transpiration and conservation of leaf water content (Della Sala et al., 2021). The morphological development of seedlings is directly linked to shading; environments with partial shade promote the development of larger leaves and a thin cuticle, increasing the photosynthetic surface area (Saavedra et al., 2020).

Substrate composition can provide favorable conditions for seedling emergence and root development. It ensures structural support for the plant and provides adequate levels of water, aeration, nutrients, and texture, producing high-quality seedlings (Santos et al., 2011). In the production of *Theobroma cacao* seedlings, the incorporation of organic materials such as peat, peat mixed with fresh cattle manure, rice husk, chicken manure, or palm oil mill sludge into acid-sulfate soils has been shown to reduce aluminum toxicity (Shamshuddin et al., 2004). Similarly, applying biochar and organic fertilizers has increased the availability of phosphorus and reduced aluminum levels (Sasmita et al., 2017). However, substrates must be formulated using readily available materials that possess biological, physical, and chemical characteristics compatible with the specific needs of the cultivated species.

Cacao seedlings require a substrate with high fertility. In this context, using commercially available peat-based substrates promotes the growth of high-quality seedlings (Tonetto et al., 2020; Ferraz et al., 2023). However, in producing forest species seedlings, using a single substrate type may not always be sufficient to ensure favorable conditions for proper development. Therefore, it is essential to formulate a substrate that guarantees both the quantity and quality of the seedlings (Caldeira et al., 2013). Vermiculite is one of the most commonly used materials in substrate mixtures due to its advantageous properties, including high porosity, good drainage capacity, low density, and near-neutral pH (Costa et al., 2015).

Early germination and rapid growth of *Theobroma cacao* seedlings were achieved using a substrate containing poultry manure, river sand and sawdust in a ratio of 3:1:2 (Odoemelam et al., 2023), as well as the use of the substrate composed of 100% oil palm compost, which promoted excellent growth and adequate nutrition by improving the substrate conditions (Tuesta et al., 2024). Substrate inoculation with arbuscular mycorrhizal fungi increased the quality of *Theobroma cacao* seedlings (Djenatou et al., 2020), the application of 100 mg L^{-1} of Pectimorf® in a substrate composed of 70% soil + 20% sand + 10% rice husk produced stronger *Theobroma cacao* seedlings with a higher survival percentage (Reyes-Perez et al., 2022) and the exogenous application of the biostimulant D'Raz® to the substrate, at a concentration of 4 mL per plant, provided high-quality cocoa seedlings (Freitas et al., 2024).

When growing *Theobroma cacao* L. seedlings, substrates containing high levels of cadmium should be avoided (Correa et al., 2021; Argüello et al., 2023; Ortiz-Álvarez et al., 2023), a toxic heavy metal. Its absorption depends highly on soil characteristics, as there is a strong relationship between soil chemical and physical characteristics and the cadmium available for growing *Theobroma cacao* L. seedlings (Correa et al., 2021), demonstrating that these relationships are driven by both soil physical properties (bulk and particle densities) and chemical attributes (concentrations of iron, sand, magnesium, potassium, sodium, and copper) (Correa et al., 2021). Studies have shown that there was greater Cd accumulation in *Theobroma cacao* under water stress (Ortiz-Álvarez et al., 2023), and soil amendments by liming significantly reduced Cd in leaves, but not in cocoa beans in the field (Argüello et al., 2023). Other studies have shown that the uptake of divalent cadmium ions (Cd^{2+}) in *Theobroma cacao* L. seedlings can be controlled and adjusted through remediation with maghemite (γ-Fe_2O_3) nanoparticles (NPs) and involves three steps: electrostatic exchange, Fe oxide adsorption, and complexation and precipitation of substrate-γ-Fe_2O_3 NPs (Arias-Contreras et al., 2024).

However, information on how substrate composition interacts with shading levels during cacao seedling production remains limited. Therefore, understanding these responses is essential to support the production of high-quality seedlings. This study aimed to evaluate gas exchange and early growth parameters of *Theobroma cacao* under different shading levels and substrate compositions under tropical conditions.

MATERIALS & METHODS

The experiment was conducted at Mato Grosso do Sul State University (UEMS), Cassilândia University Unit (UUC), in Cassilândia, Brazil (19°07'21" S, 51°43'15" W; elevation 516 m), from March 10 to June 25, 2022.

The study evaluated two factors: cultivation environment, with three shading levels (0%, 30%, and

50%), and substrate, with five different substrate compositions. The experiment followed a completely randomized design in a 3 × 5 factorial arrangement, with six replicates. Each plot consisted of four seedlings, and two seedlings per plot were measured for each response variable. The environments included a full-sun treatment (0% shading) and two shade-house structures providing 30% and 50% shading, hereafter referred to as Env30 and Env50, respectively. The shade houses measured 18.0 m × 8.0 m × 3.5 m (144 m^2) and were covered with black monofilament mesh installed at a 45° angle.

Within each cultivation environment, five substrate compositions (Sub1, Sub2, Sub3, Sub4, and Sub5) were evaluated, formulated using the commercial substrate Carolina Soil® and vermiculite as follows: Sub1 = 100% Carolina Soil®; Sub2 = 80% Carolina Soil® + 20% vermiculite; Sub3 = 60% Carolina Soil® + 40% vermiculite; Sub4 = 40% Carolina Soil® + 60% vermiculite; and Sub5 = 20% Carolina Soil® + 80% vermiculite.

Carolina Soil® consists of sphagnum peat, expanded vermiculite, dolomitic limestone, gypsum, and NPK fertilizer and is suitable for coffee, forest, fruit, desert rose, and vegetable seedlings. Each 8 kg package corresponds to 45 L of substrate. The substrate was chemically analyzed (Table 1). Carolina Soil® contains high levels of essential nutrients, including nitrogen, potassium, magnesium, sulfur, copper, iron, manganese, zinc, and boron.

Table 1: Chemical characteristics of Carolina Soil®

Characteristics	Carolina Soil®
Nitrogen (N) (g kg^{-1})	14.00
Phosphorus (P_2O_5) (g kg^{-1})	3.60
Potassium (K_2O) (g kg^{-1})	11.00
Calcium (Ca) (g kg^{-1})	9.10
Magnesium (Mg) (g kg^{-1})	42.00
Sulfur (S) (g kg^{-1})	3.00
Copper (Cu) (g kg^{-1})	0.06
Iron (Fe) (g kg^{-1})	17.52
Manganese (Mn) (g kg^{-1})	2.40
Zinc (Zn) (g kg^{-1})	0.36
Boron (B) (g kg^{-1})	0.08
Organic matter (g kg^{-1})	250.00
Moisture (g kg^{-1})	45.00
Mineral matter (g kg^{-1})	300.00
pH (in water)	6.15
C:N ratio	18.80
OM (dry matter) (%)	45.50
CEC (mmol kg^{-1})	850.00
Electrical conductivity (mS cm^{-1})	0.87

*N, Ca, Mg, S, Cu, Fe, Mn, Zn, B, and Mo – Total contents; OM = Organic matter; CEC = Cation Exchange Capacity.

The seeds of Clone CCN51 were obtained from a commercial orchard in Brasil Novo, Pará, Brazil, with coordinates 3°16'28"S and 52°33'40"W. Sowing was conducted on March 10, 2022, in polyethylene bags (15.0cm × 25.0cm) with a capacity of 1.8L. Seedling emergence was observed six days after sowing (DAS). Irrigation was performed daily, using sprinklers, according to the plant requirements, avoiding substrate saturation.

At 106 DAS (days after sowing), the following variables were measured: plant height (PH, cm), stem diameter (SD, mm), number of leaves (NL), shoot dry mass (SDM, g), and root dry mass (RDM, g). Total dry mass (TDM, g) and the Dickson Quality Index (DQI) were also determined. Seedling height was measured using a graduated ruler from the base of the stem to the apical meristem. Shoot and root dry masses were determined by drying the plant material in a forced-air circulation oven at 65°C for 72 hours, followed by weighing on an analytical balance. The Dickson quality index is defined by the formula DQI = TDM/(PH/SD + SDM/RDM) (Dickson et al., 1960).

Two plants per plot were selected, and the following gas exchange measurements were performed on the most recently fully expanded leaf: internal CO_2 concentration (*Ci*, μmol CO2 m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}), CO_2 assimilation rate (*A*, μmol CO_2 m^{-2} s^{-1}), water-use efficiency [*A/E*, (μmol CO_2) (mmol H_2O)$^{-1}$)], and instantaneous carboxylation efficiency (*A/Ci*). *Ci*, *E*, *gs*, and A measurements were performed between 09:00 and 10:00 h. using a portable infrared gas analyzer - IRGA (LCi, ADC Bioscientific, Hertfordshire, United Kingdom). Subsequently, water-use efficiency (*A/E*) was calculated as the ratio between net photosynthesis and transpiration, and instantaneous carboxylation efficiency (*A/Ci*) as the ratio between net photosynthesis and intracellular CO_2 concentration.

During the gas-exchange measurements, average temperature, relative air humidity, CO_2 concentration, and photosynthetic photon flux density (PPFD) were approximately 25 °C, 80%, 440 μmol mol^{-1}, and 750 μmol m^{-2} s^{-1} in Env30 and 24 °C, 80%, 440 μmol mol^{-1}, and 430 μmol m^{-2} s^{-1} in Env50. Light and CO_2 were not supplemented and were supplied by the surrounding environment.

Photosynthetically active radiation (PAR) (μmol m^{-2} s^{-1}) was monitored in the cultivation environments using a portable digital quantum PAR sensor (Apogee®) daily at 9:30 a.m. local time (Mato Grosso do Sul) on clear-sky days with little to no cloud cover. PAR data were analyzed using a randomized block design with eight replicates, each corresponding to 13 days of the experiment.

Statistical analyses were performed using Sisvar version 5.3 (Ferreira, 2011). Data were checked for normality and homoscedasticity, and treatment means were subjected to analysis of variance (ANOVA) in a two-way factorial model. When significant, effects were evaluated using the F-test, and means were compared by the LSD test at the 5% significance level.

Multivariate analyses were performed using canonical discriminant analysis and principal component analysis (PCA). All analyses were conducted in R software (version 4.3.3; R Core Team, 2023). Canonical discriminant analysis was carried out with the *candisc* package, while PCA was performed using the *ggfortify* and *factoextra* packages. Pearson correlation analysis was also conducted using the *corrplot* package, generating a correlation matrix with color gradients representing the strength and direction of relationships among variables. Positive correlations were displayed in shades of blue, whereas negative correlations appeared in shades of red. Asterisks were used to indicate significance levels: one asterisk for 5% ($p < 0.05$), two for 1% ($p < 0.01$), and three for 0.1% ($p < 0.001$). (S_1 = shade net structure; S_2 = greenhouse).

RESULTS

Under full sunlight, seedlings showed poor development and a very low number of viable plants; therefore, this treatment was excluded from the statistical analyses.

Photosynthetically active radiation (PAR; μmol m^{-2} s^{-1}) differed significantly among shade screens, decreasing as shading intensity increased (Fig. 1). On average, the 30% (Env30) and 50% (Env50) shade nets transmitted 62% and 38% of external PAR, respectively.

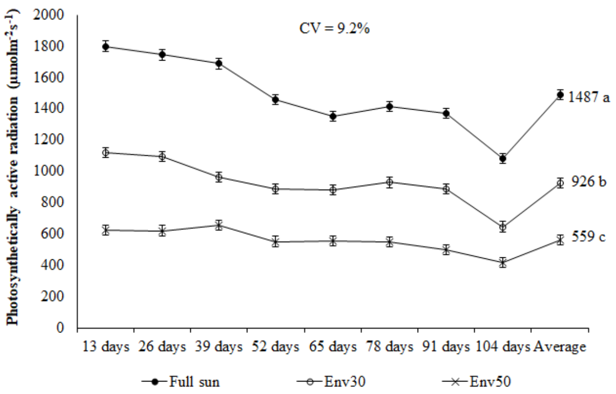

Fig. 1: Photosynthetically active radiation (PAR; μmol m^{-2} s^{-1}) across different cultivation environments. Means followed by the same letter do not differ significantly at the 5% level according to the LSD test. Env30 = 30% shade; Env50 = 50% shade. CV = coefficient of variation. Vertical bars indicate standard error.

The cultivation environments significantly influenced plant height, number of leaves, shoot dry matter, root dry matter, total dry matter, and the Dickson quality index. Stem diameter, however, was affected only by the interaction between environment and substrate (Table 2).

Table 2: Summary of the analysis of variance for plant height (PH), stem diameter (SD), number of leaves (LN), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), and Dickson quality index (DQI) of *Theobroma cacao* seedlings

	F-statistic (F)						
	PH	SD	LN	SDM	RDM	TDM	DQI
Env	144.4680	0.544	46.433	60.6630	22.2520	59.5090	5.0850
Sub	0.944	1.643	0.576	2.219	0.368	1.4430	0.554
Env X Sub	0.171	5.398	0.915	2.28	0.457	1.38	0.595
Probability (P) greater than F-statistic (P>F)							
Env	0.0000	0.4650	0.0000	0.0000	0.0000	0.0000	0.0297
Sub	0.4486	0.1824	0.6814	0.0841	0.8297	0.2376	0.6972
Env X Sub	0.9518	0.0014	0.4649	0.0775	0.7667	0.2583	0.6684
Significance							
Env	**	ns	**	**	**	**	**
Sub	ns	ns	ns	ns	ns	ns	ns
Env X Sub	ns	**	ns	ns	ns	ns	ns
Degrees of freedom (DF)							
Env	1	1	1	1	1	1	1
Sub	4	4	4	4	4	4	4
Env X Sub	4	4	4	4	4	4	4
CV	9.3	6.7	17.3	22.5	16.7	19.2	18.2

Env = environments; Sub = substrates; * significant at 5% probability (0.01 ≤ P < 0.05); ** significant at 1% probability (P < 0.01); ns = not significant (P ≥ 0.05); CV = coefficient of variation.

Theobroma cacao seedlings grown under 50% shade (Env50) exhibited the greatest plant height (Fig. 2A), number of leaves (Fig. 2B), shoot dry mass (Fig. 2C), root dry mass (Fig. 2D), total dry mass (Fig. 2E), and Dickson Quality Index (Fig. 2F).

Plants grown under 50% shade (Env50) were 38% taller (Fig. 2A), had 40% more leaves (Fig. 2B), 66% greater shoot dry mass (Fig. 2C), 25% greater root dry mass (Fig. 2D), 53% greater total dry mass (Fig. 2E), and a 12% higher Dickson Quality Index (Fig. 2F) than those grown under 30% shade (Env30).

Seedling height (Fig. 3A), number of leaves (Fig. 3B), root dry mass (Fig. 3D), and the Dickson Quality Index (Fig. 3F) of *Theobroma cacao* seedlings did not differ significantly among the tested organomineral substrates. However, plants grown in substrate Sub1 exhibited greater shoot dry mass (Fig. 3C) and total dry mass (Fig. 3E) compared to those grown in substrates Sub4 and Sub5.

There was a significant interaction between the cultivation environment and substrate for the stem diameter of Theobroma cacao seedlings. For substrates Sub1, Sub3, and Sub4, stem diameter did not differ between environments. However, in substrates Sub2 and Sub5, seedlings grown under 50% shade (Env50) showed greater stem diameter, with values 14% and 11% higher than those under 30% shade (Env30), respectively (Fig. 4). Under Env30, stem diameter did not differ significantly among substrates. In contrast, under Env50, seedlings grown in substrate Sub2 had stem diameters 16% and 14% greater than those grown in substrates Sub3 and Sub4, respectively (Fig. 4).

For the gas exchange variables, internal CO_2 concentration and water-use efficiency were affected by the main effects of environment and substrate, whereas their interaction influenced CO_2 assimilation and instantaneous carboxylation efficiency. Stomatal conductance and transpiration were not affected (Table 3).

Table 3: Summary of the analysis of variance for internal CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*), CO_2 assimilation (*A*), water-use efficiency (*A/E*), and instantaneous carboxylation efficiency (*A/Ci*) of *Theobroma cacao* seedlings

	F-statistic (F)					
	Ci	*E*	*gs*	*A*	*A/E*	*A/Ci*
Env	81.5890	0.043	0.41	34.1970	30.9950	69.4320
Sub	6.116	1.132	1.321	6.773	10.313	12.9380
Env X Sub	2.27	1.943	2.782	3.85	1.828	6.802
Probability (P) greater than F-statistic (P>F)						
Env	0.0000	0.8386	0.5291	0.0000	0.0000	0.0000
Sub	0.0022	0.3698	0.2965	0.0013	0.0001	0.0000
Env X Sub	0.0976	0.1425	0.0548	0.0177	0.1631	0.0013
Significance						
Env	**	ns	ns	**	**	**
Sub	**	ns	ns	**	**	**
Env X Sub	ns	ns	ns	*	ns	**
Degrees of freedom (DF)						
Env	1	1	1	1	1	1
Sub	4	4	4	4	4	4
Env X Sub	4	4	4	4	4	4
CV	5.9	23.2	26.7	21.8	23.5	21.8

Env = environments; Sub = substrates; * significant at 5% probability (0.01 ≤ P < 0.05); ** significant at 1% probability (P < 0.01); ns = not significant (P ≥ 0.05); CV = coefficient of variation.

Plants exhibited higher internal CO_2 concentration under the Env30 shading environment (Fig. 5A), with no significant differences among substrates (Fig. 5B). Internal CO_2 concentration under Env30 was 22% higher than

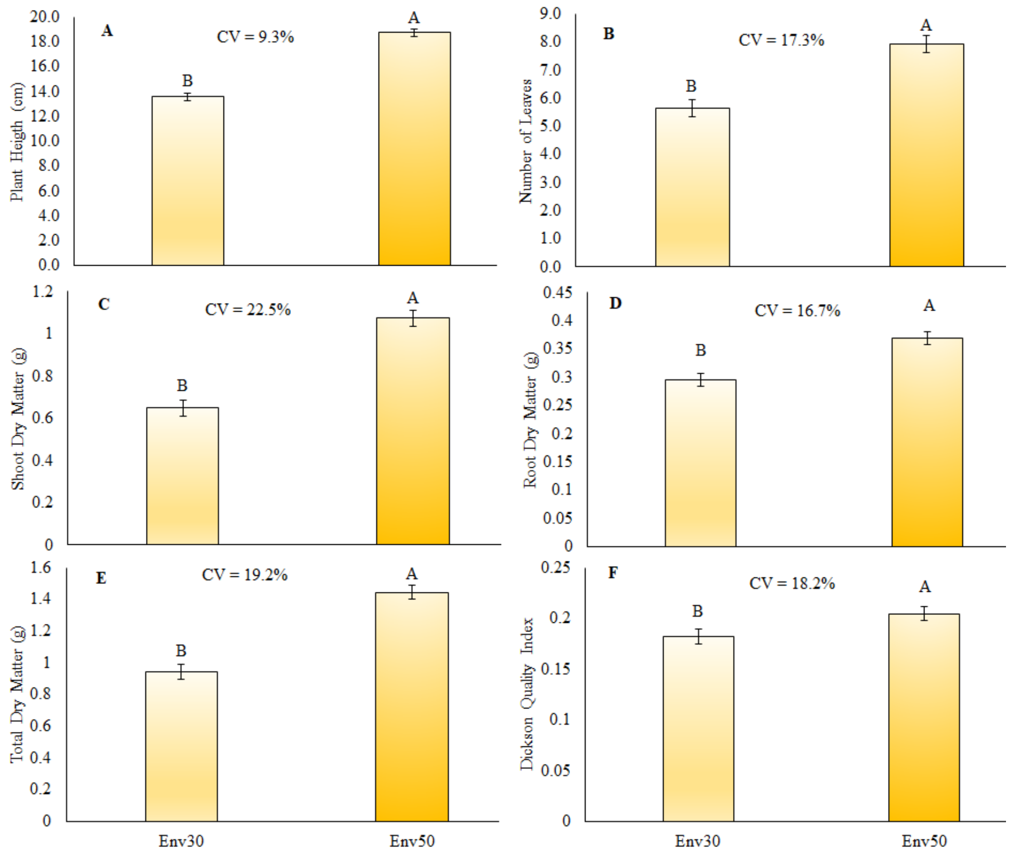

Fig. 2: Seedling height (A), number of leaves (B), shoot dry mass (C), root dry mass (D), total dry mass (E), and Dickson Quality Index (F) of *Theobroma cacao* seedlings grown under different cultivation environments. Env30 = 30% shade; Env50 = 50% shade. Means followed by the same letter do not differ significantly at the 5% level according to the LSD test for each variable. CV = coefficient of variation. Vertical bars indicate standard error.

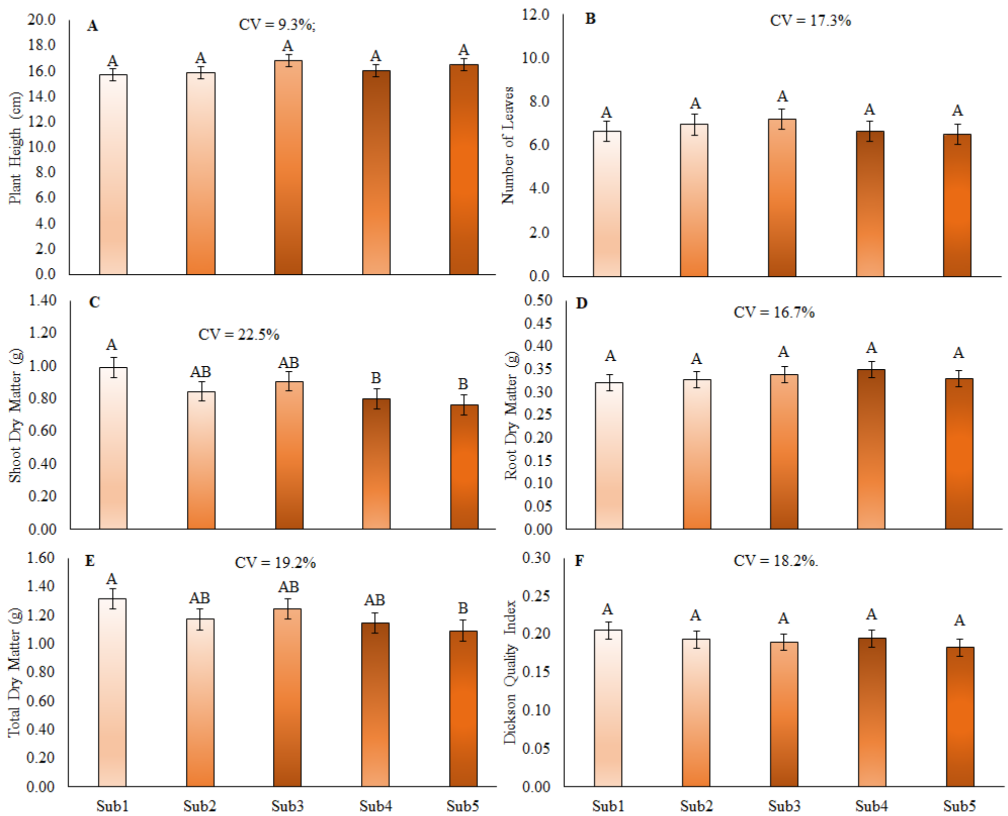

Fig. 3: Seedling height (A), number of leaves (B), shoot dry mass (C), root dry mass (D), total dry mass (E), and Dickson Quality Index (F) of *Theobroma cacao* seedlings grown in different substrates. Means followed by the same letter do not differ significantly at the 5% level according to the LSD test for each variable. CV = coefficient of variation. Sub1 = 100% Carolina Soil®; Sub2 = 80% Carolina Soil® + 20% vermiculite; Sub3 = 60% Carolina Soil® + 40% vermiculite; Sub4 = 40% Carolina Soil® + 60% vermiculite; Sub5 = 20% Carolina Soil® + 80% vermiculite. Vertical bars indicate standard error.

under Env50. Transpiration rates did not differ significantly between shading environments (Fig. 5C) or among substrates (Fig. 5D). Similarly, no significant differences in stomatal conductance were observed across environments (Fig. 5E) or substrates (Fig. 5F). The highest water-use efficiency (A/E) was observed in plants grown under Env50 (Fig. 5G) and in substrate Sub1 (Fig. 5H). *A/E* under Env50 was 63% higher than under Env30, and in substrate Sub1, it was 63%, 63%, 86%, and 101% higher than in substrates Sub2, Sub3, Sub4, and Sub5, respectively.

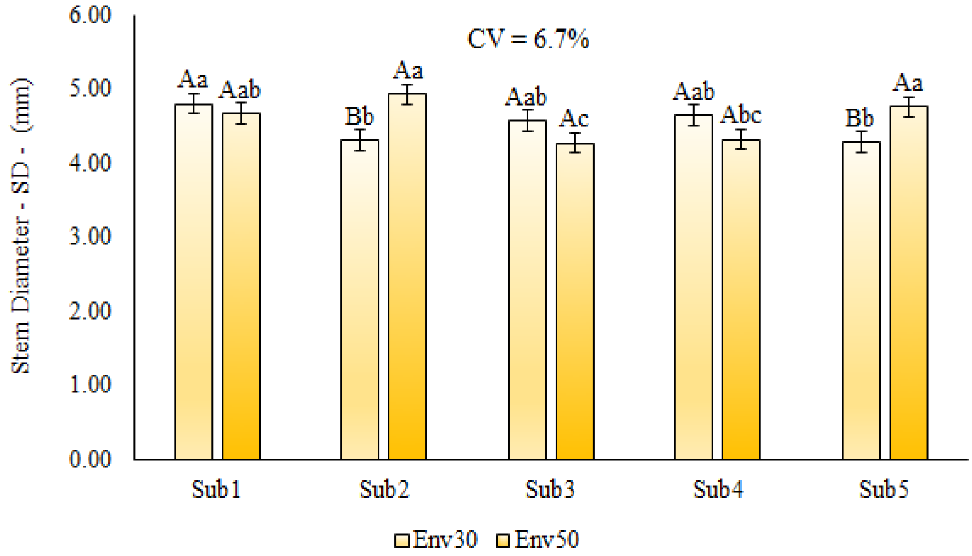

Fig. 4: Stem diameter of *Theobroma cacao* seedlings under different cultivation environments and substrate compositions. Uppercase letters indicate comparisons between shading environments, and lowercase letters indicate comparisons among substrates. Means followed by the same letter do not differ significantly at the 5% level according to the LSD test. CV = coefficient of variation. Env30 = 30% shade; Env50 = 50% shade. Sub1 = 100% Carolina Soil®; Sub2 = 80% Carolina Soil® + 20% vermiculite; Sub3 = 60% Carolina Soil® + 40% vermiculite; Sub4 = 40% Carolina Soil® + 60% vermiculite; Sub5 = 20% Carolina Soil® + 80% vermiculite. Vertical bars indicate standard error.

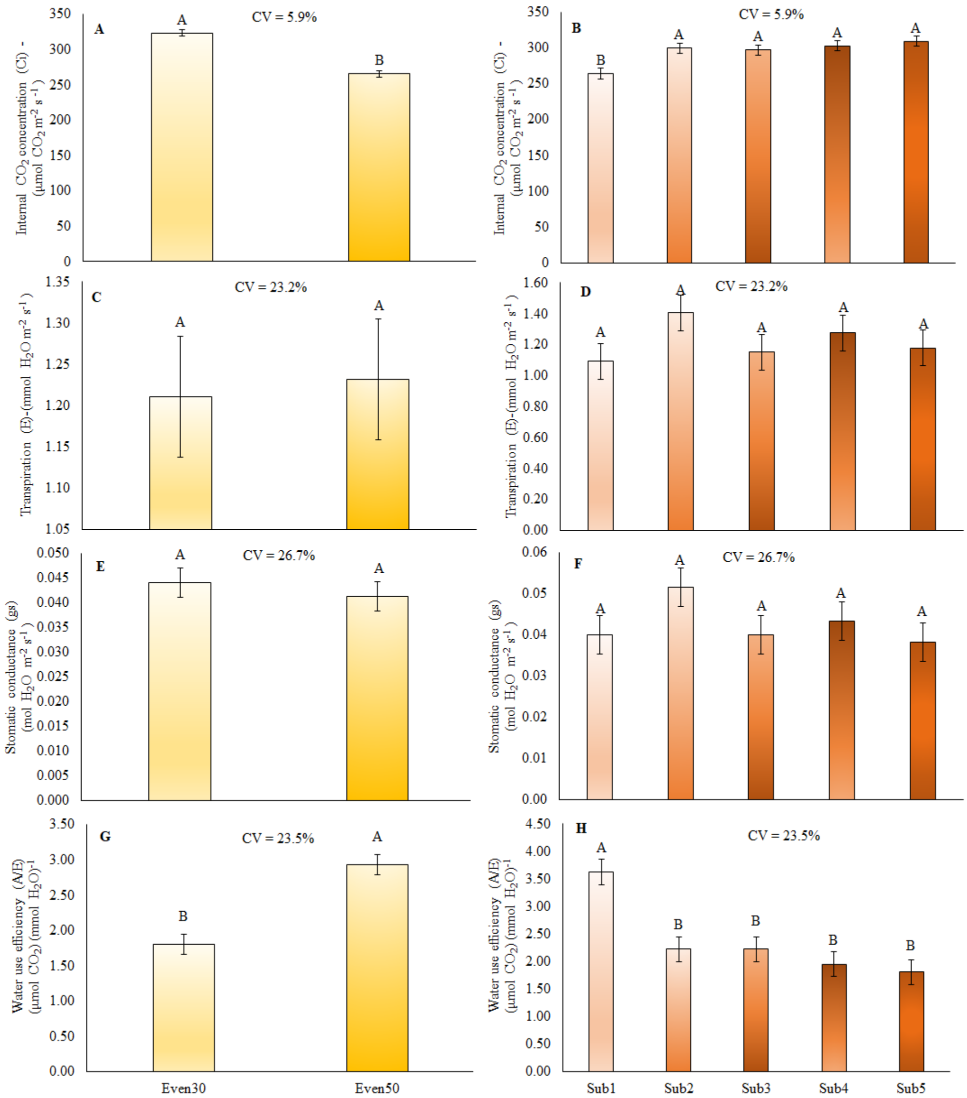

Fig. 5: Internal CO_2 concentration across environments (A) and substrates (B); transpiration rates across environments (C) and substrates (D); stomatal conductance across environments (E) and substrates (F); and water-use efficiency (A/E) across environments (G) and substrates (H) in *Theobroma cacao* seedlings. Means followed by the same letter do not differ significantly at the 5% level according to the LSD test for each variable. CV = coefficient of variation; Env30 = 30% shade; Env50 = 50% shade; Sub1 = 100% commercial substrate; Sub2 = 80% commercial substrate + 20% vermiculite; Sub3 = 60% commercial substrate + 40% vermiculite; Sub4 = 40% commercial substrate + 60% vermiculite; Sub5 = 20% commercial substrate + 80% vermiculite. Vertical bars indicate standard error.

The CO_2 assimilation rate (Fig. 6A) and instantaneous carboxylation efficiency (Fig. 6B) showed a significant interaction between cultivation environment and substrate. In substrate Sub1, plants grown under 50% shade (Env50) exhibited CO_2 assimilation rates 88% higher than those under 30% shade (Env30). In substrate Sub2, CO_2 assimilation rates in Env50 were 89% higher than in Env30. In substrates Sub3 and Sub4, CO_2 assimilation rates did not differ significantly across shading environments. In substrate Sub5, plants under Env50 exhibited CO_2 assimilation rates 121% higher than those under Env30 (Fig. 6A).

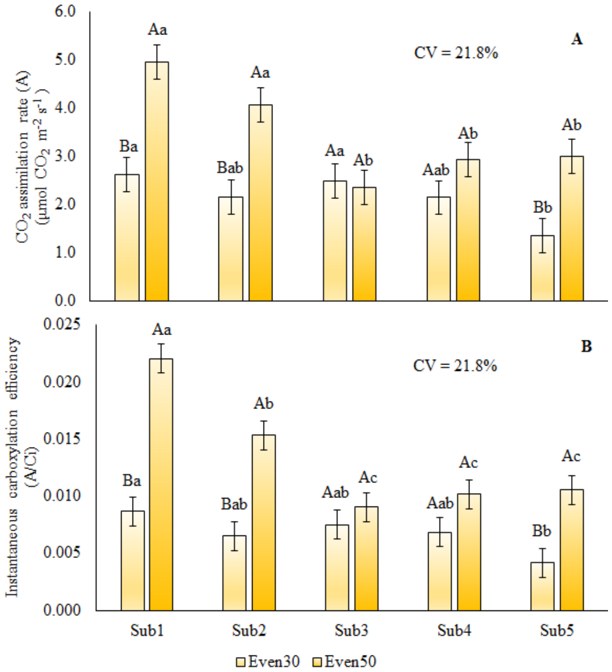

Fig. 6: CO_2 assimilation rate (A) and instantaneous carboxylation efficiency (B) of *Theobroma cacao* seedlings. Uppercase letters indicate comparisons between shading environments, and lowercase letters indicate comparisons among substrates. Means followed by the same letter do not differ significantly at the 5% level according to the LSD test for each variable. CV = coefficient of variation; Env30 = 30% shade; Env50 = 50% shade; Sub1 = 100% commercial substrate; Sub2 = 80% commercial substrate + 20% vermiculite; Sub3 = 60% commercial substrate + 40% vermiculite; Sub4 = 40% commercial substrate + 60% vermiculite; Sub5 = 20% commercial substrate + 80% vermiculite. Vertical bars indicate standard error.

Under the Env30 shading environment, CO_2 assimilation rates of cacao seedlings did not differ significantly among the substrates. Under the Env50 environment, seedlings grown in substrate Sub1 exhibited CO_2 assimilation rates of 110%, 70%, and 66% higher than those grown in substrates Sub3, Sub4, and Sub5, respectively (Fig. 6A).

In substrate Sub1, plants grown under 50% shade (Env50) exhibited the highest instantaneous carboxylation efficiency, 154% higher than those under 30% shade (Env30). In substrate Sub2, instantaneous carboxylation efficiency in Env50 was 136% greater than in Env30. In substrates Sub3 and Sub4, no significant differences in instantaneous carboxylation efficiency were observed across shading environments. In substrate Sub5, plants grown under Env50 had an instantaneous carboxylation efficiency 154% higher than those under Env30 (Fig. 6B).

Under the Env30 shading environment, the instantaneous carboxylation efficiency of cacao seedlings did not differ significantly among the substrates. Under the Env50 environment, seedlings grown in substrate Sub1 exhibited instantaneous carboxylation efficiency 44%, 144%, 117%, and 109% higher than those grown in substrates Sub2, Sub3, Sub4, and Sub5, respectively (Fig. 6B).

According to the Pearson correlation analysis (Fig. 7), growth and gas exchange variables generally showed a negative correlation with internal CO_2 concentration (Ci). In other words, lower *Ci* values were associated with greater growth and enhanced gas exchange. These results indicate that a higher CO_2 assimilation rate (*A*) led to improved water-use efficiency (*A/E*) and enhanced seedling quality, as reflected by the Dickson Quality Index (DQI).

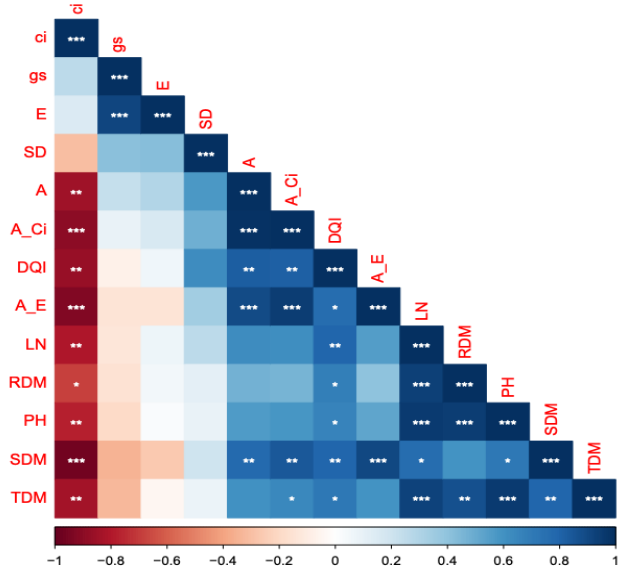

Fig. 7: Pearson correlation matrix for plant height (PH), stem diameter (SD), number of leaves (LN), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), Dickson Quality Index (DQI), internal CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*), CO_2 assimilation (*A*), water-use efficiency (*A/E*), and instantaneous carboxylation efficiency (*A/Ci*) in *Theobroma cacao* seedlings. * indicates significance at $p < 0.05$; ** at $p < 0.01$; *** at $p < 0.001$.

According to the canonical variate analysis (Fig. 8), in the 50% shade environment, substrates Sub1 and Sub2, characterized by higher nutrient content, enhanced seedling growth and gas exchange performance. Substrate Sub1 tended to produce seedlings with higher water-use efficiency and greater photosynthetic assimilation capacity, while Sub2 promoted greater biomass accumulation, resulting in seedlings of superior quality.

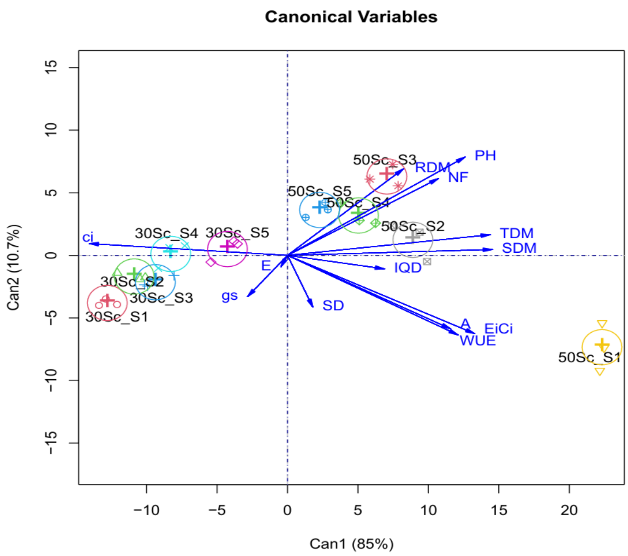

Fig. 8: Canonical variate analysis of plant height (PH), stem diameter (SD), number of leaves (LN), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), Dickson Quality Index (DQI), internal CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*), CO_2 assimilation (*A*), water-use efficiency (*A/E* = *WUE*), and instantaneous carboxylation efficiency (*A/Ci*) in *Theobroma cacao* seedlings.

DISCUSSION

Solar radiation is a key abiotic factor because it provides the energy necessary for photosynthesis (Kong et al., 2016). Nonetheless, excessive irradiance can negatively affect many crops, especially during summer when radiation intensity is highest. In high-irradiance environments, shade nets constitute an effective strategy to improve crop performance (Salles et al., 2024), as they reduce direct light interception, mitigate tissue damage, and influence plant developmental processes (Paula et al., 2017; Silva et al., 2021). In *Theobroma cacao* seedlings, intense solar radiation compromises the photosynthetic apparatus, leading to photoinhibition (Lewis et al., 2021), a condition strongly associated with reduced photosynthetic efficiency. Excess light interferes with leaf formation and damages key components of the photosynthetic machinery, particularly photosystem II (Mateus-Rodríguez et al., 2023; Zhou et al., 2022).

Our results showed that shading markedly altered the photosynthetically active radiation (PAR) reaching *Theobroma cacao* seedlings (Fig. 5 and 6). The reduction in PAR with increasing shade levels indicates that excessive irradiance can be detrimental to early seedling development, as 50% shading improved all evaluated variables (Fig. 2, 4, 5, 6, 7, and 8). These findings agree with previous research showing that adequate PAR is essential for optimizing growth under controlled conditions (Dapont et al., 2016). Cacao is highly sensitive to high light intensities during its early developmental stages; thus, shading is crucial for successful seedling establishment. The 50% shade screen provided an optimal balance between light attenuation and thermal regulation, creating a microclimate (Visscher et al., 2024) that favored initial plant growth.

Cacao plants cultivated under 50% shading exhibited greater vegetative growth and higher biomass accumulation (Fig. 2 and 4), a response consistent with findings in tamarind (Salles et al., 2024) and in cacao (Branco et al., 2017; Arévalo-Gardini et al., 2021). This improvement in seedling growth under reduced photosynthetically active radiation, as observed in the 50% shade treatment (Fig. 1), indicates that light intensity was not a limiting factor for growth in this environment (Silva et al., 2018). The enhanced vegetative growth and biomass accumulation under 50% shading are associated with adequate partial shade, which created favorable micrometeorological conditions for cacao seedlings, promoting greater leaf expansion and reduced cuticle thickness, ultimately improving photosynthetic efficiency (Saavedra et al., 2020).

Plants grown under shaded conditions acclimate their photosynthetic apparatus to low light intensities, allowing efficient capture and conversion of available light into chemical energy. They also exhibit morphological and physiological adaptations that are suited to low-light environments, contributing to a microclimate that is favorable for cacao seedling development (Taiz et al., 2017; Mensah et al., 2022).

When evaluating seedlings based on quality parameters, it became evident that the 50% shade screen had a positive influence on the quality of cacao seedlings (Fig. 2F). This effect is associated with the environmental conditions created by the shade screen, which directly influence seedling physiology and morphological development (Grossnickle et al., 2020). Improved seedling quality under 50% shading is also associated with reduced oxidative stress, which is largely triggered by leaf dehydration under high radiation and temperature. Consequently, this environment reduced reactive oxygen species (ROS) accumulation (Lahive et al., 2021), contributing to the production of high-quality, vigorous seedlings.

Dry biomass production was greatest in seedlings grown under 50% shading (Salazar et al., 2018) (Fig. 2). Dry matter accumulation under shaded conditions is closely associated with net photosynthetic rate, which is influenced by both respiration and photorespiration (Fig. 7), particularly in C3 plants. These processes are also affected by morphophysiological adaptations (Taiz et al., 2017). In C3 species such as cacao, photorespiration increases substantially under high light conditions, reducing CO_2 assimilation (Fig. 5A) and, consequently, lowering dry matter accumulation (Fig. 2).

The substrate with the highest proportion of sphagnum peat favored cacao seedling development (Fig. 3), likely due to its composition, which included peat (Trani et al., 2004), agro-industrial organic residue, and vermiculite. However, the substrate composed of 40% Carolina Soil® and 60% vermiculite produced the highest root dry mass (Fig. 3D). This response is likely attributable to the role of vermiculite in improving substrate physical properties (Kim & Kim, 2011), as it increases porosity and aeration while also contributing to water retention. The substrate used (Table 1) provided adequate nutrition (Odoemelam et al., 2023), containing high levels of essential nutrients such as nitrogen, potassium, magnesium, sulfur, copper, iron, manganese, zinc, and boron. These nutritional and physicochemical characteristics (Tuesta et al., 2024) were suitable for the initial growth of cacao seedlings. The substrate did not contain cadmium (Table 1), which is essential to prevent its accumulation in leaves (Correa et al., 2021; Argüello et al., 2023; Ortiz-Álvarez et al., 2023). Cadmium is highly toxic and strongly influenced by soil physicochemical characteristics, which determine its availability for plant uptake.

Cacao seedlings typically exhibit optimal physiological performance under low-radiation conditions (Salazar et al., 2018; Almeida et al., 2014), particularly at moderate PAR levels around 500 µmol m^{-2} s^{-1}, which contrasts with the results of the present study. Our results indicate that cultivating Theobroma cacao under 50% shading increased transpiration, water-use efficiency, CO_2 assimilation, and instantaneous carboxylation efficiency (Fig. 5, 6, 8). Similar physiological responses have been reported for Coffea seedlings (Baliza et al., 2012) and *Theobroma cacao* (Jaimez et al., 2018).

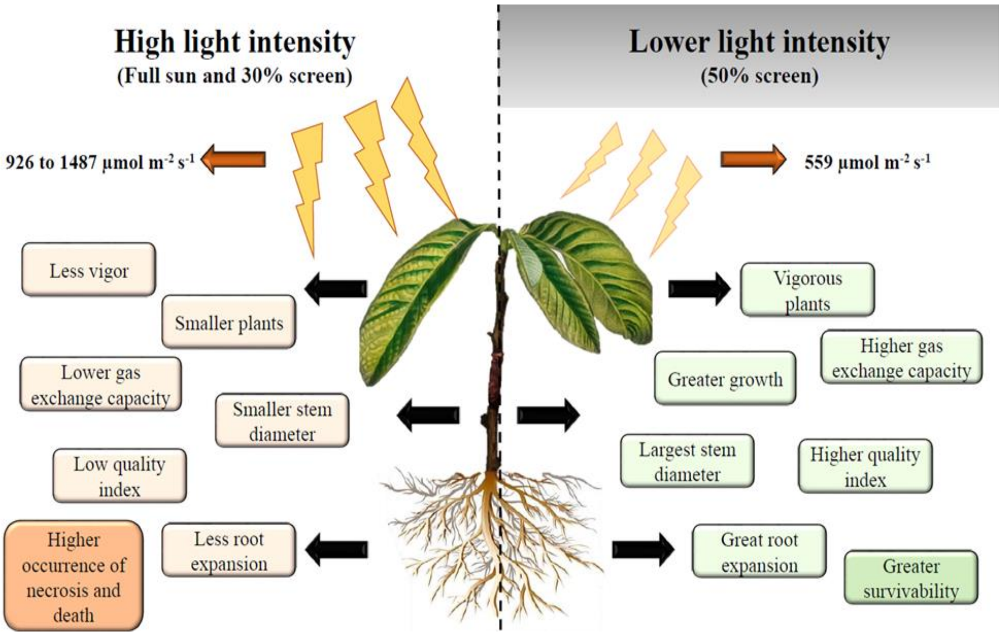

Fig. 9: Summary of the main effects of cultivation environments on *Theobroma cacao* seedlings grown in different substrates.

Based on the results of this study, we conclude that in tropical regions, moderate shade levels combined with peat-based substrates favor the development of *Theobroma cacao* seedlings. Furthermore, adopting technologies that improve environmental conditions can promote successful seedling establishment, allowing plants to express their genetic potential while minimizing physiological stress and mortality (Fig. 9).

Conclusion

Under tropical conditions, producing *Theobroma cacao* seedlings under full sunlight is not recommended.

Variations in light availability and substrate composition significantly affected seedling growth, quality, and gas exchange parameters.

Seedling production under 50% shade proved to be the most effective strategy, resulting in taller plants with greater shoot, root, and total dry mass, as well as improved overall quality. In this environment, seedlings grown in substrates containing 80% organomineral material exhibited larger stem diameters, whereas those cultivated in 100% organomineral substrate showed higher CO_2 assimilation and greater instantaneous carboxylation efficiency.

The use of a substrate composed entirely of organomineral material also promoted greater shoot and total dry mass accumulation compared to substrates containing only 20% organomineral material.

DECLARATIONS

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Conflict of Interest: None.

Data Availability: All the data is available in the article.

Ethics Statement: This article does not contain research on humans or animals; thus, ethical approval was not required.

Author's Contribution: Conceptualization: IFV, EC, TD, FFSB, EPV. Methodology: IFV, EC, FFSB. Investigation: IFV, TD, EPV, FPAPB. Resources: EC, FFSB, GHCV, EDCB. Data curation: IVF, TD, EC, JSS, FFSB, EPV. Writing-original draft: TD, JJS, EC, EPV. Writing-review & editing: TD, EC, FFSB, JJS. Project administration: EC, FFSB, GHCV, EPV. Funding acquisition: EC, FFSB, GHCV, EDCB. All co-authors reviewed the final version and approved the manuscript before submission.

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