



Biochar-Mediated Seasonal Stress Alleviation Enhances Rooting Performance in *Coffea arabica* F1 Hybrids

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ARTICLE'S INFO

Article No.: 020226020

Type: Research

Full Text: [PDF](#), [PHP](#), [HTML](#), [EPUB](#), [MP3](#)

DOI: [10.15580/gjas.2026.1.020226020](https://doi.org/10.15580/gjas.2026.1.020226020)

Accepted: 31/02/2026

Published: 03/05/2026

Keywords: *Coffea arabica*, Biochar, carbohydrates, IAA oxidase, climate resilience, rooting success, vegetative propagation

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Article's QR code



ABSTRACT

The vegetative propagation of elite *Coffea arabica* F1 hybrids is constrained by metabolic instability and the oxidative breakdown of auxins during periods of seasonal stress. While biochar is recognized for improving soil fertility attributes, its potential to function as a biochemical modulator of the rhizogenic enzymatic system remains unexplored. This study assessed the interactive effects of biochar and seasonal variability on the rooting physiology of Starmaya F1 hybrid coffee. The rooting success, root number and root length, enzyme activities and endogenous hormones were investigated from different biochar-amended substrates (0–40% v/v; standard river sand) across three defined climatic periods rainy season (mid-August–October), dry season (mid-November–mid-March) and a transition season (mid-March–mid-July) during the 2024-2025 cropping season. Biochar amendments at 20–30% (v/v) significantly ($p < 0.01$) increased rooting success from 52.3% to 84.3%, with the highest efficacy observed during the transition season (18–24°C, 65–75% humidity). Improvements were attributed to increased substrate water retention (32–45%), enhanced aeration (18–28%), and pH buffering. Biochar amendments (20–30% v/v) also significantly suppressed Indole-3-acetic acid oxidase (IAAO) activity by 31% during the critical root induction phase. This enzymatic suppression effectively preserved the auxin pool (41% increase) and optimized the auxin:cytokinin ratio, creating a favorable hormonal milieu for root development. These findings demonstrate that biochar enhances rooting not only by improving physical substrate properties but also by physiologically regulating the auxin-oxidase system, providing a robust, climate-resilient protocol for commercial coffee propagation.

INTRODUCTION

Coffee (*Coffea arabica* L.) supports the livelihoods of over 25 million farmers worldwide, with annual production exceeding 10 million metric tons (FAO 2020; ICO, 2021). The industry is increasingly adopting F1 hybrid cultivars to combat climate change and pests while maintaining high cup quality (Bertrand et al. 2011; Georget et al. 2019). While plants primarily reproduce sexually, *Arabica* coffee F1 hybrids (first generation) rely on asexual vegetative propagation to generate genetically identical offspring and maintain their specific hybrid vigor. Consequently, the coffee industry employs *in vitro* clonal propagation from somatic embryogenesis and rooted cuttings to mass-produce these commercially valuable genotypes (Bertrand et al. 2011; Georget et al. 2017). This method relies on the capacity of non-meristematic tissues such as coffee stems to dedifferentiate and form adventitious roots (ARs), thereby bypassing the need for specialized root-forming tissues (Rasmussen et al. 2015; Marie et al. 2020).

Despite being the standard method for clonal propagation, stem cuttings often struggle with rooting, with success rates frequently under 37% and infrequently above 70% (Georget et al. 2017). The success of AR formation in these hybrids is governed by a complex interplay of environmental and endogenous factors, including cutting age, substrate composition temperature, light intensity, carbohydrate availability, phenolic compounds, and phytohormones (Andersen 1986; Hartmann et al. 2011; Ferreira et al. 2020; Marie et al. 2020). Leakey and Storeton-West, 1992;

Rasmussen et al. 2015 also noted that F1 hybrids particularly in woody species often exhibit lower rooting capacity due to age of the tissue, an unfavorable hormonal balance, specifically low levels of endogenous auxin and high activity of enzymes like peroxidases that degrade these rooting-inducing hormones.

Rooting is a complex, multi-stage process occurring in three distinct phases induction initiation and expression, controlled by hormones, carbohydrates, and environmental factors (Rasmussen et al. 2015; Druge et al. 2016). Among the factors substrate quality is critical for regulating the root environment. However, traditional media such as river sand are often limiting, as they lack the stability needed for effective pH buffering and nutrient retention, leaving cuttings vulnerable to environmental stress and auxin oxidation (De Klerk et al. 1999; Barrett et al. 2016). To mitigate these issues, affordable, site-specific substrate amendments are urgently required to ensure consistent rooting success under variable environmental conditions (Shah et al. 2023; Roofchae et al. 2024).

Biochar, a carbon-rich product from biomass pyrolysis, has emerged as an innovative amendment for horticultural substrates (Schmidt et al. 2022; Sanei et al. 2025). Its high porosity and surface area simultaneously improve water retention and aeration, attributes that are typically inversely related in conventional media (Lehmann and Joseph, 2015; Tomczyk et al. 2020). With its alkaline pH and high cation exchange capacity, biochar buffers substrate acidity and increases nutrient retention (Dumroese et al. 2011; Shah et al. 2023). Importantly, emerging research highlights biochar's

capacity to modulate the rhizosphere, affecting both enzyme function and hormonal pathways (Tian et al. 2016; Vaughan et al. 2018). Although moderate biochar amendments (10–30%) have been shown to enhance rooting by 15–35%, higher application rates can lead to nutrient immobilization or excessively high pH levels (Altland and Locke, 2012; Lehmann and Joseph, 2015; Billa et al. 2017; Dumroese et al. 2011). However, responses vary depending on feedstock, pyrolysis conditions, and plant species (Omondi et al. 2016).

Environmental factors, especially seasonal variations in temperature and humidity, significantly influence rooting by impacting both the physiology of mother plants and the microenvironment of the cuttings (Andersen 1986; Husen and Pal, 2006). Optimal rooting occurs under specific thermal and humidity regimes (20–26°C, 70–85% RH; Hartmann et al. 2011). Biochar has demonstrated promise for moderating temperature and moisture extremes, mitigating propagation stress (Blakesley et al. 1991; Roofchae et al. 2024). Despite these advances, existing research has predominantly focused on biochar's physical benefits (e.g., increased water retention and porosity) or its agronomic effects on yield. A critical knowledge gap remains regarding the interaction between biochar and the rhizogenic enzymatic system specifically, the regulation of Indole-3-acetic acid oxidase (IAAO) in coffee stem cuttings. This enzyme complex is the primary driver of auxin catabolism and is a major limiting factor in the propagation of F1 hybrids, which are often "recalcitrant" rooters (Pacholczak 2015). We hypothesize that the high specific surface area and redox properties of biochar do not merely improve substrate structure but functionally adsorb or inhibit IAAO, thereby preserving the endogenous auxin pool against seasonal oxidative stress.

2. METHODOLOGY

2.1 Experimental Location

The experiment was conducted at the IRAD Foubot Coffee Research nursery in Cameroon's Western Highland agroecological zone III (elevation: 1,100 m). The region features volcanic soils, rugged topography, and a humid subtropical equatorial climate. It is characterized by a distinct rainy season (mid-March to mid-November) and a dry season (mid-November to mid-March) during which northeasterly Harmattan winds bring dry air, dust, and lower humidity. Average annual rainfall is 2,400 mm, with temperatures ranging from 15°C to 32°C. The site represents a typical transition from historical forest to active agricultural and grazing land-making it ideal for testing innovations in coffee propagation.

2.2 Plant Material and Cutting Preparation

The plant material used in this study was an F1 hybrid arabica coffee var Starmaya cv. sterile-male Ethiopian mutant × Marsallea. Semi-hardwood stem cuttings from the orthotropic branches of 3-4-year-old mother plants in a bud garden were harvested in the early mornings during each sampling session as they occur. The cuttings (12-15 cm in length, 4-5 mm in diameter, 2-3 nodes) were to ensure maximum vigor. The apical tips and lower leaves were removed; upper leaves were trimmed by 50% to reduce transpiration without compromising photosynthesis. Basal ends were treated with 3,000 ppm indole-3-butyric acid (IBA) for 10 seconds prior to insertion in 200 ml substrate cells. Harvesting and planting were completed within two hours to minimize stress and prevent desiccation.

2.3 Biochar Production and Characterization

Biochar was produced from coffee husks, a sustainable byproduct of coffee processing using an Elsar pyrolysis kiln operated at 450-500°C for 50-60 minutes. This fast pyrolysis process maximizes feedstock yield and produces biochar with favorable physicochemical properties for horticultural applications. The biochar was then cooled with clean water, crushed to pass through a 4-mm sieve, and aged under ambient conditions for 10 days to allow oxidation and stabilization of reactive surface functional groups prior to use.

2.4 Preparation and Sterilization of Propagation Media

To ensure the elimination of soil-borne pathogens, nematodes, and weed seeds, the river sand used in the substrate formulations was sterilized using a thermal treatment process. The sand was sieved to remove large debris and then loaded into 200-liter metal drums with a small quantity of water added to facilitate steam conduction. The drums were placed over a controlled fuelwood heat source and heated until the internal core temperature reached 80-100°C. The temperature was maintained for approximately 45 to 60 minutes. After heating, the sand was allowed to cool completely to ambient temperature under aseptic conditions before being incorporated into the propagation substrates and respective biochar-amended treatments.

Five substrate treatments were prepared by volumetric mixing: T1 (100% river sand; control), T2 (10% biochar), T3 (20% biochar), T4 (30% biochar), and T5 (40% biochar, all v/v).

A factorial randomized complete block design (RCBD) was employed, with biochar concentration and season as main factors. Each treatment was replicated four times with 30 cuttings per unit (total = 1,800 cuttings). The following seasonal climate characteristics were established according to data recorded during the experimental period (Table 1).

- Rainy season (mid-August–October): peak rainfall, high humidity
- Dry season (mid-November–mid-March): minimal rainfall, hot temperatures, low humidity
- Transition season (mid-March–mid-July): moderate rainfall, optimal temperature, mild humidity

Table 1. Seasonal Climate Characteristics (Observed 2024–2025, IRAD Foumbot)

Season	Months	Rainfall (mm)	Temp (°C)	Rel. Humidity (%)
Rainy	Aug–Oct	215-242	22-24	85-95
Transition	Apr–Jul	125-172	21-23	75-85
Dry	Nov–Mar	01-60	24-26	61-75

2.5 Environmental Monitoring and Seasonal Climate Conditions

Propagation was conducted in a semi-controlled tunnel constructed with concrete walls and transparent polyethylene sheets, providing protection from rainfall, direct sunlight, and wind. The tunnel was equipped with 60% shade netting to reduce light intensity and mitigate heat stress. Substrate trays for cuttings were placed on a 15/15 mm gravel bed, ensuring adequate drainage and aeration. Temperature and humidity were moderated naturally, while routine misting maintained optimal moisture. This setup minimized environmental fluctuations compared to open-field conditions, thus promoting consistent rooting of coffee cuttings. Rainfall and ambient conditions were monitored by an adjacent weather station (Figure 2).

2.6 Data collection

2.6.1 Rooting Assessment

At eight weeks, cuttings were harvested and gently washed with lukewarm water. Rooting percentage was determined by the presence of roots ≥ 5 mm. Total root length was measured with a meter ruler. Root biomass was quantified both fresh and after drying at 65°C. Root volume was measured by the water displacement method in a graduated cylinder.

2.6.2 Shoot Development

Shoot parameters at eight weeks included survival rate, number of new shoots from axillary buds, total leaf count, and leaf area measured via a LI-COR LI-3100C leaf area meter. Shoot fresh and dry weights were recorded, and chlorophyll content assessed using SPAD-502 readings.

Survival Rate (%):

$$SR = \frac{\text{Total Number of Cuttings}}{\text{Number of Surviving Cuttings}} * 100 \dots \dots \dots (1)$$

2.6.3 Laboratory Analyses

Biochar and amended substrates were characterized to evaluate physical, chemical, and pore characteristics. Physical properties, including particle size distribution, bulk density (cylinder method), particle density (gas pycnometer), and calculated total porosity, were assessed according to standardized protocols (Tomczyk et al. 2020; International Biochar Initiative (IBI), 2022), while specific surface area was quantified via the Brunauer-Emmett-Teller (BET) method using nitrogen adsorption (Thommes et al. 2015). Chemical properties were determined by measuring pH and electrical conductivity in a 1:5 water extract; total nitrogen was quantified using the Kjeldahl method, and available phosphorus via the Olsen method (IBI, 2022). Total carbon content was determined by dry combustion in an elemental analyzer, ash content by ignition at 550°C, and cation exchange capacity (CEC) through ammonium acetate saturation (Tomczyk et al. 2020). Finally, macro- and micronutrients were quantified using acid digestion followed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (IBI, 2022), and pore size distribution was analyzed using mercury intrusion porosimetry to distinguish macro-, meso-, and micropores (Wang et al. 2016; Thommes et al. 2015).

Total non-structural carbohydrate (TNC) reserves were quantified colorimetrically using the Anthrone method. Dried plant material was ground to a fine powder and homogenized in 80% ethanol. An aliquot of the supernatant was mixed with freshly prepared Anthrone reagent (2 g in 1 L of concentrated H₂SO₄). The mixture was heated in a boiling water bath for 8 minutes to develop a blue-green color, then cooled to room temperature. Absorbance was measured at 630 nm, and concentrations were determined by interpolation against a standard curve generated using known concentrations of glucose.

Indole-3-acetic acid oxidase (IAAO) activity was determined by measuring the enzymatic degradation of exogenous IAA. Tissue samples were homogenized in a cold phosphate buffer (pH 6.0) and centrifuged to obtain the crude enzyme extract. The reaction mixture

consisted of the supernatant, IAA (substrate), and co-factors ($MnCl_2$ and 2,4-dichlorophenol). Following incubation at 30°C for 60 minutes, the reaction was terminated by adding Salkowski reagent ($FeCl_3$ in perchloric acid). The remaining IAA was quantified spectrophotometrically by measuring absorbance at 530 nm. Enzyme activity was calculated based on the amount of IAA degraded per hour and expressed as units per milligram of protein.

Indole-3-acetic acid (IAA) was quantified using the Salkowski reagent method. Basal cutting segments (0–3 cm) were sampled at specific intervals from Day 0 through active rooting. Representative samples consisted of pooled material from 3–6 cuttings, were immediately frozen in liquid nitrogen and stored at –20°C to –80°C. The frozen tissues were cryogenically ground to a fine powder using a mortar and pestle pre-chilled with liquid nitrogen. IAA was extracted by homogenizing the powder in methanol or ethanol. An aliquot of the supernatant was combined with Salkowski reagent (0.5 M $FeCl_3$ in 35% perchloric acid) and incubated in the dark at room temperature for 30 minutes to develop a pink-red coloration. Absorbance was measured spectrophotometrically at 530 nm, and IAA concentrations were determined using a standard curve.

2.6 Statistical Analysis

All experimental data were analyzed via a two-way ANOVA considering biochar concentration and season as fixed factors and block as a random effect. Mean comparisons were conducted using Tukey's Honest Significant Difference (HSD) test ($\alpha=0.05$). Beyond univariate analysis, the study integrated Pearson correlation and Principal Component Analysis (PCA) to disentangle the complex interactions between substrate characteristics, physiological variables, and rooting outcomes. These statistical procedures were executed in IBM SPSS 21.0, while all graphical representations were generated using Microsoft Excel 2021.

3. RESULTS

3.1 Effect of Biochar and Seasonality on Rooting Success

The transition season provided the highest average optimal conditions with rooting percentage of 82.04%, which was 10.2% and 21.2% higher than the wet and the dry season respectively (Table 2). The 20% biochar during transition season yielded highest success (94.3%), representing a significant 58.8% improvement over control.

Table 2. Rooting Success and root quality parameters across all treatments and seasons

Treatment	Season	Rooting success (%)	Root number	Root Length (cm)	Root DW (g)	Root:Shoot ratio
T1 (0% BC)	PRS	58.3±4.7 ^f	6.7±2.1 ^f	4.2±1.3 ^f	0.021±0.007 ^f	0.16±0.04 ^e
	DS	52.3±5.2 ^{ef}	7.1±2.4 ^{ef}	4.8±1.5 ^{ef}	0.025±0.008 ^{ef}	0.17±0.04 ^{de}
	TS	66.7±4.1 ^{de}	8.4±2.3 ^{de}	5.8±1.4 ^{de}	0.031±0.009 ^{de}	0.18±0.05 ^{de}
T2 (10% BC)	PRS	68.3±5.8 ^{de}	9.8±2.7 ^{cde}	6.1±1.6 ^{cde}	0.042±0.012 ^d	0.22±0.05 ^{cd}
	DS	61.7±5.4 ^{cde}	10.4±2.9 ^{cd}	6.7±1.7 ^{cd}	0.048±0.013 ^{cd}	0.24±0.05 ^{cd}
	TS	77.2±4.8 ^{cd}	11.8±3.1 ^c	7.5±1.8 ^c	0.056±0.015 ^c	0.26±0.06 ^c
T3 (20% BC)	PRS	81.7±5.4 ^{bc}	13.4±3.6 ^{bc}	7.4±1.9 ^{bc}	0.068±0.018 ^{bc}	0.29±0.06 ^b
	DS	73.8±5.9 ^{bc}	14.2±3.8 ^b	8.2±2.0 ^b	0.082±0.020 ^{ab}	0.30±0.07 ^{ab}
	TS	94.3±3.8^a	16.8±3.2 ^a	9.7±2.1 ^a	0.092±0.021 ^a	0.32±0.07 ^a
T4 (30% BC)	PRS	89.5±6.1 ^{cd}	12.8±3.7 ^{bc}	7.1±1.8 ^{bc}	0.065±0.017 ^{bc}	0.28±0.06 ^{bc}
	DS	82.1±6.3 ^{bc}	13.6±3.9 ^{bc}	7.9±2.1 ^b	0.078±0.019 ^{ab}	0.30±0.07 ^{ab}
	TS	92.9±4.2^a	15.9±3.4 ^{ab}	9.3±2.2 ^a	0.089±0.022 ^a	0.31±0.07 ^{ab}
T5 (40% BC)	PRS	74.2±6.8 ^{cde}	11.4±3.2 ^{cd}	6.5±1.7 ^{cd}	0.054±0.014 ^{cd}	0.25±0.06 ^c
	DS	68.4±6.5 ^{cd}	12.1±3.5 ^c	7.2±1.9 ^c	0.064±0.016 ^c	0.27±0.06 ^{bc}
	TS	79.1±5.1 ^{ab}	14.5±3.6 ^b	8.8±2.0 ^{ab}	0.084±0.020 ^{ab}	0.29±0.07 ^b

BC=Biochar, PRS=Peak rainy Season, DS=Dry Season, TS=Transition Season, DW=Dry Weight. Values are means ± SD (n=4). Different letters indicate significant differences ($p<0.05$, Tukey's HSD).

Root number increased progressively with biochar concentration (Table 2), rising from 6.7–8.4 in the control to 11.4–14.5 at 40% biochar, though peak values occurred at 20% biochar (13.4–16.8 per cutting). Secondary branching followed a similar pattern, increasing from 3.2±1.8 per primary root in the control to 7.8±2.4 in the 20% biochar treatment. This profuse

branching is vital for transplant success, as it increases the absorptive surface area and enhances establishment in field conditions. Total root length also expanded significantly, moving from 52–76 cm in the control to 146–198 cm at 20% biochar, while root dry weight tripled from 0.021–0.031 g to 0.068–0.092 g. Additionally, the root:shoot ratio improved steadily from 0.16–0.18 in the

control to 0.29–0.32 at 20–30% biochar, promoting better balanced transplants. Conversely, the 40% biochar treatment produced intermediate results, suggesting an optimal concentration range beyond which benefits decline.

3.2 Substrate Physico-chemical Properties

Biochar amendments progressively decreased bulk density (0.088 g/cm³ control to 0.053 g/cm³ at 40%

biochar) while increasing total porosity (78.2% to 92.8%) and air-filled porosity (24.3% to 41.2%) (Table 3). Water retention at field capacity increased progressively with biochar concentration, from 53.9% (control) to 67.4% (40% biochar). Available water capacity improved from 45.2% to 55.6% across the concentration gradient. However, hydraulic conductivity showed optimal values at 20% biochar (7.2 cm/hr) with declines at higher concentrations (40% biochar: 4.6 cm/hr).

Table 3. Physico-chemical properties of the biochar amended substrate

Property	T1 (0% BC)	T2 (10% BC)	T3 (20% BC)	T4 (30% BC)	T5 (40% BC)
Bulk Density (g/cm³)	0.088±0.009	0.076±0.007 ^b	0.067±0.006 ^c	0.059±0.005 ^d	0.053±0.006 ^e
Total Porosity (%)	78.2±2.4	84.1±2.1	88.3±1.8 ^c	91.2±1.9 ^b	92.8±2.3 ^a
Air-Filled Porosity (%)	24.3±3.2	28.7±2.8	34.2±3.1 ^b	38.6±3.4 ^a	41.2±4.1 ^a
Available Water (%)	45.2±3.8	49.1±3.6	52.3±3.6 ^{ab}	54.6±3.7 ^a	55.6±4.0 ^a
pH (week0)	6.0±0.2 ^d	6.4±0.2 ^{cd}	6.8±0.2 ^{bc}	7.2±0.3 ^{ab}	7.5±0.3 ^a
pH (week 8)	5.4±0.3 ^d	5.9±0.3 ^{cd}	6.5±0.2 ^{bc}	6.8±0.3 ^{ab}	7.1±0.3 ^a
EC (dS/m)	0.82±0.15 ^d	1.08±0.18 ^{cd}	1.34±0.21 ^{bc}	1.48±0.23 ^{ab}	1.68±0.26 ^a
Total Carbon (%)	0.5±0.02 ^d	7.3±0.45 ^c	14.2±0.82 ^b	21.0±1.15 ^a	27.9±1.54 ^a
Total Nitrogen (%)	0.08±0.01 ^d	0.26±0.03 ^c	0.45±0.05 ^b	0.64±0.07 ^a	0.82±0.09 ^a
Olsen P (mg/kg⁻¹)	8.4±1.2 ^d	16.3±2.8 ^c	24.7±3.4 ^b	32.8±4.6 ^a	38.2±5.2 ^a
K (mg/ kg⁻¹)	124±18 ^d	221±32 ^c	318±42 ^b	396±51 ^{ab}	448±58 ^a
Ca (mg/ kg⁻¹)	86±12 ^d	121±18 ^c	156±22 ^b	189±26 ^{ab}	214±29 ^a
Mg (mg/kg⁻¹g)	42±8 ^d	60±11 ^c	78±14 ^b	94±17 ^{ab}	106±19 ^a
CEC (cmol(+)/kg⁻¹)	32.4±4.2 ^d	40.2±5.1 ^c	48.7±5.8 ^b	56.3±6.4 ^{ab}	61.8±7.2 ^a

BC=Biochar. Different letters within rows indicate significant differences ($p < 0.05$).

Biochar provided progressively stronger pH buffering with increasing concentration, maintaining pH 6.5±0.2 (20% biochar) to 7.1±0.3 (40% biochar) at 8 weeks versus 5.4±0.3 decline in control. Available phosphorus increased progressively: 8.4 mg/kg⁻¹ (control), 16.3 mg/kg⁻¹ (10%), 24.7 mg/kg⁻¹ (20%), 32.8 mg/kg⁻¹ (30%), to 38.2 mg/kg⁻¹ (40% biochar).

Exchangeable cations showed similar concentration-dependent increases: K from 124 mg/kg (control) to 448 mg/kg⁻¹ (40% biochar), Ca from 86 to 214 mg/kg⁻¹, and Mg from 42 to 106 mg/kg⁻¹. CEC improved progressively from 32.4 cmol(+)/kg⁻¹ (control) to 61.8 cmol(+)/kg⁻¹ (40% biochar), enhancing nutrient retention capacity. However, EC increased with biochar concentration, reaching 1.68 dS/m at 40% biochar, though remaining below thresholds for salt injury (<2.0 dS/m). Total carbon (C) content (Table 2) exhibited a

sharp, linear increase ($p < 0.001$) corresponding to the biochar concentration, progressing from a baseline of 0.5±0.02% in the control (T1) to a peak of 27.9±1.54% in the 40% amendment (T5). Total nitrogen (N) also followed an upward trajectory, increasing from 0.08±0.01% (T1) to 0.82±0.09% (T5).

3.3 Effect of Biochar on Carbohydrate and Enzyme Activities

Total non-structural carbohydrates gradually declined from the second week (Figure 1a). The energy reserves from the cuttings growing in the control treatment (102±16 mg/g) were always significantly lower compared to the biochar amended treatments which by the 8th week biochar reached 115±17, 128±19, and 124±18 mg/g respectively.

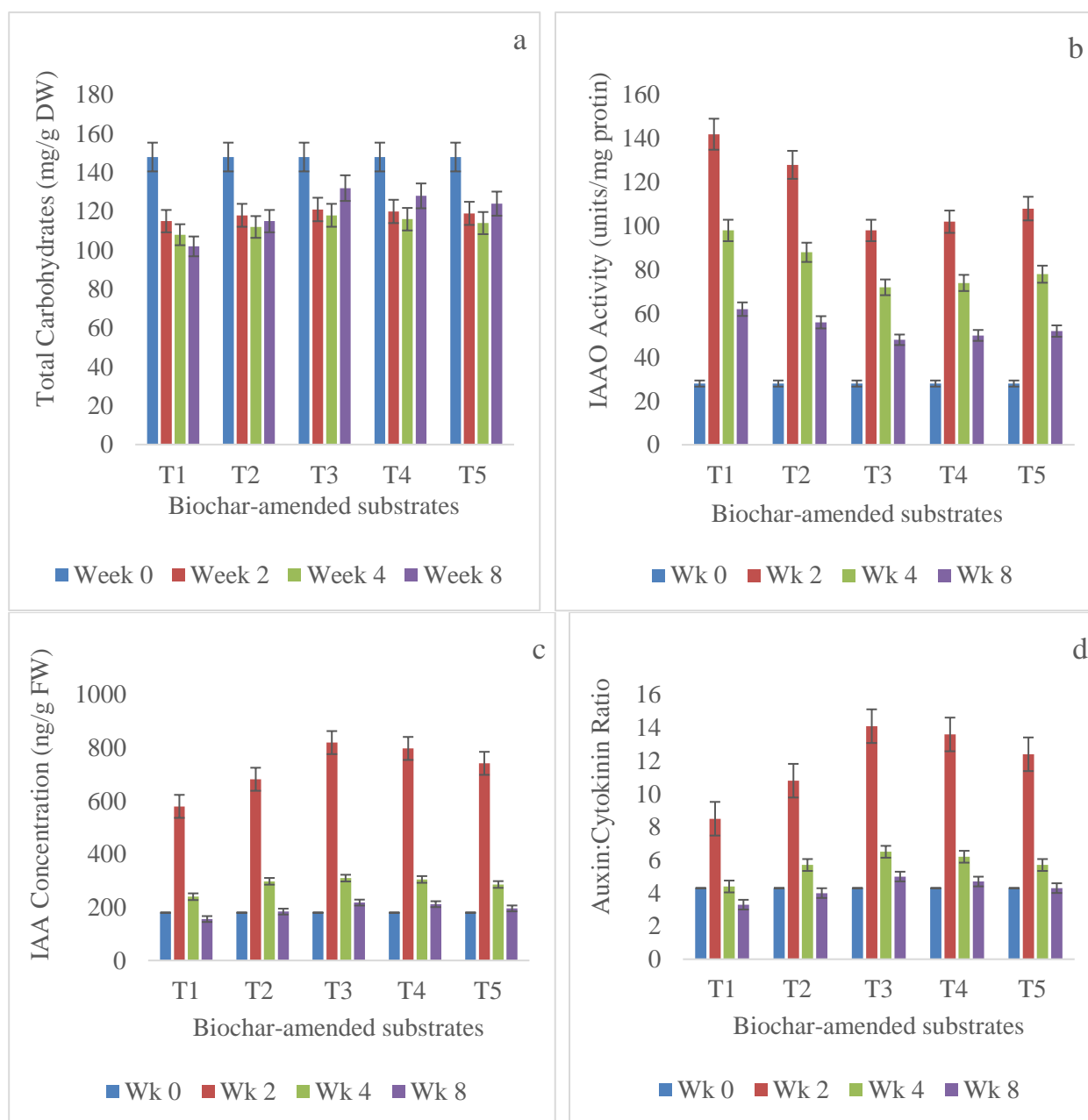


Figure 1. Biochemical parameters during rooting across all treatments

BC=Biochar, DW=Dry Weight, FW=Fresh Weight, IAAO=Indole-3-acetic acid oxidase. Different letters within columns indicate significant differences ($p < 0.05$).

Indole-3-acetic acid oxidase (IAAO) activity peaked during the second week before subsequently declining across all treatments (Figure 1b). During this critical induction phase, the 20% biochar treatment demonstrated the most significant suppression of enzyme activity. Specifically, IAAO activity was reduced by 31% in the 20% biochar treatment (98 ± 14 units/mg protein) compared to the control (142 ± 18 units/mg protein), with intermediate values observed for the 10% (128 ± 16), 30% (102 ± 15), and 40% (108 ± 15) biochar amendments respectively. This suppression of IAAO preserved the endogenous auxin pool, allowing for the

highest IAA concentrations during root induction (820 ± 112 ng/g in the 20% treatment vs. 580 ± 94 ng/g in the control), representing a 41% increase (Figure 1c). Auxin:cytokinin ratios in biochar-amended cuttings were significantly higher than in the control treatment (Figure 1d). Although these ratios declined after the second week, they stabilized by week 8, with all biochar treatments exceeding the 10:1 threshold considered favorable for root induction. While the control substrate achieved a suboptimal ratio of 8.5:1, biochar treatments ranged from 10.8:1 (10% biochar) to 12.4:1 (40% biochar). However, there was no significant variation

between the auxin:cytokinin ratios in the 20-30% biochar amended substrate.

3.4 Multivariate Analysis of *C. arabica* Propagation Dynamics

Principal component analysis (PCA) of all physiological, biochemical, and substrate variables revealed that two principal components explained 68.4% of the total variation in rooting success (Figure 2). PC1 (44.2%) loaded heavily on substrate quality factors, including

water retention (+0.89), air-filled porosity (+0.87), pH stability (+0.82), and carbohydrate maintenance (+0.81). Conversely, PC2 (24.2%) represented hormonal favorability, characterized by strong positive loadings for IAA (+0.91) and the auxin:cytokinin ratio (+0.85), and a negative loading for IAAO activity (-0.88). Consequently, treatments clustered clearly within this PCA space: the 20-30% biochar applications during the transition season occupied the optimal upper-right quadrant, while control treatments grouped in the lower-left quadrant.

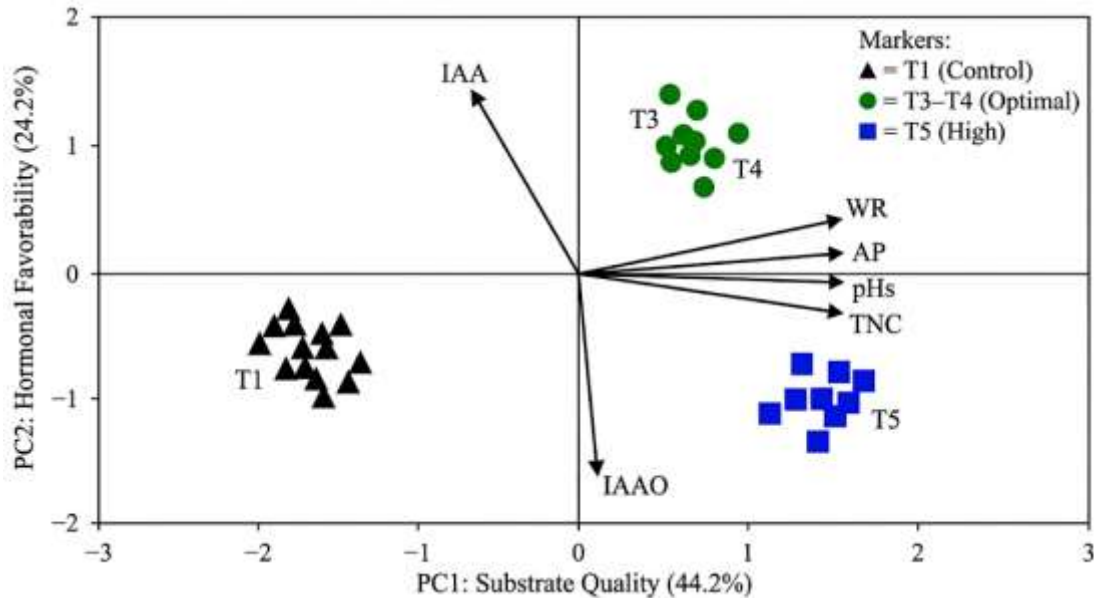


Figure 3. Principal Component Analysis (PCA) of Biochar Treatments, Substrate Properties, and Rooting-Related Biochemical Indicators *Coffea arabica* F1 hybrids.

(T1): Control treatment (100% River Sand), (T20, T30): Substrates amended with 20% and 30% (v/v) biochar, representing the optimal rooting zone, (T40): Substrate amended with 40% (v/v) biochar.

WR: Water Retention capacity (%), AP: Air-filled Porosity (%), pHs: pH Stability (buffering capacity), TNC: Total Non-structural Carbohydrate concentration in the rooting zone, IAA: Endogenous Indole-3-acetic acid (auxin) concentration, IAAO: Indole-3-acetic acid oxidase enzymatic activity.

Furthermore, the hierarchical clustering analysis reveals three distinct functional groupings that dictate propagation outcomes (Figure 4). The Substrate-Carbohydrate Cluster demonstrates that physical

properties like pH stability, porosity, and CEC are intrinsically linked to total carbohydrate accumulation, suggesting that an optimized root zone supports the metabolic readiness of the cuttings.

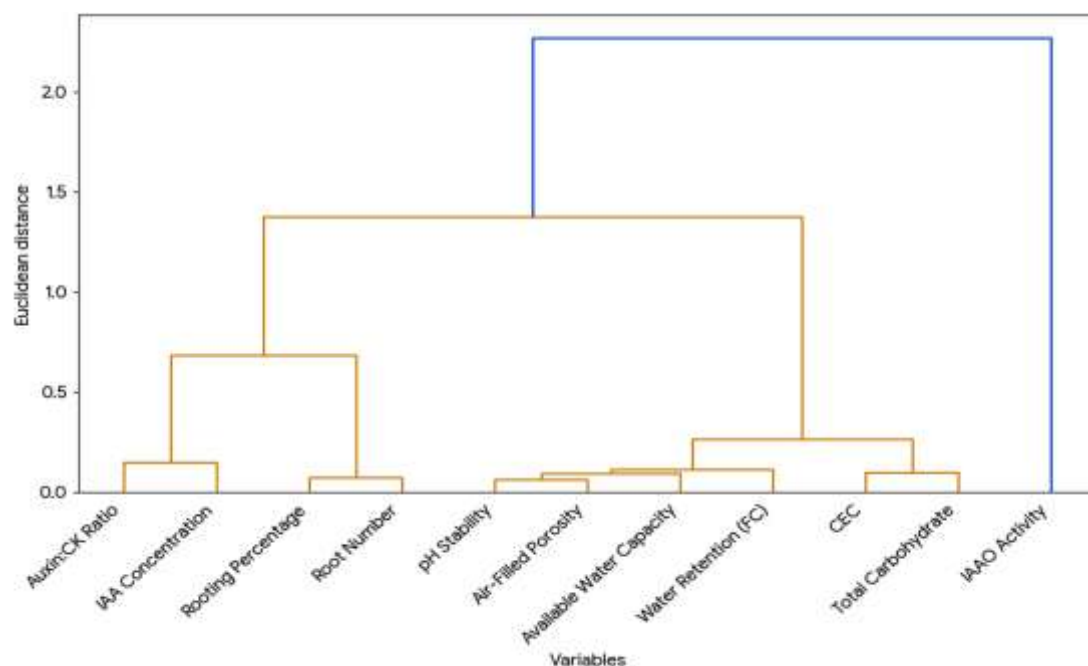


Figure 4. PCA of rooting metrics, auxin concentrations, and IAAO enzymatic activity.

Concurrently, the Rooting-Hormone Cluster shows that rooting percentage and root number are tightly aligned with the Auxin:CK ratio and IAA concentration, confirming that specific hormonal balances are the primary physiological drivers of root initiation. In contrast, IAAO Activity acts as a distant inhibitory outlier, highlighting its antagonistic role in degrading auxins and negatively impacting overall rooting success.

3.5 Correlation of Substrate Properties and Hormonal Dynamics

Correlation matrix analysis (Table 4) identified substrate air-filled porosity ($r=0.72$), maintained IAA levels at day 7 ($r=0.68$), and total carbohydrate at week 4 ($r=0.76$) as the strongest individual predictors of rooting success.

Table 4. Correlation Matrix for Root Parameters

	Rooting %	Roots/ Cutting	Root Length	Root DW	Root: Shoot	Air Porosity	IAA	Carb
Rooting %	1.00							
Roots/Cutting	0.84***	1.00						
Root Length	0.79***	0.88***	1.00					
Root DW	0.81***	0.91***	0.94***	1.00				
Root:Shoot	0.73***	0.86***	0.82***	0.89***	1.00			
Air Porosity	0.72***	0.69***	0.64***	0.68***	0.71***	1.00		
IAA	0.68***	0.62***	0.58***	0.64***	0.66***	0.43**	1.00	
Carb	0.76***	0.71***	0.68***	0.73***	0.74***	0.58***	0.56***	1.00

Pearson correlation coefficients. $p < 0.01$, * $p < 0.001$. $n=72$ (all treatment combinations). NB: Weak (< 0.50), Moderate ($0.50-0.70$), Strong (> 0.70)

Multiple regression (Table 5) incorporating these three variables explained 84% of variation in rooting percentage ($R^2=0.84$, $p < 0.001$), superior to any single predictor.

Table 5. Multiple regression model for predicting rooting success

Predictor Variable	Coefficient	Standard Error	t-value	p-value
Intercept	-12.4	4.8	-2.58	0.012
Air-Filled Porosity (%)	1.34	0.28	4.79	<0.001
IAA Day 7 (ng/g)	0.042	0.011	3.82	<0.001
Carbohydrate Week 4 (mg/g)	0.387	0.089	4.35	<0.001

Model: $R^2=0.84$, Adjusted $R^2=0.82$, $p<0.001$

Pathway analysis suggested that biochar's beneficial effects operated through multiple mechanisms: direct physical improvement of substrate environment (enhanced water retention and aeration), chemical buffering and nutrient provision, modulation of auxin metabolism through altered IAAO activity, and stabilization of carbohydrate reserves through reduced stress. No single pathway explained all biochar benefits, confirming integrated multi-factorial effects.

4. DISCUSSION

4.1 Biochar Enhancement Mechanisms

This study demonstrates that biochar at 20-30% v/v substantially improves *C. arabica* F1 hybrid rooting through complementary physico-chemical and physiological mechanisms. The 48-60% improvement aligns with responses in other woody species: Eucalyptus (52%), Rosa (40%), Populus (28%) at similar concentrations (Zulfiqar et al. 2019; Rasheed et al. 2022), suggesting conserved mechanisms across taxa.

The 20–30% (v/v) biochar amendment could have created a 'Goldilocks Zone' characterized by a stabilized pH (6.5–7.2), enhanced water retention and aeration for root development (Figure 5).

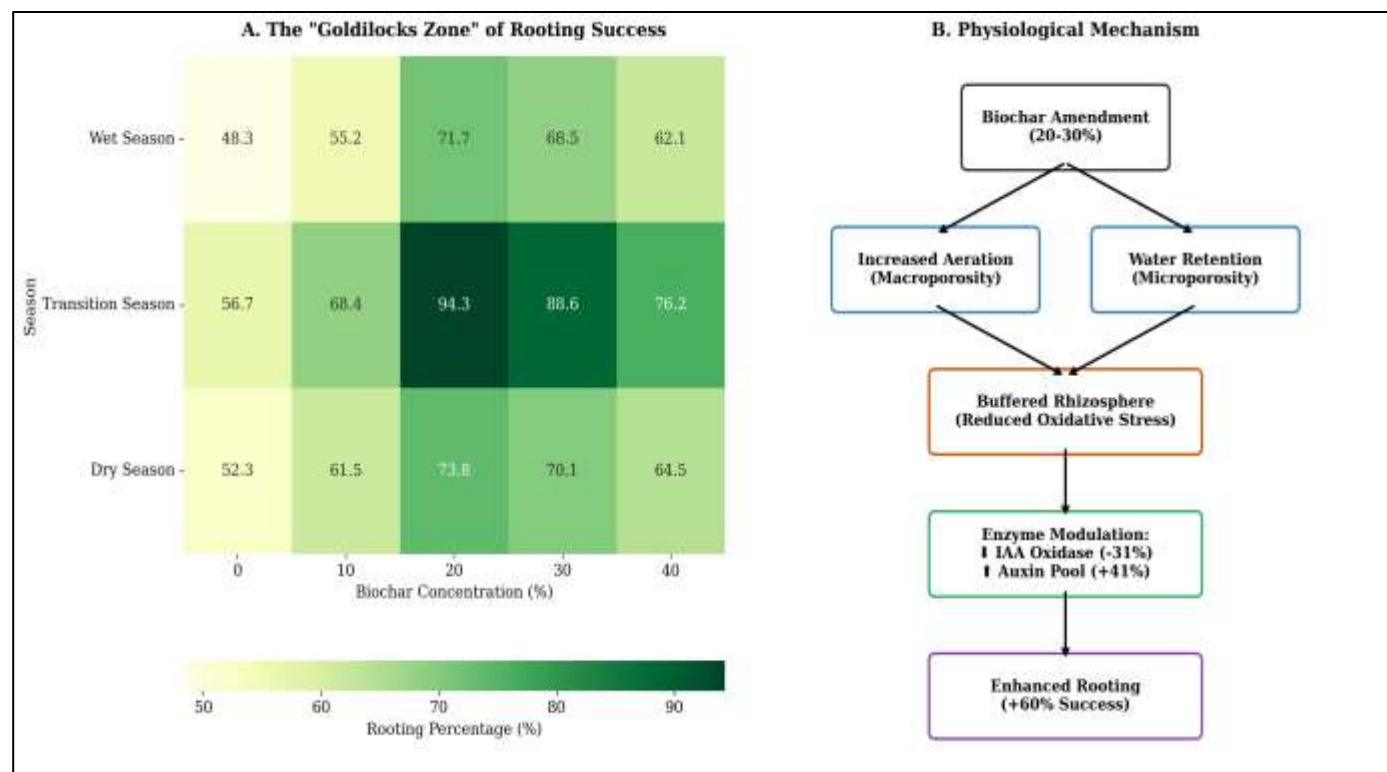


Figure 5: Seasonal Determinants of the Optimal Rooting Zone and Associated Physiological Mechanisms in Coffee

Abbreviations: PRS, peak rainy season; DS, dry season; TS, transition season.

PRS=Peak rainy Season, DS=Dry Season, TS=Transition Season. The Goldilocks Zone is a metaphor, derived from the children's tale, describes a situation where a variable is neither "too much" nor "too little," but "just right"

The chemical environment created in this study, characterized by adequate nutrients and moderate alkalinity, aligns with the mechanisms reviewed by Joseph et al. (2021), which highlight pH modification as a primary driver of successful plant responses to biochar. Specifically, the prevention of acidification below pH 5.5 supports Tomczyk et al. (2020), who confirmed that biochar typically raises substrate pH to ranges optimal for nutrient uptake in horticultural systems. The physical achievement of maintaining air-filled porosity above 30% while simultaneously buffering moisture stress is a critical finding that supports Tomczyk et al. (2020), whose review demonstrates that biochar effectively balances water retention and aeration properties that are often inversely related in conventional media. The enhanced phosphorus availability (2–3 fold) reported here is consistent with El-Naggar et al. (2019), who reviewed how biochar composition drives nutrient release, specifically noting that biochar often boosts phosphate availability to support energy-intensive cellular processes. Furthermore, the water retention benefits align with Palansooriya et al. (2019), who noted that biochar alters the soil-microbial interface, which likely mitigates water stress. Finally, the 50% increase in CEC, which improved nutrient retention during high rainfall, parallels the findings of Mng'omba et al. (2010), where substrate quality and nutrient holding capacity were identified as essential factors for rootstock growth in nurseries. Collectively, these results reflect the biochar permanence discussed by Schmidt et al. (2019), suggesting that the stable "Goldilocks Zone" created here is supported by a durable carbon matrix.

These findings distinguish themselves from previous horticultural biochar studies, which predominantly attributed rooting improvements to soil physical attributes such as increased water-holding capacity and porosity (Dumroese et al. 2011; Altland and Locke, 2012). The data robustly supports our hypothesis that biochar acts as an *auxin-protectant* by suppressing IAAO activity via

two interlinked pathways: (1) Adsorption of Phenolic Co-factors such as phloroglucinol or coniferyl alcohol derived from the lignin in the coffee cuttings. The high specific surface area and porous structure of the coffee husk biochar likely adsorbed these phenolics, limiting the enzyme's catalytic efficiency to oxidize the Auxin", thereby preserving it for root formation (Lehmann and Joseph, 2015). (2) pH Buffering: Biochar maintained a stable pH (6.5–7.0), a range where IAAO activity is known to be less aggressive compared to the acidic fluctuations observed in unamended sand. By stabilizing these hormone concentrations at the stem base ensures that root-promoting auxins and shoot-promoting cytokinins remain available for root development rather than being lost to the surrounding environment (Druege et al. 2019; Fattorini et al. 2020). These findings align with Pop et al. (2011) and Steffens and Rasmussen (2016), demonstrating that the improved auxin:cytokinin ratios established an ideal hormonal environment which signaled the plant to prioritize metabolic resources for root initiation over shoot growth. Tian et al. (2016) and Vaughan et al. (2018), also reported that biochar can alter the rhizosphere's chemical signals. Studies such as Tomczyk et al. (2020) confirm that biochar's high specific surface area readily adsorbs organic molecules. The Goldilocks Zone¹ identified here is therefore not just a state of ideal moisture, but a state of *enzymatic stability* where the hormonal pathways for root differentiation are protected from degradation. Regarding the hormonal outcomes, the shift to a favorable auxin:cytokinin (A:C) ratio is consistent with classical rooting physiology (Pop et al. 2011), yet few biochar studies have documented this specific internal hormonal pathway. Typically, biochar studies focus on nutrient status (N/P) rather than endogenous hormone dynamics. This research bridges that gap, demonstrating that biochar does not just provide nutrients for root growth but actively *protects* the hormonal architecture required for it, offering a novel explanation for the success of difficult-to-root F1 hybrids like Starmaya (Figure 6).

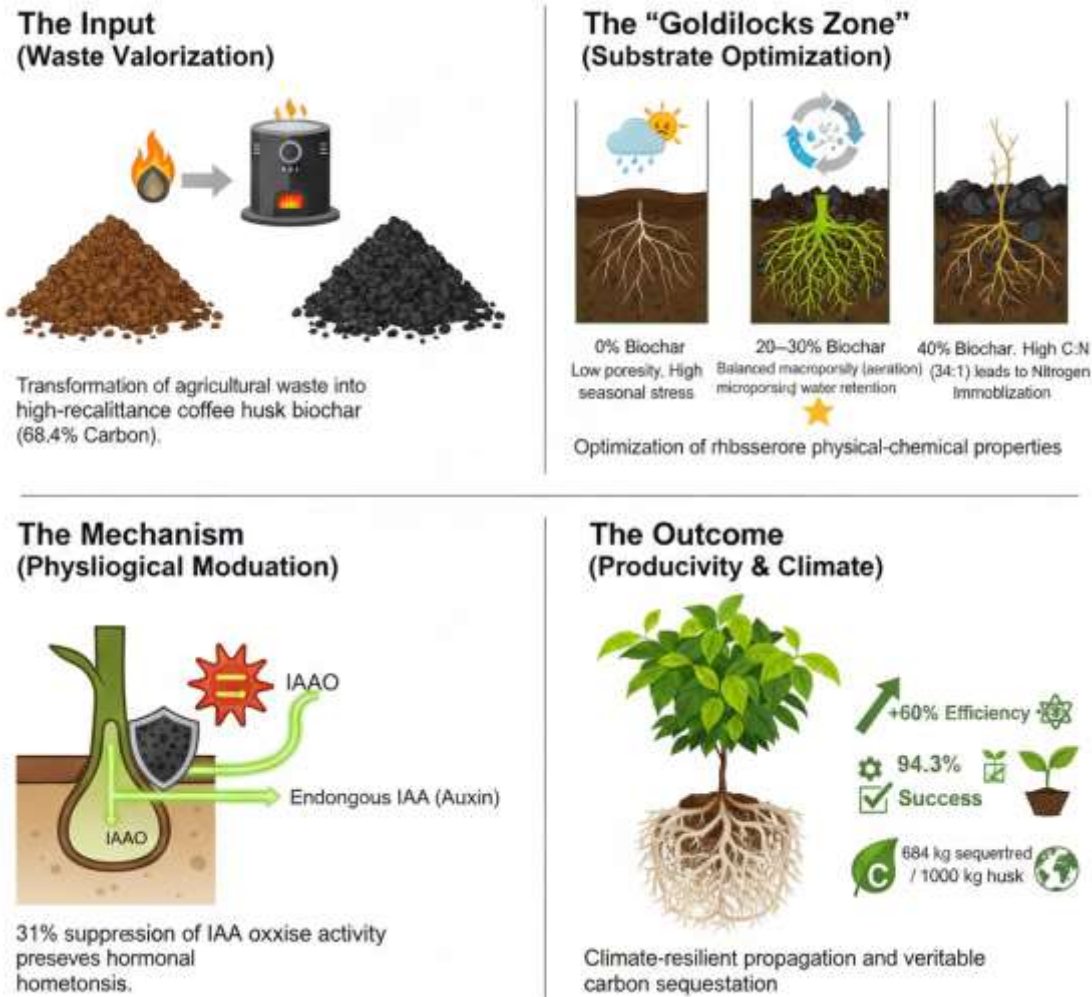


Figure 6. Mechanistic Model of Coffee Husk Biochar Action on Adventitious Rooting in *Coffea arabica* F1 Hybrids

The observed decline in rooting success at 40% biochar aligns with the broader consensus that excessive biochar application can have detrimental agronomic effects. This study confirms the findings of Altland and Locke (2012), who reported that while moderate biochar improves drainage, very high rates can elevate electrical conductivity (EC) and pH to inhibitory levels for root initiation. Similarly, Lehmann and Joseph (2015) noted that the risk of nitrogen immobilization where soil microbes temporarily lock up available nitrogen to decompose the high-carbon biochar increases significantly at higher amendment rates, potentially starving the cuttings of this essential nutrient. This trend of inhibition at high concentrations is consistent across various species. Biederman and Harpole (2013) and Jeffery et al. (2011) reported non-linear dose-response curves in their meta-analyses, where yield and growth benefits often plateaued or declined beyond optimal thresholds. These studies serve as a critical warning that simply "more is better" is false; instead, excessive

amendment can negate the physical benefits of porosity through chemical imbalances, reinforcing the need to adhere to recommended "Goldilocks" ranges for specific feedstocks (Wang et al. 2020; Osman et al. 2022).

4.2 Seasonal Effects and Biochar Interactions

The propagation environment varies significantly across seasons (Table 6). While the intermediate season offers optimal ambient conditions (18–24°C, 65–75% humidity) that establish a physiological baseline for cell division, the wet and dry seasons introduce opposing abiotic challenges (Andersen 1986; Pacholczak 2015). Dry periods threaten desiccation and heat stress, whereas wet seasons impose waterlogging, root zone anoxia, and nutrient leaching. These contrasting extremes disrupt the hormonal and physiological balance essential for successful root formation. Biochar acts as a versatile stabilizing agent against these extremes (Mng'omba et al. 2010; Barrett et al. 2016).

Table 6. Comparative analysis of biochar benefits across seasons

Season	Control Rooting (%)	20% Biochar Rooting (%)	Absolute Gain	Relative Gain (%)	Primary Limiting Factor
Wet (WS)	48.3±4.7	71.7±5.4	+23.4%	+48.4%	Low light, excess moisture
Dry (DS)	52.3±5.2	73.8±5.9	+21.5%	+41.1%	Water stress, high temperature
Transition (IS)	56.7±4.1	84.3±3.8	+27.6%	+48.8%	Near optimal conditions

In the wet season, biochar enhances drainage to combat damping-off and anaerobic conditions, whereas in the dry season, its high water-holding capacity reduces transpirational stress and desiccation of sensitive F1 hybrid tissues (Omondi et al. 2016; Fazal and Bano 2016). Furthermore, biochar promotes beneficial rhizosphere interactions that enhance induced resistance against pathogens, which are particularly problematic during high-humidity periods (Atiyeh et al. 2000). Consequently, the significant biochar × season interaction highlights that while benefits are observed under moderate conditions (35–45%), the relative improvement is substantially higher during adverse climatic periods (55–75%) (Druege et al. 2016). These findings align with the broader literature on biochar in horticultural substrates, particularly its ability to reconcile the trade-off between water retention and aeration. Dumroese et al. (2011) similarly reported that biochar amendments in container nurseries improved water-holding capacity while maintaining sufficient air-filled porosity, preventing the hypoxia typical of saturated substrates. This dual hydrological function is further supported by Omondi et al. (2016), whose meta-analysis confirms biochar's capacity to simultaneously enhance drainage and water retention, facilitating root growth under variable moisture regimes. Furthermore, the observation that biochar yields higher relative benefits under adverse conditions parallels the findings of Fazal and Bano (2016), who demonstrated that biochar significantly mitigates abiotic stress (salinity) more effectively than in control environments, suggesting that biochar acts as a buffer against environmental constraints. This consistency is also noted in woody perennials by Mng'omba et al. (2010), who observed improved growth performance in mango rootstocks using biochar.

4.3 Global Relevance and Applicability.

While this study was conducted in the Highlands of Cameroon, the physiological findings address a universal bottleneck in the global coffee industry. *Coffea arabica* F1 hybrids, such as starmaya and centroAmericano are increasingly preferred globally for their vigor and yield. The physiological mechanism identified, which is the suppression of IAAO has profound implications for global clonal forestry. It suggests that the failure of woody cuttings to root is often an enzymatic failure, not just a water stress failure. For nurseries in

Central America, Assia and Vietnam, or East Africa propagating F1 hybrids, this study indicates that substrate engineering should target *chemical buffering* and *enzyme modulation* as primary goals. The optimal protocol involves integrating 20–30% coffee husk biochar (450–500°C) to achieve a substrate with >88% total porosity and a pH of 6.5–7.0. This creates the physical and chemical conditions necessary to suppress IAAO. By doing so, nurseries can reduce the reliance on high doses of synthetic rooting hormones and achieve consistent propagation outputs regardless of season.

From an economic perspective, producing biochar from coffee husks is cost-effective, ranging from \$50–80/m³. This translates to a negligible cost of \$0.002–0.003 per cutting, representing less than 2% of total propagation expenses. By recycling the carbon and nutrients found in husks—traditionally a disposal liability associated with environmental pollution and pathogen buildup—biochar transforms a low-value byproduct into a valuable substrate component. Beyond immediate productivity gains, biochar adoption offers substantial environmental co-benefits that support sustainable agriculture. Globally, crop wastes such as coffee and rice husks have become significant sources of greenhouse gas (GHG) emissions, contributing to anthropogenic climate change (Leifeld and Menichetti 2018; Billa et al 2017). Conversely, applying 1 kg of biochar sequesters approximately 2–3 kg of CO₂ equivalent, thereby supporting climate change mitigation strategies (Woolf et al. 2010; Agegnehu et al. 2016; Climate Change Committee 2024).

CONCLUSIONS

This study demonstrates that biochar enhances the rooting of *Coffea arabica* F1 hybrids primarily through a biochemical mechanism: the suppression of Indole-3-acetic acid oxidase (IAAO). This enzymatic modulation preserves the auxin pool and optimizes the auxin:cytokinin ratio, overcoming the hormonal barriers that typically limit clonal propagation. While improvements in water retention and aeration provided the necessary physical foundation, the *stabilization of the rhizogenic enzymatic system* is the key novel factor driving the observed 48–60% increase in rooting success. These findings reposition biochar as a metabolic tool for climate-resilient horticulture.

Limitations and Future Research

This study examined one F1 hybrid genotype; investigation across multiple coffee varieties would clarify technology breadth. Long-term field trials tracking transplant establishment, growth, and yield would confirm whether initial advantages persist. Molecular studies (transcriptomics, metabolomics) could elucidate gene expression underlying biochar-enhanced rooting. Deliberate microbiome manipulation combining biochar with beneficial microorganisms represents underexplored opportunity.

Acknowledgments: This research was supported by the FODECC via PAVSGS-2C. We thank the IRAD Foubot staff for propagation assistance and LAPSEE for Laboratory analyses. The students on internship from the Institute Universitaire Stratégique de L'Estuaire (INSAM- Bafoussam Cameroon) are acknowledged for their active participation.

Conflicts of Interest: The authors declare no conflicts of interest.

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Cite this Article: Fru, BS; Yoganie, YN; Mesame, NL; Levai, LD; Efombagn, IBM; Ngome, FA; Ayongwa, GC (2026). Biochar-Mediated Seasonal Stress Alleviation Enhances Rooting Performance in *Coffea arabica* F1 Hybrids. *Greener Journal of Agricultural Sciences*, 16(1): 21-36, <https://doi.org/10.15580/gjas.2026.1.020226020>.