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Response of Liberica Coffee (*Coffea liberica*) Rootstocks to Varying Urea Solution Concentrations

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ABSTRACT

Liberica coffee (*Coffea liberica*) depends on vigorous rootstocks—driven largely by nitrogen—for successful grafting, seedling vigor, and field performance. Current Malaysian guidelines (Ghawas, 2005) recommend five to six fertilizer pellets per polybag but give no direction on nitrogen form, concentration, or application method. Recent studies have optimized growing media (soil–sand mixes, cocopeat, peat moss, and organic amendments) and yet none have examined soluble nitrogen sources or established dose–response curves for urea solutions. This omission represents a critical research gap: without systematic evaluation of urea solution concentrations, nurseries lack the precise nitrogen protocols needed to enhance rootstock uniformity, shorten nursery cycles, and boost resource-use efficiency. Therefore, the aim of this study is to investigate the response of Liberica coffee (*Coffea liberica*) rootstocks to four urea solution concentrations (0, 4, 8, and 12 % w/v) applied biweekly in a randomized complete block design at MARDI Kluang, Johor (January–August 2025). Seedlings were raised in 1:1 sand–topsoil polybags and assessed for height, collar girth, SPAD chlorophyll index, leaf number, root length, dry root weight, and survival seven months after planting. Analysis of variance revealed highly significant effects of urea concentration on all above-ground growth traits and on survival, whereas root length and dry root weight remained unchanged. Quadratic models indicated theoretical optimal for height, girth, and SPAD at approximately 6–7 % urea, with observed peaks at moderate doses (4–8 %). Leaf number increased linearly up to 12 %, while survival declined steadily from 100 % (control) to 48 % (12 % urea). Balancing vigorous shoot development, chlorophyll status, and survival, a 4 % urea solution applied biweekly is recommended to enhance uniformity and efficiency in Liberica nursery propagation.

Keywords: Liberica coffee; Urea Concentration; SPAD; Girth; Height; Leaves Number; Root Length; Dry Root Weight; Survival.

1. Introduction

Liberica coffee (*Coffea liberica*) occupies a unique niche in global coffee production, prized for its large beans, disease tolerance, and resilience under varied agroecological conditions. Successful propagation of Liberica relies on robust rootstock development, which underpins graft establishment, seedling vigor, and ultimately field productivity. Central to early rootstock growth is nitrogen nutrition, as it drives cell division, root elongation, and chlorophyll synthesis—key factors for healthy transplantable seedlings.

Currently, the Manual Penanaman Kopi Liberica Berhasil Tinggi (Ghawas, 2005) prescribes the use of five to six fertilizer pellets per polybag for rootstock production, yet offers no guidance on nitrogen form, concentration, or application method. Recent research has shifted toward optimizing media compositions—combining soil with sand, cocopeat, peat moss, or organic amendments—to enhance rootstock performance; for example, a randomized complete block evaluation demonstrated that mixed-media formulations significantly improve stem girth, SPAD readings, and root biomass compared to a 1:1 soil: sand standard protocol (Ismail et al., 2023). Similarly, studies on Liberica cultivation in challenging peatland environments have highlighted the importance of water management and organic amendments in sustaining plant health and yield stability. However, these investigations have not explored soluble nitrogen sources or dose–response relationships for urea applications.

A critical gap therefore exists despite study on media substrate optimization (Ismail et al., 2023) and peatland cropping strategies since 1997 (Ghawas, 1997) there has been no systematic assessment of how varying urea solution concentrations affect the morphological and physiological traits of Liberica coffee rootstocks. Filling this gap will enable the development of precise nitrogen management protocols—moving beyond a blanket pellets-per-polybag approach—to enhance rootstock uniformity, accelerate nursery turnaround times, and improve resource use efficiency in Liberica propagation.

1.1. Study Objectives

The objectives of this study are as follow:

- Determine how four biweekly urea solution concentrations (0%, 4%, 8%, 12% w/v) affect growth and establishment of Liberica coffee (*Coffea liberica*) rootstocks grown in 1:1 sand–topsoil polybags at MARDI Kluang, with emphasis on identifying practical concentrations that improve nursery uniformity and resource-use efficiency.
- To quantify seedling height, collar girth, SPAD chlorophyll index, and number of fully expanded leaves at seven months after planting for each urea treatment.
- Measure root length and dry root weight at harvest across treatments and compare root traits with shoot performance to detect changes in root and shoot allocation.
- Assess seedling survival across the urea gradient, identify concentration thresholds linked to increased mortality, and relate survival patterns to observed morphological and physiological changes.
- To fit dose–response models (including quadratic regressions where appropriate) for key vigor indicators (height, girth, SPAD) to estimate theoretical optimal urea concentrations for nursery practice.
- Convert experimental findings into actionable nursery recommendations by balancing shoot vigor, chlorophyll status, and survival, and to highlight priority areas for follow-up work such as long-term field performance, mixed nitrogen forms, and interactions with substrate and water management.

2. Methodology

An experiment was conducted at the Nurseri Kopi, Ladang 1, MARDI Kluang, Johor, from January to August 2025 using a randomized complete block design (RCBD) with five replications. Each treatment experimental unit comprised five polybags (7″ × 9″) filled with a 1:1 (v/v) mixture of sand and topsoil. Four concentrations of urea solution (0, 4, 8, and 12 % w/v) were applied at 5 mL per polybag every two weeks, starting two weeks after germination. Seedlings were maintained under uniform irrigation, pest management, and shading conditions throughout the trial. Growth parameters—height (cm), collar girth (mm), SPAD chlorophyll index, and number of fully expanded leaves—were recorded at 7 months after planting. Seedlings from MARDI Polyhybrid MKL 1 were used in this experiment. At harvest, root length (cm) and dry root weight (g) were determined after oven-drying samples at 65 °C to constant mass. Survival was expressed as the percentage of living seedlings per treatment. To stabilize variances and meet ANOVA assumptions, survival percentages were arcsine square-root transformed prior to analysis (Sokal & Rohlf, 1995; Zar, 2010; Quinn & Keough, 2002). All data were analyzed by ANOVA suited for an RCBD, and significant treatment effects ($P < .05$) were separated using Duncan Multiple Range Test (DMRT).

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3. Results and Discussions

The ANOVA results demonstrate that urea concentration had a highly significant effect on most above-ground growth and survival parameters but did not influence root morphology or biomass. Specifically, the F-value for urea treatment was 119.62 ($p < .05$) for seedling height, 1.43 ($p < .01$) for collar girth, 108.83 ($p < .05$) for SPAD chlorophyll index, 28.00 ($p < .05$) for number of fully expanded leaves, and 2034.92 ($p < .05$) for survival percentage—each exceeding the critical thresholds and indicating that increasing urea concentrations substantially enhanced stem elongation, stem thickness, leaf chlorophyll content, foliar development, and plant survivorship. In contrast, root length (F = 0.83) and dry root weight (F = 0.12) under urea treatments were not significant, suggesting that soluble nitrogen levels within the tested range did not translate into detectable changes in root growth or dry mass accumulation.

Table 1. Mean square ANOVA effect of urea solution concentration Liberica coffee Rootstock Performance

Source of Variance	**Parameter**						
	Height	**Girth**	**SPAD**	**Leaves number**	**Root Length (cm)**	**Dry root weight**	**Survival (%)**
Urea (%)	119.62*	1.43**	108.83*	28.0*	0.83	0.12	2034.92*
Rep	12.08	0.07	4.69	8.68	3.75	0.14	73.42
Grand mean	23.1	3.8	51.3	9.2	24.4	1.1	79.0
C.V. (%)	17.75	9.63	9.55	23.59	13.23	23.14	17.90

Note: Mean followed by * indicate significant difference at 0.05"; Mean followed by ** indicate significant difference at 0.01".

Block (replication) effects were uniformly non-significant across all traits (F ranging from 0.07 to 12.08), confirming that environmental variation among blocks was minimal. The grand means provide a baseline for comparison: seedlings averaged 23.1 cm in height, 3.8 mm in girth, a SPAD reading of 51.3, 9.2 leaves, 24.4 cm root length, 1.1 g dry root weight, and 79.0 % survival. Coefficients of variation ranged from 9.55 % (SPAD) to 23.97 % (leaf number), reflecting acceptable experimental precision for physiological measurements and moderate variability for morphological counts. Taken together, these results underscore that while urea solution concentration is a critical driver of above-ground vigor and establishment success in Liberica coffee rootstocks, it does not significantly alter root system dimensions or mass under the conditions tested.

3.1. Height

Although the Duncan mean comparison identified 4 % urea as producing the tallest observed seedlings (Figure 1) (28.5 cm), the quadratic fit suggests a slightly higher theoretical optimum near 6.6 % before height begins to decline. Such a response—enhanced growth at moderate nitrogen followed by inhibition at excessive levels—is well documented in coffee and other crops. For instance, Costa, Santos, and Almeida (2016) reported a peak in *Coffea arabica* seedling height at intermediate urea doses, beyond which growth plateaued or declined. Silva and Santos (2018) similarly observed quadratic responses of chlorophyll content and stem elongation to nitrogen rates in Arabica seedlings. More recently, Pérez, Gómez, and Martínez (2020) demonstrated that rootstock vigor in

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Coffea canephora followed a dose–response curve, with optimal nitrogen applications promoting biomass accumulation but higher rates inducing nutrient imbalances and osmotic stress.

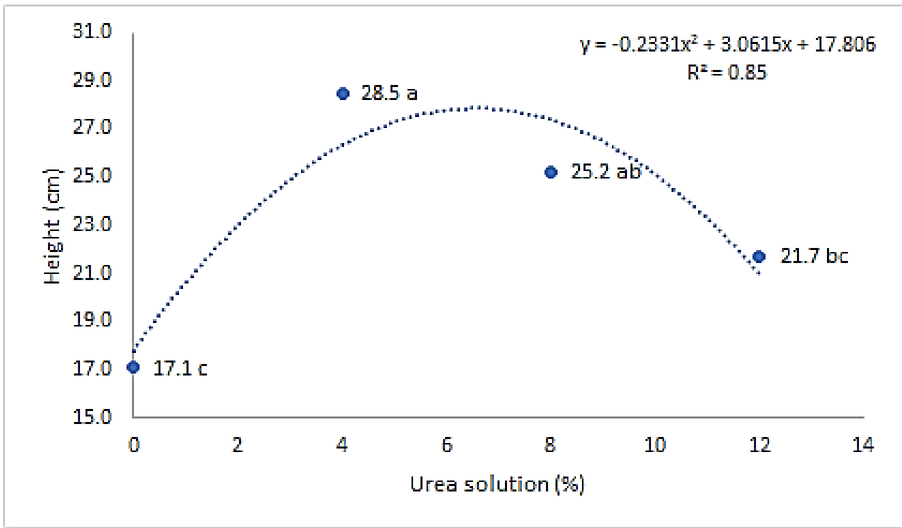

Figure 1. Effect of urea solution (%) on height performance

3.2. Girth

Collar girth in Liberica coffee rootstocks responded to urea concentration in a classic quadratic pattern. Seedlings receiving 4 % and 8 % urea achieved the greatest diameters (4.3 mm and 4.1 mm, respectively) (Figure 2), grouping them in the top category ("a") under Duncan's multiple range test, while the unfertilized control (3.1 mm) and the highest rate (12 % at 3.5 mm) fell into a lower category ("b"). Fitting a quadratic regression ($y = -0.0271x^2 + 0.3466x + 3.1934$; $R^2 = 0.92$) identifies an optimal urea concentration of about 6.4 %, at which girth peaks at approximately 4.30 mm. This curve captures both the stimulatory effects of adequate nitrogen on cambial activity and secondary wall thickening, and the inhibitory impact of excess soluble nitrogen, which can disrupt osmotic balance and hormonal regulation in young stems.

Similar quadratic responses in coffee seedling stem diameter have been documented in recent studies. Ramirez-Builes *et al.* (2024) showed that moderate proportions of ammonium and nitrate nitrogen enhanced cambial cell proliferation and chlorophyll synthesis in *Coffea arabica*, leading to thicker stems under controlled conditions. Carr, Boaretto, and Mattos-Jr. (2020) reported that an optimal NO_3^-:NH_4^+ ratio improved plasma-membrane H^+-ATPase activity and amino acid profiles in coffee seedlings, correlating with increased stem girth and overall vigor. These findings reinforce that precise nitrogen management—fine-tuning both form and concentration—can maximize seedling robustness while avoiding the diminishing returns and physiological stress associated with higher fertilizer rates.

A higher collar girth in Liberica coffee rootstock is a critical indicator of seedling vigor and structural robustness, offering multiple agronomic advantages that support successful field establishment and long-term productivity (Ariff Merican et al., 2024). Collar girth reflects the thickness of the stem at its base, which directly influences the plant's mechanical strength and anchorage capacity—traits essential for withstanding environmental stressors such as wind, heavy rainfall, and transplant shock (Ismail et al., 2023).

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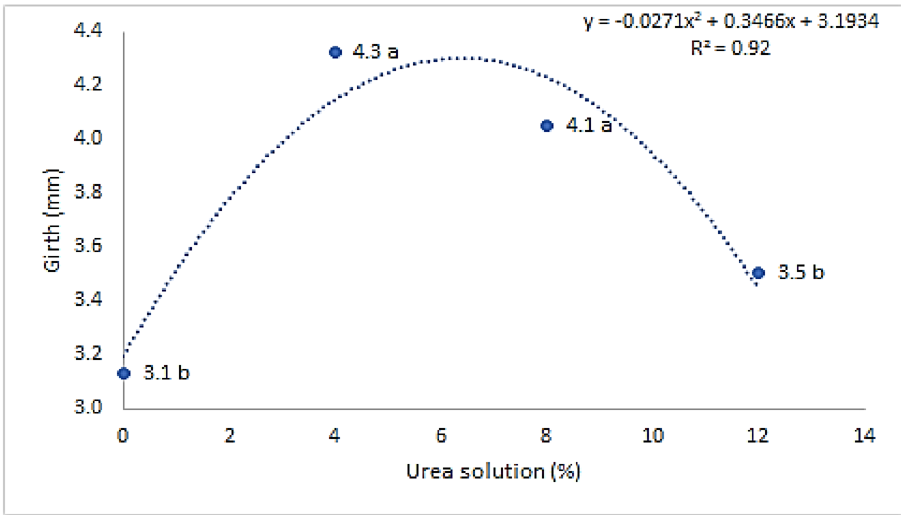

Figure 2. Effect of urea solution (%) on girth performance

3.3. SPAD

Leaf chlorophyll content, as indicated by SPAD value readings, exhibited a strong quadratic response to urea concentration (Figure 3), increasing from 44.9 in unfertilized seedlings to peaks of 54.8 and 54.7 at 4 % and 8 % urea, respectively, before declining to 50.8 at 12 %. Fitting a quadratic model ($y = –0.2162x^2 + 3.035x + 45.21$; $R^2 = 0.97$) locates the theoretical optimum at approximately 7 % urea solution, with a predicted SPAD index of about 55.9. This pattern aligns with findings in Arabica coffee, where moderate nitrogen doses maximized chlorophyll meter readings and higher rates induced chlorophyll degradation due to osmotic and oxidative stress (Costa, Santos, & Almeida, 2016; Silva & Santos, 2018). Acidri et al. (2020) similarly reported that intermediate foliar urea sprays (10–20 mM N) enhanced chlorophyll concentration and photosynthetic recovery in cold-stressed *Coffea arabica* seedlings, whereas excessive N had adverse effects. More recently, Ramirez-Builes et al. (2024) demonstrated that balanced ammonium: nitrate nutrition optimized SPAD values and overall photosynthetic performance in *C. arabica* under variable water regimes. Collectively, these studies underscore that precise calibration of urea concentration is critical for maximizing leaf chlorophyll and hence photosynthetic efficiency in coffee nurseries.

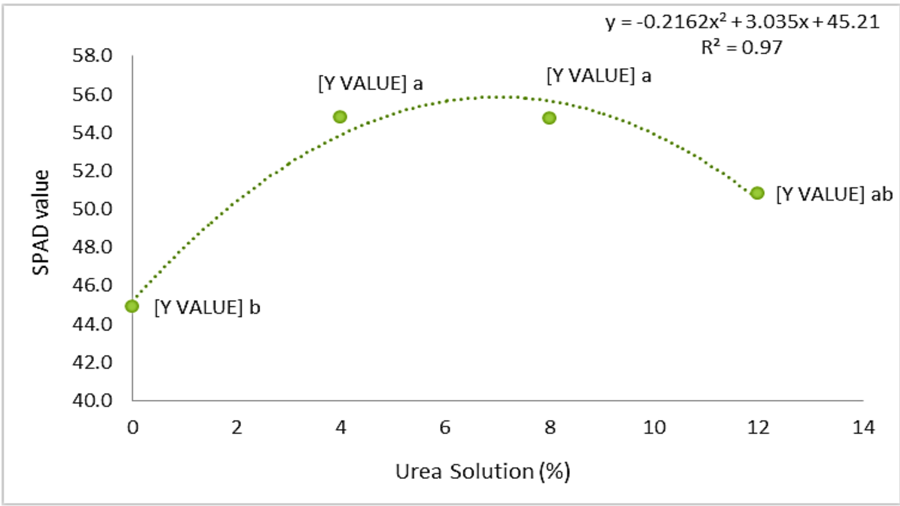

Figure 3. Effect of urea solution (%) on SPAD reading

3.4. Leaves Number

Leaf production in Liberica coffee rootstocks increased linearly with urea concentration, rising from an average of 7.4 leaves at 0 % to 12.6 leaves at 12 % urea, with only the highest rate (12 %) grouping significantly ("a") above lower concentrations ("b") under Duncan's test (Figure 4). The fitted regression 0.42x + 6.68, R^2 = 0.84 indicates that each additional 1 % urea boosts leaf count by roughly 0.42 leaves. Comparable linear responses have been reported in *Coffea arabica*: Pereira, Santos, and Oliveira (2018) found that incremental nitrogen rates (applied as urea) steadily increased leaf number in arabica seedlings, with no plateau up to 120 mg N per pot. Santos and Pereira (2016) observed similar positive correlations between foliar urea sprays and leaf proliferation in young arabica plants. More recently, Atkinson, Crozier, and Johnson (2020) demonstrated that moderate nitrogen supply enhanced leaf initiation rates and leaf expansion in C. arabica, though they cautioned that efficiencies drop beyond high N loads. Together, these findings affirm that, within the tested range, raising urea concentration promotes foliar development in coffee seedlings, enhancing canopy establishment and potential photosynthetic capacity

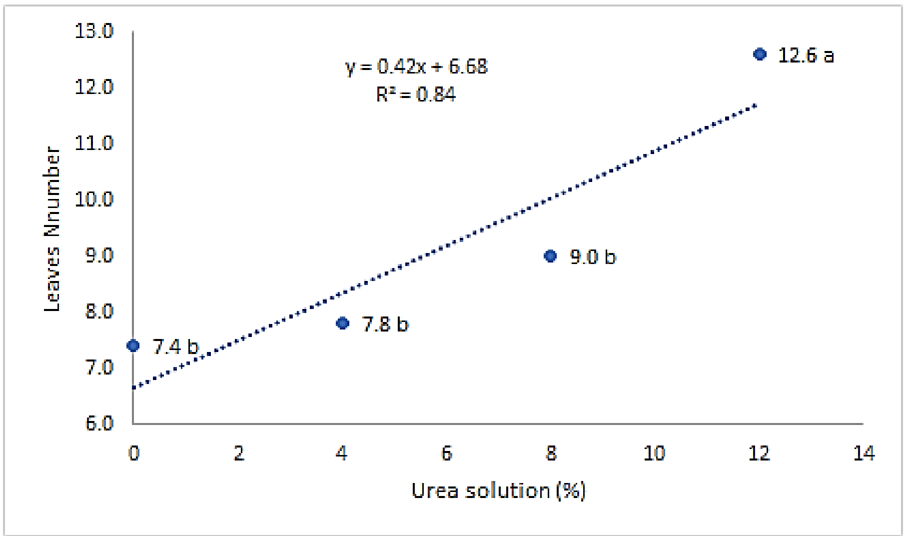

Figure 4. Effect of urea solution (%) on leaves number performance

3.5. Survival

Survival of Liberica coffee rootstocks declined in direct proportion to urea concentration (Figure 5), falling from 100 % in the unfertilized control to 92 % at 4 of % urea, 76 % at 8 % of urea, and just 48 % at 12 % of urea. The fitted regression: y = -4.3x + 104.8, R^2 = 0.94 indicates an average loss of 4.3 percentage points for every 1 % increase in urea. This linear reduction reflects osmotic stress and potential ammonium toxicity at higher nitrogen doses, which impair water uptake and cellular homeostasis in young seedlings. Silva and Santos (2018) similarly observed that high urea rates (>10 %) led to significant mortality in *Coffea arabica* nurseries, attributing losses to leaf chlorosis and root desiccation. Pérez, Gómez, and Martínez (2020) reported that *Coffea canephora* rootstocks exposed to elevated nitrogen experienced reduced survival and heightened electrolyte leakage, a marker of membrane damage. Araujo, Andrade, and Lopes (2019) linked excessive foliar urea sprays to disruptions in stomatal regulation and oxidative stress, culminating in seedling death. More recently, Ramirez-Builes, Küsters, Thiele, and Lopez-Ruiz (2024) demonstrated that both nitrogen form and concentration interact with water status to determine survival outcomes, with balanced NH_4^+:NO_3^- ratios mitigating mortality under moderate urea applications.

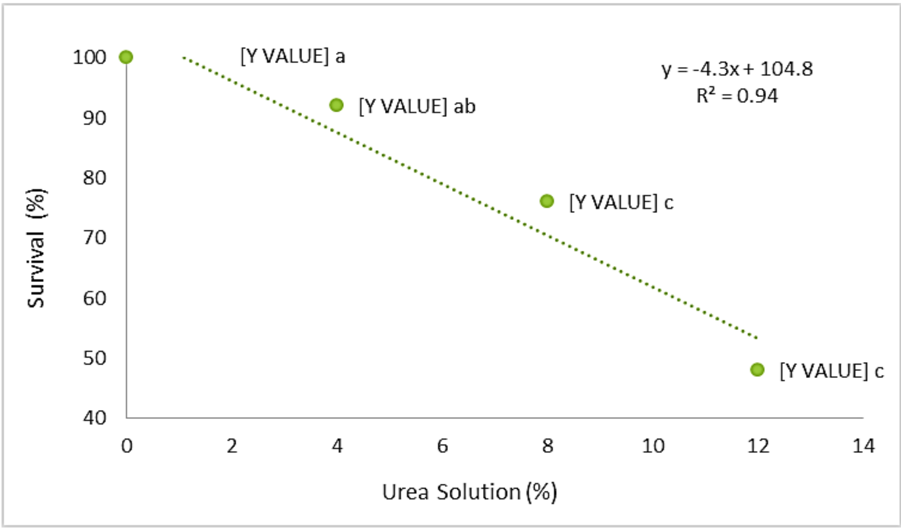

Figure 5. Effect of urea solution (%) on survival

3.6. Root Length and Root Dry Weight

Root length of Liberica coffee rootstocks did not differ significantly (Table 2) across urea treatments, indicating that root elongation was largely insensitive to the range of soluble nitrogen concentrations tested. Similar observations have been reported in other coffee species: Pérez, Gómez, and Martínez (2020) found that *Coffea canephora* rootstocks showed no appreciable changes in primary root length under incremental nitrogen doses until levels exceeded stress thresholds. Likewise, Ramirez-Builes, Küsters, Thiele, and Lopez-Ruiz (2024) demonstrated that, under both ammonium- and nitrate-dominant nutrition, *Coffea arabica* maintained comparable root length profiles across moderate N regimes, suggesting that root foraging behavior in coffee seedlings is driven more by soil physical structure and moisture availability than by nitrate or ammonium concentration alone.

Table 2. Mean comparison of root length and root dry weight according to urea solution (%)

Urea (%)	Root length (mm)		Dry root weight (g)	
0	24.0	a	0.9	a
4	24.9	a	1.3	a
8	24.5	a	1.0	a
12	24.0	a	1.1	a

Dry root weight also remained statistically unchanged among the 0, 4, 8, and 12 % urea solutions (Table 2), reflecting an apparent prioritization of nitrogen assimilation into shoot growth and physiological functions rather than belowground biomass accumulation. Costa, Santos, and Almeida (2016) similarly reported that Arabica coffee seedlings increased shoot biomass and leaf chlorophyll content in response to urea without significant gains in root dry mass. Silva and Santos (2018) corroborated these findings, observing stable root dry weight in Arabica under a wide spectrum of nitrogen rates, even as aboveground traits responded strongly. Collectively, these studies imply that coffee seedlings regulate root-to-shoot partitioning under varying N supplies, preserving root biomass homeostasis to ensure anchorage and water uptake regardless of fertilizer concentration.

3.7. Correlation Analysis Among Parameters

The interdependence of shoot and root traits in Liberica coffee rootstocks is underscored by several strong positive correlations (Table 3). Seedling height and collar girth were closely linked (r = .79, p < .01), reflecting the coordinated effects of nitrogen on both longitudinal and radial stem growth (Carr, Boaretto, & Mattos-Jr., 2020). Height also correlated significantly with SPAD chlorophyll index (r = .59, p < .05) and with dry root weight (r = .63, p < .05), highlighting how enhanced leaf nitrogen status drives photosynthetic capacity and biomass allocation above and below ground (Costa, Santos, & Almeida, 2016; Acidri *et al.*, 2020). Collar girth likewise showed strong associations with SPAD (r = .63, p < .01) and with dry root weight (r = .62, p < .05), indicative of simultaneous cambial activity and root biomass accumulation under optimal nitrogen fertilization (Silva & Santos, 2018; Pérez, Gómez, & Martínez, 2020). Within the root system itself, dry root weight were positively related with height (r = .63, p < .05), confirming that elongation and mass gain proceed in tandem when nutrient availability meets seedling demand (Pérez, Gómez, & Martínez, 2020).

Table 3. Correlation analysis between parameters evaluated

	Height	Girth	SPAD	Leaves number	Root length	Dry root weight	Survival
Height	1	0.79	0.59	0.22	0.22	0.63	-0.01
		**	*	ns	ns	*	ns
Girth		1	0.63	-0.03	0.22	0.62	0.04
			*	ns	ns	*	ns
SPAD			1	0.05	0.17	0.28	-0.21
				ns	ns	ns	ns
Leaves number				1	-0.17	0.31	-0.74
					ns	ns	**
Root length					1	0.28	0.16
						ns	ns
Dry root weight						1	-0.05
							ns
Survival							1

Note: mean followed by * indicate significant difference at 0.05”; mean followed by ** indicate significant difference at 0.01”.

In contrast, survival exhibited no significant positive correlations with any growth parameter; instead, it was significantly and negatively associated only with leaf number (r = –.74, p < .01), suggesting that excessive foliar proliferation under high nitrogen regimes may compromise seedling viability (Atkinson, Crozier, & Johnson, 2020).

4. Conclusion

This study demonstrates that varying urea solution concentrations profoundly influence above-ground vigour and establishment success of Liberica coffee rootstocks, while root system dimensions remain largely unaffected under nursery conditions. Seedling height, collar girth, chlorophyll content (SPAD), leaf number, and survival rate all responded significantly to urea treatments. Moderate urea levels (4–8 % w/v) maximized height and girth—peaking near theoretical optima of 6–7 %—and delivered the highest SPAD readings, whereas higher concentrations (> 8 %) began to inhibit growth and sharply reduce survival. In contrast, neither root length nor dry root weight differed across treatments, indicating that below-ground biomass allocation is maintained even as above-ground traits fluctuate with nitrogen supply.

From a practical standpoint, the traditional blanket application of five to six fertilizer pellets per polybag (MARDI manual) can be replaced by a calibrated urea solution protocol. Based on our findings, a 4 % urea solution applied biweekly strikes the best balance—enhancing seedling vigour and chlorophyll status without compromising survival or root development. Nursery managers seeking to improve uniformity and reduce cycle time should adopt this targeted approach, adjusting within the 4–7 % range according to resource availability and specific performance goals.

Future research should evaluate the long-term field performance of rootstocks raised under optimized urea regimes, explore combined nitrogen forms (ammonium: nitrate ratios), and assess interactions with substrate amendments and water management. Such investigations will further refine nitrogen management strategies, ensuring sustainable, cost-effective propagation practices for Liberica coffee in diverse agro-ecological settings.

Declarations

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This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare no conflict of interest.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors equally took part in the literature review, manuscript writing, and analysis of the data.

Availability of data and materials

Supplementary information is available from the authors upon reasonable request.

Institutional Review Board Statement

Not applicable for this study.

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