



Effects of Leaf Reserved and clipped on Axillary Bud Quality in Umbrella-Shaped *Hevea brasiliensis* ‘Reken 628’ Bud-sticks

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Abstract—The insufficient supply of bud-sticks from traditional single-stem rubber trees (*Hevea brasiliensis*) remains a bottleneck for mini-seedling budding in commercial rubber plantations. To address this issue, we investigated the impact of leaf reserved versus leaf clipped on axillary bud development in umbrella-shaped ‘Reken 628’ rubber trees. After removing apical dominance by topping, the plants developed multiple branches, yielding 4–6 times more bud-sticks than conventional methods. Bud-sticks were harvested when petioles detached naturally and leaf scars turned brown. Axillary buds (scale buds and petiole buds) from the second (2nd) and third (3rd) leaf whorls were analyzed for quantity, moisture content, and morphological traits (bud scar dimensions, bud eye size). Leaf reserved 2nd buds exhibited superior quality, with significantly higher moisture content (9.40–10.69%), larger bud scar width (43.21%), and thicker bud scars (19.69%) compared to their clipped counterparts ($P < 0.05$). Leaf-clipped reduced physiological consistency, increasing variability in leaf length (CV: 21.40–25.86%), leaf width (20.79–23.36%), and stem moisture (5.89%). Correlation analysis revealed strong synergies between leaves reserved, stem thickness, and bud moisture, critical for grafting success. We conclude that reserved leaves on 2nd whorls of umbrella-shaped trees optimizes bud-sticks quality for mini-seedling budding. Post-topping management should prioritize frequent irrigation and balanced fertilization to sustain nutrient supply. This strategy enhances bud-sticks yield, grafting efficiency, and survival rates, offering a scalable solution for high-demand rubber nurseries.



Keywords—*Hevea brasiliensis*, umbrella – shaped bud sticks, leaf reserved, axillary bud, quality.

I. INTRODUCTION

In recent years, the persistently low price of natural rubber and its suboptimal economic returns have significantly diminished the willingness of rubber farmers and cultivation entities to invest in new rubber saplings. This has precipitated a sharp decline in demand for rubber seedlings, thereby disrupting the sales dynamics of rubber nursery stocks. Concurrently, reduced market demand has further eroded the enthusiasm of nursery production units

to cultivate rubber seedlings. As a critical agricultural commodity and strategic resource, natural rubber production in China faces constraints from resource limitations and socioeconomic development. Notably, the national self-sufficiency rate of natural rubber has steadily declined from approximately 50% in the 1990s to 13.7% by 2020, with a sustained deficit below 20% over seven consecutive years, positioning China as a major importer of natural rubber [1]. This insufficient self-sufficiency poses strategic security risks, prompting governmental

interventions. The 2018 No.1 Central Document mandated the establishment of a 12-million-acre natural rubber production protection zone across Hainan, Yunnan, and Guangdong provinces to safeguard domestic supply. Subsequent policies, including the 14th Five-Year Plan for Natural Rubber Production Capacity Development (2021) and the 2023 No.1 Central Document, reinforced support mechanisms. In December 2023, the Comprehensive Insurance Policy for Natural Rubber was jointly issued to stimulate production incentives. The 2024 agricultural policy further emphasized industrial consolidation through intelligent harvesting technologies, aging plantation renewal, and specialized rubber garden development, driving nationwide standardization of 58,000 acres of specialized plantations to enhance production efficiency.

The surging demand for rubber seedlings has exposed critical bottlenecks in conventional propagation methods. Natural rubber cultivation remains a vital economic pillar for tropical regions like Hainan, Yunnan, and Guangdong, sustaining millions of livelihoods [4]. However, traditional nursery practices—characterized by extended production cycles (≥ 18 months for rootstock-to-grafted seedling development), escalating land use costs, and labor-intensive operations—urgently require modernization to improve efficiency and reduce costs.

Mini-seedling budding technology has emerged as a pivotal industrial propagation method [5–6]. This technique employs nutrient-rich rubber seeds for early-stage indoor grafting, enabling bud union on juvenile rootstocks prior to full leaf expansion. Compared to conventional approaches, it offers multiple advantages: shortened cultivation cycles (miniaturized seedlings), reduced labor inputs, higher spatial efficiency, enhanced root system development, and improved post-transplantation survival rates with earlier tapping maturity [7–9]. Consequently, it has gained widespread adoption in Yunnan and Guangdong [10–12]. Nevertheless, Hainan's rubber industry faces mounting pressures from stagnant rubber prices, rising labor costs, and infrastructure demands from the Hainan Free Trade Port initiative. Technical challenges persist, including inconsistent survival rates due to variable rootstock/scion quality and environmental factors, which escalate production costs through wasted materials and additional management labor. Furthermore, suboptimal post-grafting care often yields inferior seedlings, diminishing market acceptance.

Current bud-sticks production methods exhibit limitations: (1) Repeated pruning of field-grown plants yields green bud-sticks with dimensional incompatibility to rootstocks [13]; (2) Micro-propagation via anther culture incurs high technical and capital costs [14]; (3) Shoot tipping of field plants produces limited quantities of juvenile bud-sticks

[15]; (4) Root-restricted greenhouse cultivation achieves small-stem bud-sticks at elevated costs [16]. Empirical evidence suggests defoliation enhances budding success in rubber trees [7–8], though analogous practices in other tree species may induce stress responses [17–18]. Existing studies have investigated external factors influencing grafted seedling growth [19–21], bud-stick selection criteria [22–23], and apical dominance disruption for multi-branch bud-sticks production [24–25]. However, systematic analyses of pre-grafting axillary bud quality—a critical determinant of success—remain lacking.

To address these gaps, this study innovates a protocol for efficiently producing small-stem defoliated bud-sticks compatible with mini-seedling grafting. Using *Hevea brasiliensis* clone 'Reken 628' as propagation material, we developed umbrella-shaped bud-sticks by tipping single-stem plants at four-whorl maturity to induce stable three-whorl lateral branches. Defoliation was performed on secondary and third whorl petiole buds, followed by bud-stick collection after petiole abscission and scar lignification. Comprehensive metrics—including bud counts, moisture content, scar dimensions (length, width, thickness), and bud eye morphology were quantified and compared to non-defoliated controls. This investigation elucidates the relationship between defoliation practices and axillary bud quality, aiming to optimize mini-seedling budding productivity, survival rates, and economic viability while providing theoretical and practical guidance for industrial propagation.

II. MATERIAL AND METHODS

2.1 plant materials and experimental site

The experiment utilized *Hevea brasiliensis* clone 'Reken 628', planted in December 2023 at the Rubber Research Institute Nursery Base (109°29'62"E, 19°30'12"N; elevation: 116.9 m) of the Chinese Academy of Tropical Agricultural Sciences in Danzhou, Hainan Province, China.

2.2 treatments and growth management

In June 2024, single-stem shoots with four stabilized leaf whorls and actively elongating apical buds underwent manual apical dominance removal (de-topping). By September 2024, umbrella-shaped shoots with three stabilized leaf whorls had developed from the de-topped positions.

2.3 physiological measurements

In August 2024, phenological stages of the top whorl leaves on umbrella-shaped 'Reken 628' shoots were monitored. Leaf length and width were measured using a transparent ruler. Chlorophyll content, nitrogen

concentration, leaf surface humidity, and temperature were quantified with a SPAD meter (Jinkelida TYS-4N). Plant height and stem diameter were recorded using a measuring tape (1 mm precision) and digital vernier caliper (0.01 mm precision), respectively.

2.4 experimental design and sampling

Six treatments were established in a randomized complete block design with three replicates per treatment. Upon stabilization of the top whorl leaves, defoliation treatments were applied: leaves on petiole buds of the second and third whorls—excluding densely noded buds—were excised. After petiole scars transitioned from green to brown, stems from 2nd and 3rd positions were harvested. Leaf blades, petiole buds, and scale buds from these positions were collected separately.

2.5 biometric and hydration analyses

Fresh weights of leaves, buds, and stems were recorded using an analytical balance (0.01 g). Leaf dimensions were measured with a ruler (1 mm), while bud scar dimensions (length, width, thickness) and bud eye morphology (length, width) were quantified via digital vernier caliper (0.01 mm). Stem diameter and length were assessed using calipers and a tape measure, respectively. Following fresh weight measurements, samples were oven-dried to constant weight at 65°C for dry mass determination and water content calculation.

2.6 statistical analysis

Data were processed using DPS software (v20.05 Advanced Edition) for Student's t-tests (two-sample comparisons) and one-way ANOVA with Duncan's multiple range test ($\alpha = 0.05$). Graphical representations were generated using GraphPad Prism (v8.3.0), and correlation analyses were performed via the Tutools Platform (<http://www.cloudtutu.com>).

III. RESULT AND DISCUSSION

3.1 Plant growth performance (leaf length, leaf width, leaf number, leaf water content, plant height, stem diameter, stem water content)

3.1.1 Umbrella-shaped non-defoliated 2nd and 3rd leaf whorls

In the non-defoliated umbrella-shaped treatment, the 2nd leaf whorl exhibited significantly greater leaf width

(Fig. 1B, +9.32%, $P < 0.05$), leaf number (Fig. 1C, +18.92%, $P < 0.05$), leaf water content (Fig. 1D, +6.30%, $P < 0.05$), and stem water content (Fig. 1E, +8.33%, $P < 0.05$) compared to the 3rd leaf whorl. Conversely, the 2nd whorl showed significantly reduced plant height (Fig. 1F, -34.83%, $P < 0.05$) and stem diameter (Fig. 1G, -2.36%, $P < 0.05$). No significant differences were observed in other parameters. These results indicate that the 2nd leaf whorl outperformed the 3rd in leaf width, leaf number, and water retention, while the 3rd whorl exhibited superior vertical growth (plant height) and stem thickening.

3.1.2 Umbrella-shaped Defoliated 2nd and 3rd Leaf Whorls

Under defoliation treatment, the 2nd leaf whorl displayed significantly greater leaf length (Fig. 1A, +6.56%, $P < 0.05$), leaf width (Fig. 1B, +9.60%, $P < 0.05$), and stem water content (Fig. 1E, +3.78%, $P < 0.05$) compared to the 3rd whorl. However, the 2nd whorl exhibited a significantly smaller stem diameter (Fig. 1G, -19.75%, $P < 0.05$). Other parameters showed no significant differences. This suggests that defoliation amplified physiological advantages in the 2nd whorl for leaf expansion and water retention but compromised stem thickening.

3.1.3 Comparative Analysis: Non-Defoliated vs. Defoliated 2nd and 3rd Leaf Whorls

Leaf- reserved 2nd whorls exhibited significantly higher leaf length (Fig. 2A, +11.29%, $P < 0.05$), leaf width (Fig. 2B, +12.25%, $P < 0.05$), leaf number (Fig. 2C, +36.22%, $P < 0.05$), leaf water content (Fig. 2D, +15.54%, $P < 0.05$), and stem water content (Fig. 2E, +13.12%, $P < 0.05$) compared to defoliated counterparts. Similarly, non-defoliated 3rd whorls showed superior performance in leaf length (+15.64%, $P < 0.05$), leaf width (+12.52%, $P < 0.05$), leaf number (+39.34%, $P < 0.05$), leaf water content (+10.96%, $P < 0.05$), plant height (Fig. 2F, +28.26%, $P < 0.05$), and stem water content (+8.81%, $P < 0.05$). These findings collectively demonstrate that non-defoliated treatments consistently outperformed defoliated treatments across key growth metrics, highlighting the critical role of intact foliage in maintaining physiological stability and growth vigor.

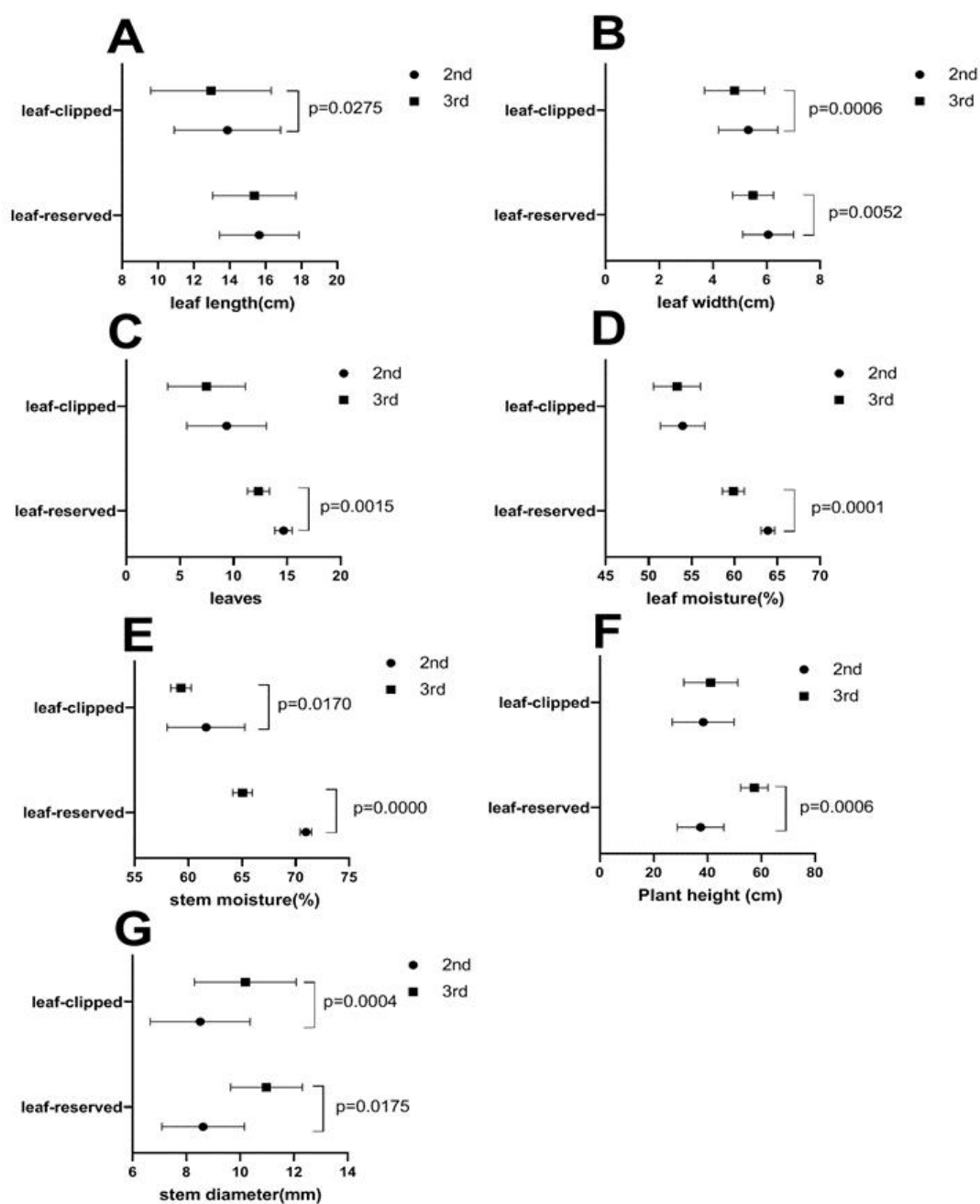


Fig. 1. Comparison of Leaf length, leaf width, leaves, leaf moisture, stem moisture, plant height, and stem diameter between 2nd and 3rd leaf whorl

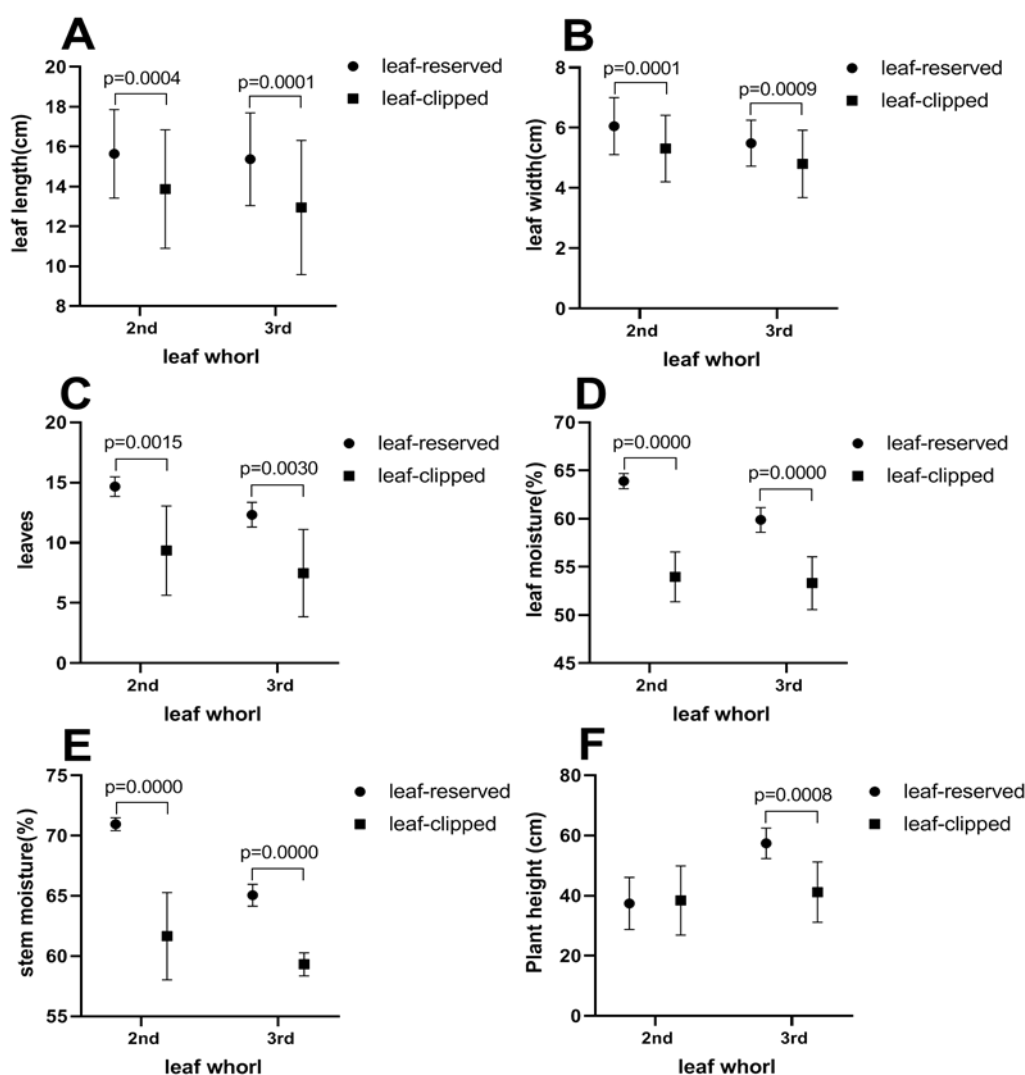


Fig. 2. Comparison of leaf length, leaf width, leaves, leaf moisture, stem moisture, plant height between leaf reserved and clipped treatment in the same leaf whorl

3.2 axillary bud quality

3.2.1 Comparison of Petiole and Scale Bud Numbers between leaf -reserved and clipped Treatments

In leaf- reserved plants, the 2nd leaf whorl produced significantly fewer petiole buds (Fig. 3A, -31.11%, $P < 0.05$) but more scale buds (Fig. 3B, +27.59%, $P < 0.05$) compared to the 3rd whorl. Notably, non-defoliated 2nd whorls exhibited 15.17% more scale buds than defoliated 2nd whorls (Fig. 3C, $P < 0.05$). These trends suggest a whorl-specific trade-off between petiole and scale bud development, with defoliation preferentially suppressing scale bud proliferation.

3.2.2 Axillary Bud Quality (Scar Dimensions, Bud Eye Morphology, Moisture)

3.2.2.1 Leaf reserved Treatment: Intra-Plant Variation between 2nd and 3rd Leaf Whorls

For scale buds (Fig. 4), the 2nd whorl exhibited significantly larger scar width (Fig. 5A, +43.21%, $P < 0.05$), scar thickness (Fig. 5B, +19.69%, $P < 0.05$), and bud eye width (Fig. 5C, +20.19%, $P < 0.05$) but shorter scar length (Fig. 5D, -24.99%, $P < 0.05$) compared to the 3rd whorl. For petiole buds, the 2nd whorl demonstrated reduced scar length (Fig. 5a, -23.61%, $P < 0.05$) and width (Fig. 5b, -31.85%, $P < 0.05$) but greater scar thickness (Fig. 5c, +11.89%, $P < 0.05$) and bud eye length (Fig. 5d, +24.47%, $P < 0.05$). These contrasting patterns underscore the complexity of bud quality assessment, as no single morphological parameter reliably predicts overall bud viability.

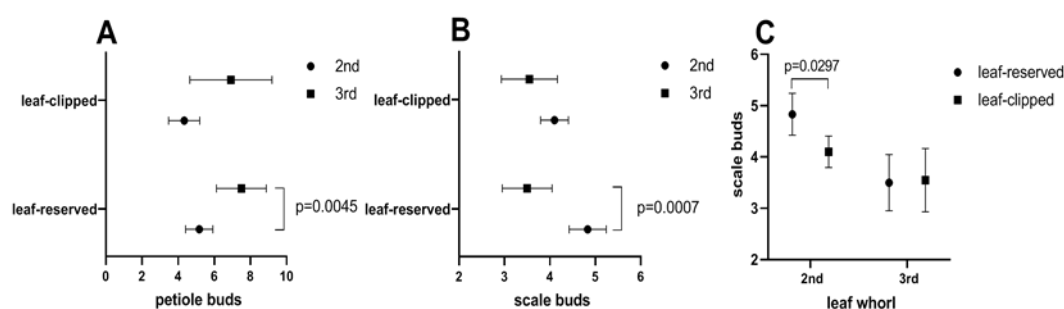


Fig. 3. Comparison of the number in petiole buds and scale buds between the 2nd and 3rd leaf whorl, and scale buds between leaf reserved and clipped treatment in the same leaf whorl

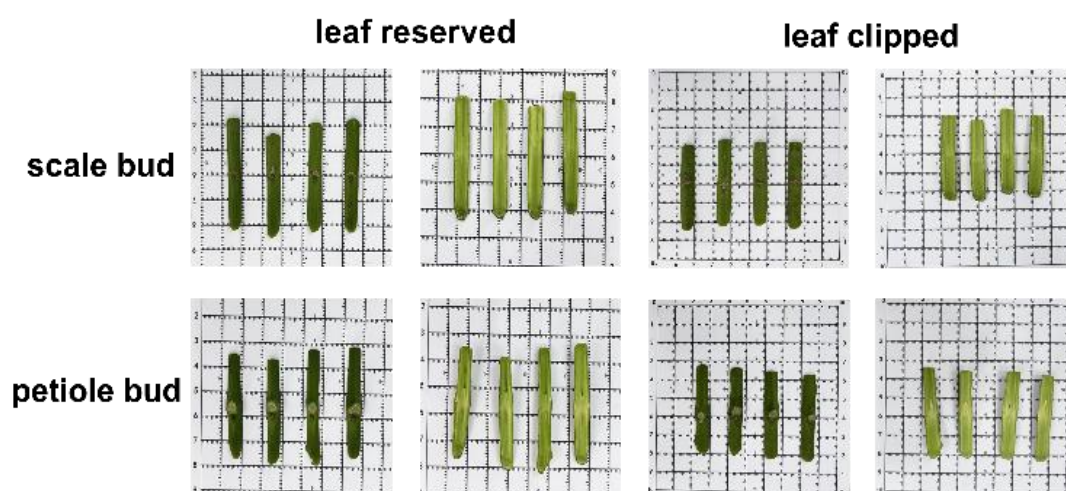


Fig. 4. Positive and negative side view of petiole bud and scale bud on 2nd leaf whorl

3.2.2.2 Comparison of umbrella-shaped leaf-clipped among different leaf whorls of the same plant

In the umbrella-shaped leaf clipping treatment, the bud scale scar width (Fig. 5A) and thickness (Fig. 5B) of scale buds in the 2nd leaf whorl (2nd) were significantly larger than those in the 3rd leaf whorl (3rd) by 24.14% ($P < 0.05$) and 14.27% ($P < 0.05$), respectively. Conversely, the bud eye width (Fig. 5C), scar length (Fig. 5D), and eye length (Fig. 5E) of scale buds in 2nd were significantly smaller than those in 3rd by 6.40% ($P < 0.05$), 32.21% ($P < 0.05$), and 12.06% ($P < 0.05$), respectively. For petiole buds, 2nd exhibited significantly greater bud scar width

(Fig. 5b), scar thickness (Fig. 5c), eye length (Fig. 5d), and eye width (Fig. 5e) compared to 3rd, with increases of 15.94% ($P < 0.05$), 25.98% ($P < 0.05$), 8.12% ($P < 0.05$), and 14.21% ($P < 0.05$), respectively, while other parameters showed no significant differences.

These results indicate that scale buds in 2nd displayed larger scar dimensions (width and thickness) but smaller scar length and eye dimensions compared to 3rd. In contrast, petiole buds in 2nd consistently outperformed 3rd in scar width, thickness, and eye dimensions, suggesting superior quality of petiole buds in 2nd after leaf clipping.

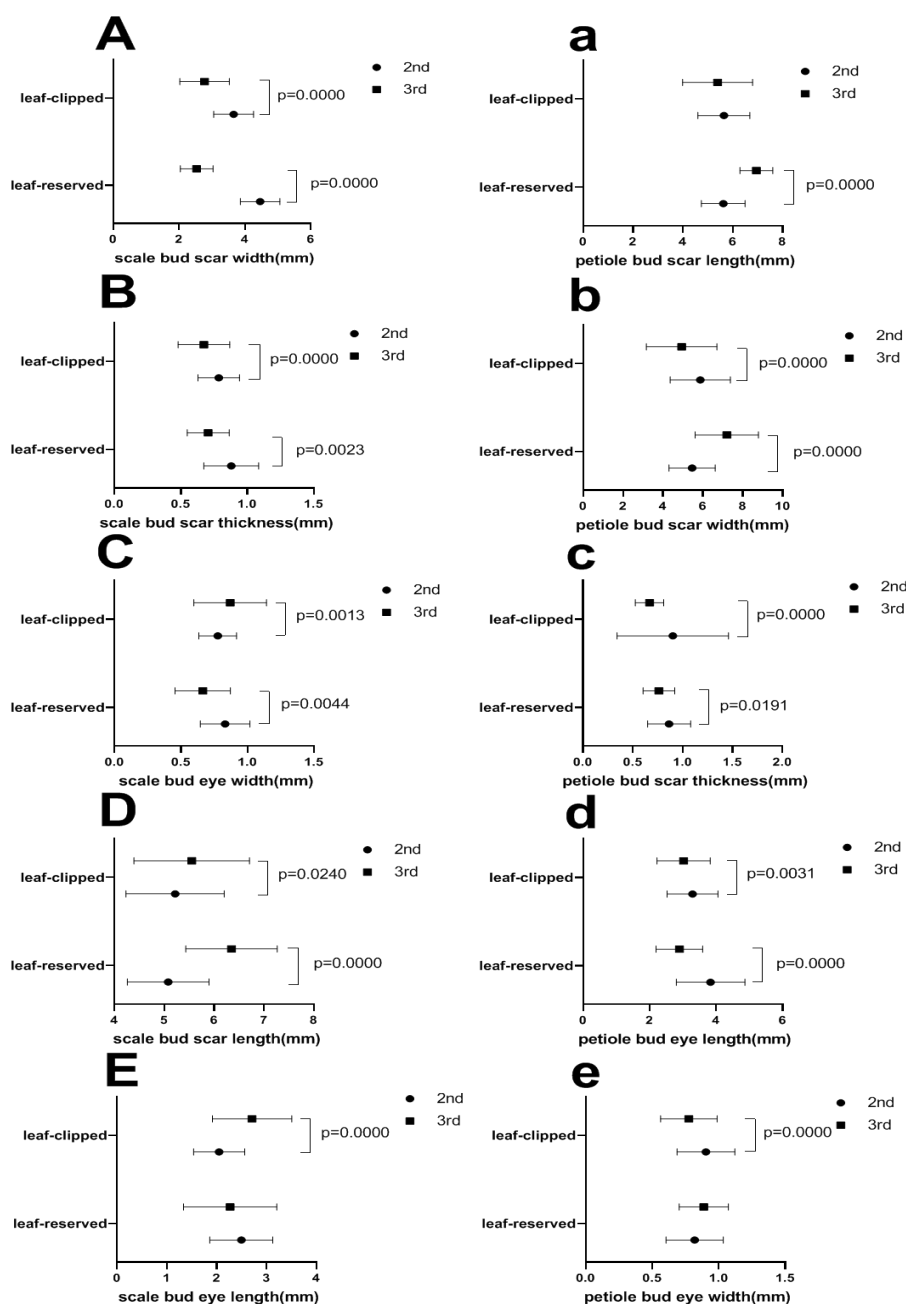


Fig. 5. Comparison of scar dimensions, bud eye morphology between the 2nd and 3rd leaf whorl

3.2.2.3 Comparison of umbrella-shaped leaf-reserved vs. leaf-clipped within the same leaf whorl

For scale buds in 3rd, the scar length of reserved leaves (Fig. 6A) was 12.55% larger than that of clipped leaves ($P < 0.05$). In 2nd, reserved leaves exhibited 18.03%, 10.63%, and 17.80% greater scar width (Fig. 6B), thickness (Fig. 6C), and eye length (Fig. 6D), respectively, compared to clipped leaves ($P < 0.05$). Conversely, the eye length and width of reserved 3rd scale buds (Fig. 6E) were 19.41% and 31.06% smaller than those of clipped 3rd ($P < 0.05$). For petiole buds, reserved

3rd showed 22.38%, 31.47%, and 12.34% increases in scar length (Fig. 6a), width (Fig. 6b), and thickness (Fig. 6c) compared to clipped 3rd ($P < 0.05$), while reserved 2nd exhibited a 14.16% increase in eye length (Fig. 6d) relative to clipped 2nd ($P < 0.05$).

Leaf- reserved generally enhanced morphological development in petiole buds. Reserved 2nd demonstrated larger scar width, thickness, and eye length in scale buds, while reserved 3rd showed longer scars but smaller eyes compared to clipped counterparts.

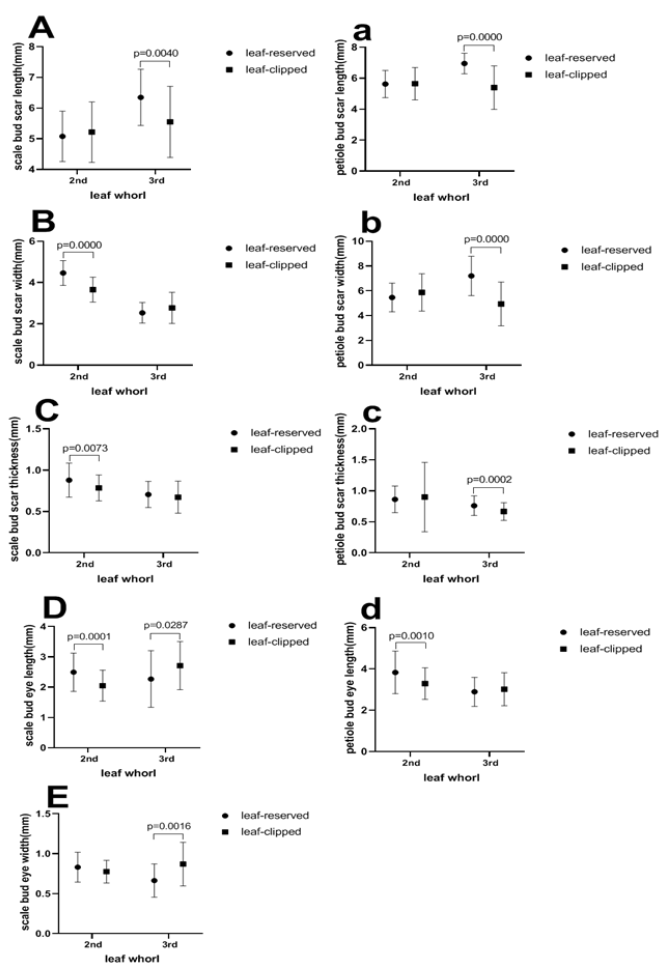


Fig. 6. Comparison of scar dimensions, bud eye morphology within the same leaf whorl

3.2.2.4 Moisture content comparison between leaf-reserved and leaf-clipped treatments

The moisture content of scale buds in reserved 2nd (Fig. 7A) and 3rd was 9.40% and 8.28% higher, respectively, than in clipped treatments ($P < 0.05$).

Similarly, petiole bud moisture content in reserved 2nd (Fig. 7B) and 3rd exceeded clipped treatments by 10.69% and 8.55% ($P < 0.05$). These findings confirm that leaf-reserved positively maintains moisture levels in both bud types.

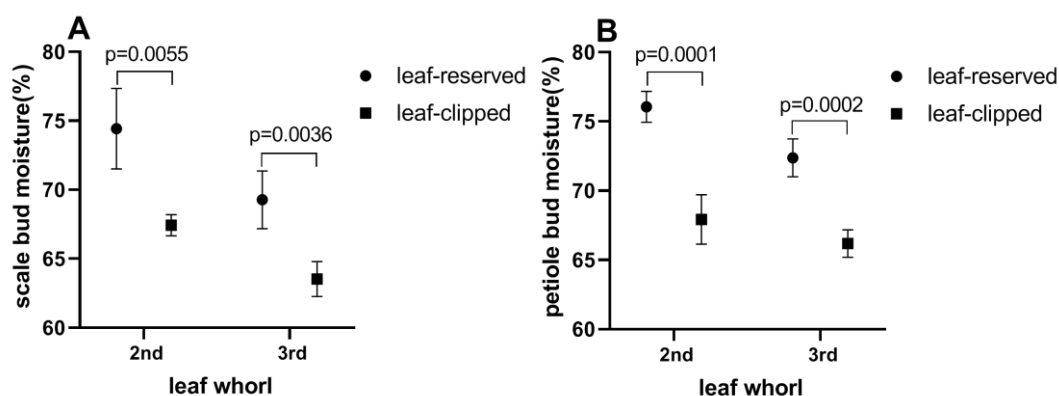


Fig. 7. Moisture content comparison between leaf-reserved and leaf-clipped treatments

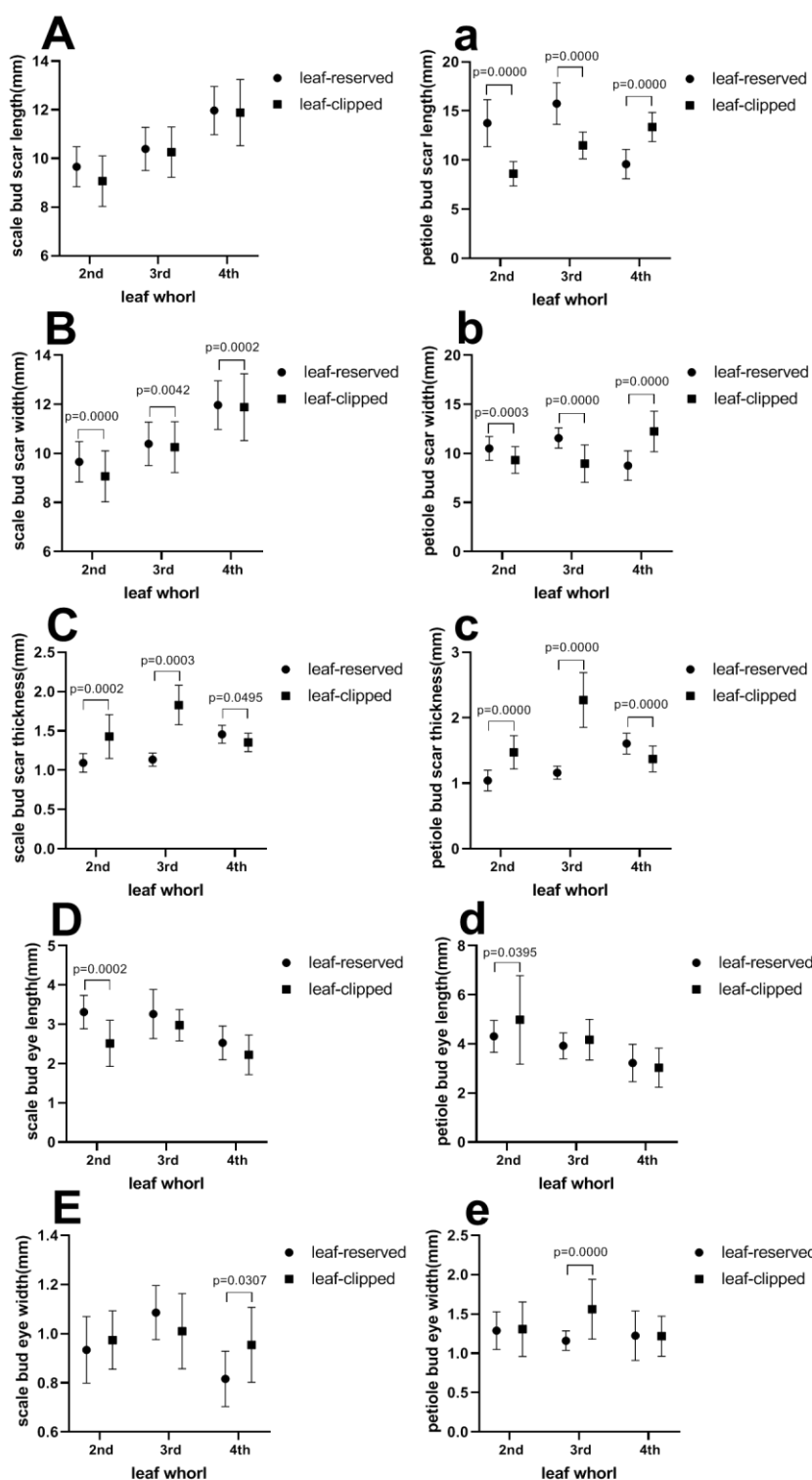


Fig. 8. The quality of axillary buds at different leaf whorl on the same plant under leaf reserved and clipped treatment

3.3 Coefficient of variation analysis

Leaf clipping increased CV values for leaf length, width, number, moisture content, and stem moisture in both whorls (2nd: 21.40%, 20.79%, 39.67%, 4.80%, 5.89%; 3rd: 25.86%, 20.79%, 48.39%, 5.14%, 24.41%), indicating reduced uniformity. Reserved 2nd exhibited lower variability in petiole bud number (CV = 14.57%)

and scale bud number (CV = 8.45%) compared to 3rd (18.38% and 15.65%, respectively). Moisture-related CVs for reserved treatments (scale buds: 1.13–3.92%; petiole buds: 1.46–2.62%) were consistently lower than clipped treatments. Morphological CVs for buds (scar/eye dimensions) ranged from 9.47% to 62.08%, with clipped treatments generally showing higher variability.

Table 1. Coefficient of variation (%) between all parameters of umbrella shaped under leaf reserved and clipped treatment

parameter		reserved leaves		parameter		clipped leaves	
		2 nd	3 rd			2 nd	3 rd
growth index	leaf length	14.21	15.12	growth index	leaf length	21.40	26.00
	leaf width	15.62	13.92		leaf width	20.79	23.36
	plant height	23.18	8.84		plant height	29.92	24.41
	stem diameter	17.79	12.17		stem diameter	21.82	18.58
number	leaves	5.57	8.37	number	leaves	39.67	48.39
	scale bud	8.45	15.65		scale bud	7.47	17.38
	petiole bud	14.57	18.38		petiole bud	19.88	32.88
moisture	leaf	1.24	2.13	moisture	leaf	4.80	5.14
	stem	0.75	1.39		stem	5.89	1.62
	scale bud	3.92	3.01		scale bud	1.13	2.00
	petiole bud	1.46	1.89		petiole bud	2.62	1.50
scale bud	bud scar length	16.16	14.45	scale bud	bud scar length	18.90	20.87
	bud scar width	13.46	19.67		bud scar width	16.58	27.20
	bud scar thickness	23.44	22.39		bud scar thickness	19.89	28.83
	bud eye length	25.31	41.25		bud eye length	24.92	29.31
	bud eye width	22.45	31.39		bud eye width	18.24	31.41
petiole bud	bud scar length	15.61	9.47	petiole bud	bud scar length	18.45	25.99
	bud scar width	21.20	22.03		bud scar width	25.68	35.80
	bud scar thickness	24.94	20.76		bud scar thickness	62.08	21.33
	bud eye length	26.91	24.23		bud eye length	23.27	26.44
	bud eye width	26.21	20.82		bud eye width	24.09	27.43

3.4 Correlation analysis

Strong positive correlations ($p < 0.01$) were observed between leaf number and petiole bud moisture, stem diameter and scale bud moisture, and scale bud number with petiole eye length. Significant positive correlations ($p < 0.05$) included leaf width with scale bud moisture

and multiple morphological parameters between bud types. Negative correlations emerged between scale eye width and petiole scar width ($p < 0.01$), as well as scale eye length and petiole eye width ($p < 0.05$), suggesting coordinated growth regulation.

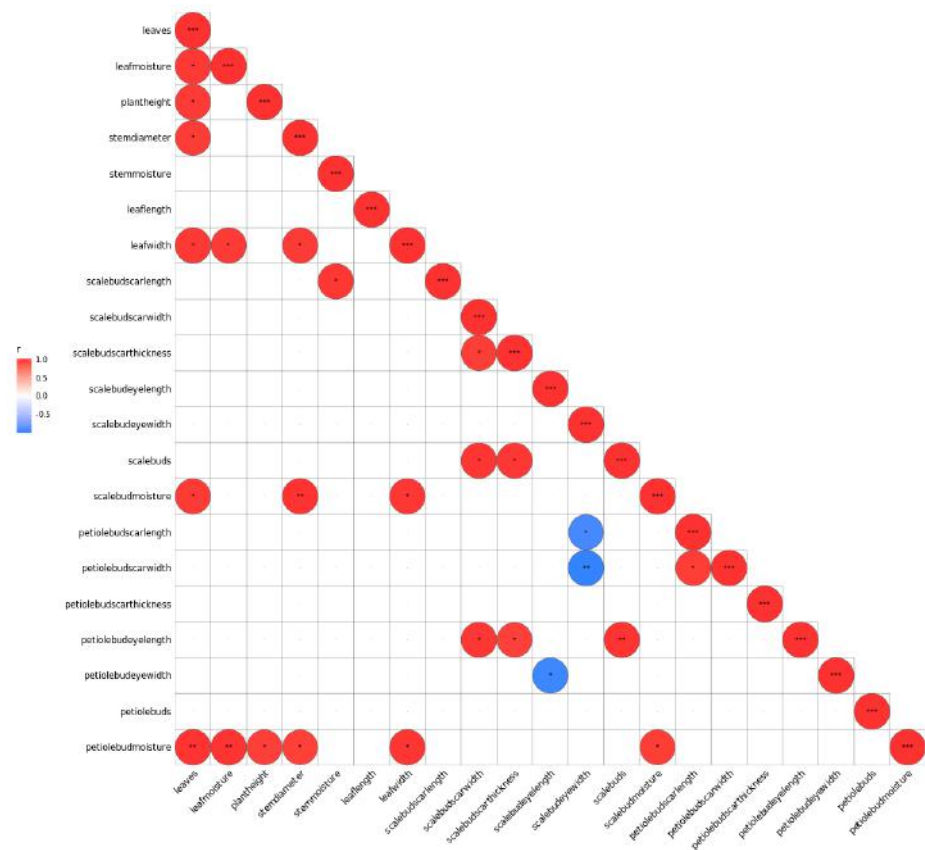


Fig. 9. Correlation analysis of all growth indexes observed

3.5 Comprehensive analysis

Using Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) with stem diameter and plant height as low-priority indicators, the ranking of

treatments was: reserved 2nd > reserved 3rd > clipped 2nd > clipped 3rd. Reserved 2nd demonstrated optimal bud quality, supported by superior physiological metrics (leaf number, stem thickness, moisture content).

Table 2. Comprehensive analysis based on growth index

Leaf whorl - leaf status	Statistic CI	Rank
2 nd -leaf reserved	0.6773	1
3 rd -leaf reserved	0.4963	2
2 nd - leaf clipped	0.4197	3
3 rd -leaf clipped	0.3711	4

CI, approximation to the Optimal Vectors.

IV. CONCLUSION

In *Hevea brasiliensis* clone Reken 628, leaf-reserved buds from the 2nd leaf whorl (2nd) exhibited the highest quality for seedling grafting. Leaf-reserved promotes moisture retention and morphological development in both scale and petiole buds, while the clipped increases phenotypic variability. For practical propagation, apical pruning without leaf removal is recommended to enhance

branching. Priority should be given to 2nd-derived buds due to their stability and positive correlations with key growth parameters (leaf number, stem thickness, plant height), which collectively improve grafting success rates.

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