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Effects of Defoliation and Organic Fertilizers on the Budding Success of Rubber (*Hevea brasiliensis* Muell Arg) Seedlings

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ABSTRACT

This study aimed to evaluate the budding success of rubber in response to budwood defoliation and organic fertilizer application. The experiment followed a 3 × 4 factorial arrangement in a randomized complete block design (RCBD), with budwood defoliation as Factor A and both chemical and organic fertilizers as Factor B. A morpho-anatomical analysis was also conducted to assess bud-union development between the stock and scion of rubber seedlings. Defoliating budwood two weeks before budding significantly increased budding success (71.15%) and led to earlier sprouting—12.83 d after cutback—compared to non-defoliated budwood. It also promoted the development of the longest shoots at 60 d post-cutback. Organic fertilizers significantly enhanced budding success, early sprouting of scion buds, and scion shoot development compared to the non-defoliated control. Among the fertilizers, vermicast resulted in the highest budding success (69.72%), comparable to chicken manure (63.24%). In contrast, seedlings fertilized with 16-20-0 achieved a budding success rate of 45.80%, similar to the control (43.66%). At 14 d after budding, cross-sectional analysis revealed partial bud-union formation with profuse callus development at the cambial contact, leaving a visible gap at the center of the bud-union. Seedlings treated with vermicast exhibited superior bud-union proliferation compared to those fertilized with ammonium phosphate. Additionally, chicken manure application led to greater callus formation at cambial contacts than the control. By 28 d after budding, cross-sections of all treated seedlings displayed an almost complete bud-union, regardless of treatment, indicating successful budding.

Introduction

Rubber (*Hevea brasiliensis* Muell. Arg) is an economically important perennial plant, primarily valued for its sap-like extract known as latex or natural rubber (Venkatachalam et al., 2006). This natural product has a wide range of industrial, technological, and domestic applications. Its unique technical properties make it preferable over synthetic rubber for various uses (Cornish, 2014). The market for natural rubber remains robust and attractive due to the ever-increasing demand for rubber-based products. In the Philippines, rubber production is considered one of the most profitable agri-industrial ventures (Sappalani et al., 2021). The potential of the natural rubber industry in the Philippines can be assessed through domestic and export market requirements. Furthermore, with the continuous rise in rubber prices on the global market and the presence of vast, highly suitable areas for rubber cultivation—

particularly in Tawi-Tawi—farmers are increasingly encouraged to engage in rubber tree planting. However, one of the key constraints in expanding rubber production is the limited availability of planting materials, especially in areas undergoing agricultural conversion for rubber farming. Commercial rubber propagation is primarily done through budding, a technique widely practiced by local nursery operators. The success of budding for vegetative multiplication depends on the appropriate physiological stage of both green and brown budwood, as well as the condition of the rootstocks, to ensure high bud-take rates (Sappalani, 2020). Some propagators have reported that defoliating budwood one to two weeks before budding can enhance budding success by promoting faster stock-scion union and encouraging early bud sprouting (LT Dayondon & Silao, personal communication, November 20, 2011). However, scientific studies validating this practice remain limited. Currently, there is a lack of studies and available information on the role of nutrient application in the budding success of rubber and its developmental processes leading to complete bud-take in seedlings. This gap in knowledge highlights the necessity of conducting further research on rubber propagation under local conditions. To date, no studies have specifically evaluated how budwood defoliation and fertilizer application influence budding success in rubber rootstocks. Fertilization—whether chemical, organic, or biological—has been shown to significantly impact plant growth performance and developmental processes (Serri et al., 2021; Ebrahimi et al., 2021). However, while budwood defoliation is a common practice among local rubber seedling propagators, the precise timing for optimal scion production has not yet been established in the region. Given these considerations, this study was conducted to determine the effects of budwood defoliation and organic fertilizer application on the budding success of rubber seedlings. Furthermore, it sought to document the developmental processes contributing to higher bud-take rates, providing insights into best practices for improving rubber seedling propagation.

Material and methods

Field experiment

The experiment was conducted in a private nursery located in Dologon, Maramag, Bukidnon, Philippines. Rubber seeds of the RRIM 600 clone were used as planting materials in this study, as this clone is highly recommended for its superior latex yield and strong resistance to pests and diseases. The seeds were sourced from the Rubber Project of CMU-BMRDO, CMU, Musuan, Maramag, Bukidnon. The field experiment followed a 3 × 4 factorial arrangement in a Randomized Complete Block Design (RCBD). Factor A represented the defoliation duration, with three levels: A1 = no defoliation (control), A2 = one-week defoliation before budding (1WBB), and A3 = two-week defoliation before budding (2WBB). Factor B consisted of different fertilizer application rates: B1 = control (no fertilizer), B2 = 2 g hill^{-1} of ammonium phosphate (16-20-0), B3 = 10 g hill^{-1} of chicken manure, and B4 = vermicast (Table 1). Each treatment was replicated three times, with ten samples per replication.

Table 1. Treatment combinations for the field experiment.

Budwood defoliation (Factor A)	**Fertilizer treatment (Factor B)**	**Treatment combination**	**Treatment code**
No defoliation (A_1)	No fertilizer application (B_1)	A_1B_1	T_1
	2 g Ammonium phosphate (16-20-0) (B_2)	A_1B_2	T_2
	10 g Chicken manure (B_3)	A_1B_3	T_3
	10 g Vermicast (B_4)	A_1B_4	T_4
Defoliation	No fertilizer application (B_1)	A_2B_1	T_5
1 week before budding	2 g Ammonium phosphate (16-20-0) (B_2)	A_2B_2	T_6
(1WBB)	10 g Chicken manure (B_3)	A_2B_3	T_7
(A_2)	10 g Vermicast (B_4)	A_2B_4	T_8
Defoliation	No fertilizer application (B_1)	A_3B_1	T_9
2 weeks before budding	2 g Ammonium phosphate (16-20-0) (B_2)	A_3B_2	T_{10}
(2WBB)	10 g Chicken manure (B_3)	A_3B_3	T_{11}
(A_3)	10 g Vermicast (B_4)	A_3B_4	T_{12}

Regular and careful hand weeding was conducted at least twice a month using a trowel, while chemical herbicide was applied once a week. Rubber seedlings used as rootstocks were grown in the field and standardized based on their height and leaf color to ensure uniformity. Semimature budwoods (green-colored clone RRIM 600) were obtained from CMU-BMRDO. Defoliation of budwood was performed one and two weeks before budding to induce dormancy, ensuring the buds were ready to sprout after budding. For non-defoliated budwood, leaves were removed only at the time of budding. Both defoliated and non-defoliated budwood were collected early in the morning on the day of budding. To protect the bud eyes from physical damage during transport, the budwood was wrapped in banana bracts. The green budding method (Marattukalam and Saraswathyamma, 1992) was employed in this experiment. Budding was performed approximately six inches above the ground on the rootstock seedlings. Two vertical, parallel cuts, each about two inches long, were made on the rootstock. The upper and lower portions of these cuts were then lifted using a budding knife and the forefinger, exposing the budding panel while leaving a one-centimeter tongue where the bud was inserted. The bud eye was carefully pulled and slipped off the budstick (budwood), aligning it with the incision on the rootstock. The green bud patch was then inserted beneath the flap of the stock. After insertion, the budded portion was wrapped tightly with polyethylene plastic strips in a spiral manner, starting from the budded area and moving upward, ensuring that the edges of the tape overlapped. Each budded seedling was tagged with the date of budding and its corresponding budwood defoliation treatment. The budded portion was opened 21 d after budding by loosening the polyethylene strips above and below the patch bud to expose it and allow shoot sprouting. Budded seedlings with green bud patches were considered successful, whereas those with brown bud patches indicated failure. Cutback was performed 7 d after opening for successful budded seedlings, at approximately five inches above the patch bud. After six weeks, successful budded seedlings were uprooted, and both lateral and taproots were pruned before transplanting them into polyethylene bags.

Morpho-anatomical development of bud-union

The morpho-anatomical microtechnique was conducted at the Agronomy Seed Laboratory and Veterinary Medicine Laboratory of Central Mindanao University (CMU) in Musuan, Maramag, Bukidnon. This experiment aimed to determine the time of completion and extent of bud-take or union development between the rootstock and scion of budded rubber seedlings using microtechnique. To assess bud union development, samples were collected at three intervals: 14, 21, and 28 d after budding. Three sets of budded rubber seedling samples were taken, ensuring that each sample was a healthy representative with minimal injury. The budded portions were cut into uniform two-inch segments and collected early in the morning. These samples were immediately labeled and placed in fixing solutions to prevent contamination. The collected samples were fixed in clean bottles containing FAA (formalin-alcohol-acetic acid) for four weeks to ensure thorough preservation and softening. To enhance fixation, 10 mL of glycerin was added per 50 mL of FAA solution. After the fixation period, samples were washed thoroughly in running water for 5 to 6 h to remove all traces of acid. Dehydration was carried out using an ethyl alcohol series: 30% and 50% for 30 min each, followed by 70%, 85%, 95%, and absolute 100% alcohol for one h at each concentration. The specimens were then cleared in a graded xylene series (25%, 50%, 75%, and 100%) with two changes per concentration for one h each. Following dehydration, the specimens were placed in bottles containing xylene and melted paraffin. The xylene was gradually decanted and replaced with melted soft paraffin (melting point: 42°C), and the specimens were maintained in an oven at 42°C for 12 h. The soft paraffin was then replaced with fresh melted soft paraffin and incubated at 42°C for an additional 24 h. Next, a 50:50 mixture of soft and hard paraffin was introduced, and the specimens were maintained in an oven at 57°C for 24 h. Finally, the specimens were embedded in pure hard paraffin at 57°C for another 24 h before embedding. For embedding, specimens were placed in hard paraffin within a paper boat coated with glycerin. The boats were floated in a basin of ice-cold water to promote rapid hardening and improve the transparency of the paraffin cake. Once hardened, the embedded specimens were trimmed to fit the microtome. Using a rotary microtome set at 10-micron thickness, the specimens were sectioned until good-quality ribbons were obtained. The ribboned sections were floated on warm water on slides pre-coated with Mayer's adhesive and mounted using a slide warmer. To ensure proper adhesion, slides were passed over a flame, and excess water was removed using absorbent tissue paper. The slides were then stored in a slide box for 2 d. To prepare the specimens for staining, the paraffin was removed by hydrating down to 100% xylene through a graded alcohol series. The slides were then stained with safranin for 48 h, followed by washing in running water to remove excess stain. Final dehydration was performed up to 95% ethanol before counterstaining with fast green. The slides were further dehydrated in absolute ethyl alcohol with two changes and cleared through a graded xylene series (50% to 100%) for about three min per step. Finally, the specimens were mounted using Canada balsam. A summarized flowchart of the procedures is presented in Figure 1.

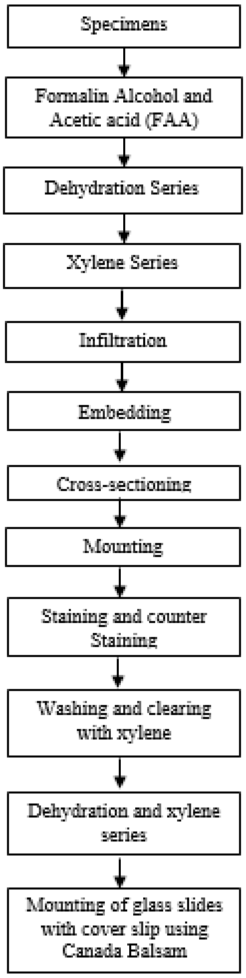


Fig. 1. Process flow for micro-technique procedure.

Data collection
The percentage of budding success was obtained after 60 d from operation. Success of budding was characterized by complete patch union as indicated by sprouting of the shoots after cutback. This was computed using the formula below:

$$Percent\ budding\ success = \frac{No.of\ successful\ budded\ seedlings}{Total\ no.of\ seedlings\ budded} \times 100$$

The average number of days to the sprouting of shoots was monitored daily and recorded from 30 sampled seedlings from each replication per treatment. Recognizable shoots served as the basis for recording the success and the computation of this parameter. The total number of leaves developed per budded seedling was counted from budding and at 30 d intervals until the termination of the study. The initial and final length of shoots of the budded seedlings was measured from the base to the terminal shoot using a meter stick. This was taken from 30 randomly sampled seedlings at the termination of the study. The difference in length was computed as the growth of scion bud and expressed as shoot length.

Statistical analysis
Data were subjected to Analysis of Variance (ANOVA). Significant differences among treatment means were compared using Duncan's Multiple Range Test (DMRT) using SPSS software.

Results
Percentage budding success
The percentage of budding success of 'RRIM 600' rubber in response to budwood defoliation and fertilizer application is presented in Table 2. Budding success at 60 d after budding was significantly influenced by both the independent and interaction effects of budwood defoliation and fertilizer application. Rubber seedlings that budded with scion buds from budwood defoliated two weeks before budding (2WBB). They exhibited the highest budding success rate of 71.15%, which was significantly higher than those from budwood defoliated one week before budding (1WBB) at 56.07%. In contrast, scions from non-defoliated budwood had the lowest budding success, with only 39.60%. The effect of fertilizer application on budding success also showed significant differences. Rootstocks treated with vermicast and chicken manure achieved statistically similar success rates of 69.72% and 63.24%, respectively—both notably higher than the control, which had a budding success rate of 43.66%. Meanwhile, the application of ammonium phosphate (16-20-0) resulted in a budding success rate of 45.80%, which was not significantly different from the control.

Results further showed that the highest budding success rate of 90.29% was achieved in rootstocks fertilized with vermicast and budded with scion budwood defoliated at 2WBB. Although this treatment had a relatively higher budding success rate compared to those fertilized with chicken manure (84.44%), the difference was not statistically significant. On the other hand, the unfertilized rootstocks with scions from non-defoliated budwood had the lowest budding success rate of 31.40%.

Number of days to sprouting of scion

Table 3 presents the significant variation in the number of days to scion sprouting from cutback in response to budwood defoliation and nutrient application. The results showed that defoliation at 2WBB led to the earliest shoot sprouting, occurring 12.83 d after cutback. This was followed by seedlings with scion buds from budwood defoliated one week before budding (13.83 d). In contrast, scions from non-defoliated budwood exhibited significantly delayed growth, sprouting at 15.33 d. Furthermore, the application of chicken manure and vermicast to rubber seedlings significantly promoted earlier sprouting of scion buds, occurring between 12.11 and 13.11 d after cutback, respectively.

Table 2. Percentage of budding success of rubber seedlings in response to budwood defoliation and nutrient application.

Defoliation	Fertilizer treatment				MEAN
	No fertilizer	16-20-0	Chicken manure	Vermicast	
Non-defoliated	31.40[f]	33.11[f]	45.01[e]	48.89[e]	39.60[c]
1WBB	46.12[de]	47.93[de]	60.26[c]	69.97[b]	56.07[b]
2WBB	53.48[cd]	56.38[c]	84.44[a]	90.29[a]	71.15[a]
MEAN	43.66[b]	45.80[b]	63.24[a]	69.72[a]	

CV= 7.55%. Means within the same columns and rows followed by a common letter are not significantly different at 5% level DMRT.

Table 3. Number of days to sprouting of shoots from cutback in response to budwood defoliation and nutrient application.

Defoliation	Fertilizer treatment				MEAN
	No fertilizer	16-20-0	Chicken manure	Vermicast	
Non-defoliated	18.33	15.67	14.33	13.00	15.33[a]
1WBB	15.33	14.33	13.00	12.67	13.83[b]
2WBB	14.33	14.33	12.00	10.67	12.83[c]
MEAN	16.00[a]	14.78[b]	13.11[c]	12.11[c]	

CV= 8.03%. Means within the same column and row followed by a common letter are not significantly different at 5% level DMRT.

Seedlings treated with 16-20-0 took 14.77 d to sprout, while the control group exhibited delayed shoot emergence at 15.33 d. Although there were no significant interaction effects between the factors, it was observed that defoliation of budwood two weeks before budding, combined with the application of vermicast on rootstocks, accelerated bud sprouting, with the earliest sprouting occurring two weeks before budding.

Number of leaves per budded seedling

The number of leaves per budded rubber seedling in response to budwood defoliation and nutrient application is shown in Table 4. The results revealed that both defoliation and nutrient application significantly influenced this parameter. However, no interaction effects between these two factors were observed. Rubber seedlings budded from budwood defoliated two weeks before budding (2WBB) produced the highest number of leaves, with an average of 22.40, which was comparable to the number of leaves produced by seedlings budded from budwood defoliated one week before budding (1WBB), which developed 21.07 leaves. Similarly, seedlings budded with scions from non-defoliated budwood had a statistically similar number of leaves to those budded with one-week defoliated budwood. Among the different fertilizer treatments, seedlings that received vermicast application on their rootstocks developed the highest number of leaves (22.93), followed by those treated with chicken manure (21.70) and ammonium phosphate (16-20-0) (20.46 leaves). The control group had the least number of leaves, with an average of 19.68.

Length of scion shoot

Table 5 presents the length of scion shoots of budded seedlings in response to budwood defoliation and nutrient application. Statistical analysis indicated that both budwood defoliation and nutrient application significantly influenced this parameter. Defoliation at 1WBB resulted in the longest scion shoot, measuring 18.10 cm, which was comparable to the length of shoots from budwood defoliated at 2WBB (17.85 cm). The shortest scion shoots were observed in seedlings budded with scions from non-defoliated budwood, with a length of only 15.39 cm.

Table 4. Number of leaves per budded rubber seedlings in response to budwood defoliation and nutrient application.

Defoliation	Fertilizer treatment				MEAN
	No fertilizer	16-20-0	Chicken manure	Vermicast	
Non-defoliated	18.67	19.67	20.93	21.50	20.11^{b}
1WBB	19.10	20.53	21.70	22.97	21.07^{ab}
2WBB	21.30	21.50	22.47	24.33	22.40^{a}
MEAN	19.69^{c}	20.47^{bc}	21.70^{ab}	22.93^{a}	

CV= 8.03%. Means within the same column and row followed by a common letter are not significantly different at 5% level DMRT.

Table 5. Length of shoots (cm) at 60 d after cutback of budded rubber seedlings in response to budwood defoliation and nutrient application.

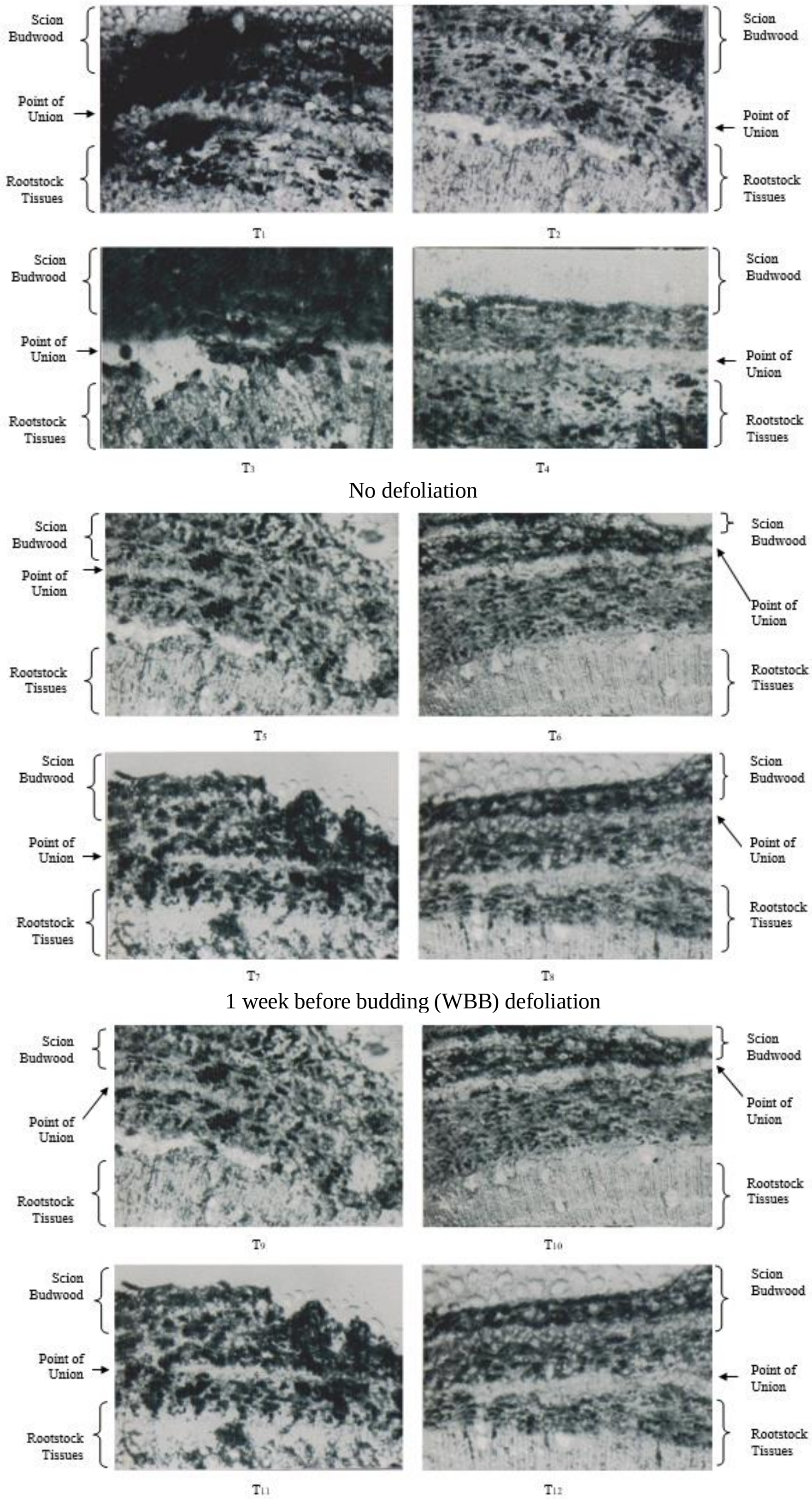
Defoliation	Fertilizer treatment				MEAN
	No fertilizer	16-20-0	Chicken manure	Vermicast	
Non-defoliated	14.10	14.98	15.93	16.57	15.39^{b}
1WBB	15.96	17.93	18.53	19.96	18.10^{a}
2WBB	16.17	16.43	18.77	20.07	17.85^{a}
MEAN	15.41^{c}	16.44^{bc}	17.74^{ab}	18.87^{a}	

CV= 7.98%. Means within the same column and row followed by a common letter are not significantly different at 5% level DMRT.

Seedlings treated with chicken manure and vermicast developed longer scion shoots, measuring 17.74 cm and 18.87 cm, respectively. This was followed by seedlings fertilized with 16-20-0, which had a mean shoot length of 16.44 cm. Unfertilized seedlings had the shortest scion shoots, measuring 15.39 cm, although this was not significantly different from the effect of 16-20-0. However, no significant interactions were observed between these two factors.

Morphoanatomical development of bud-union of rubber

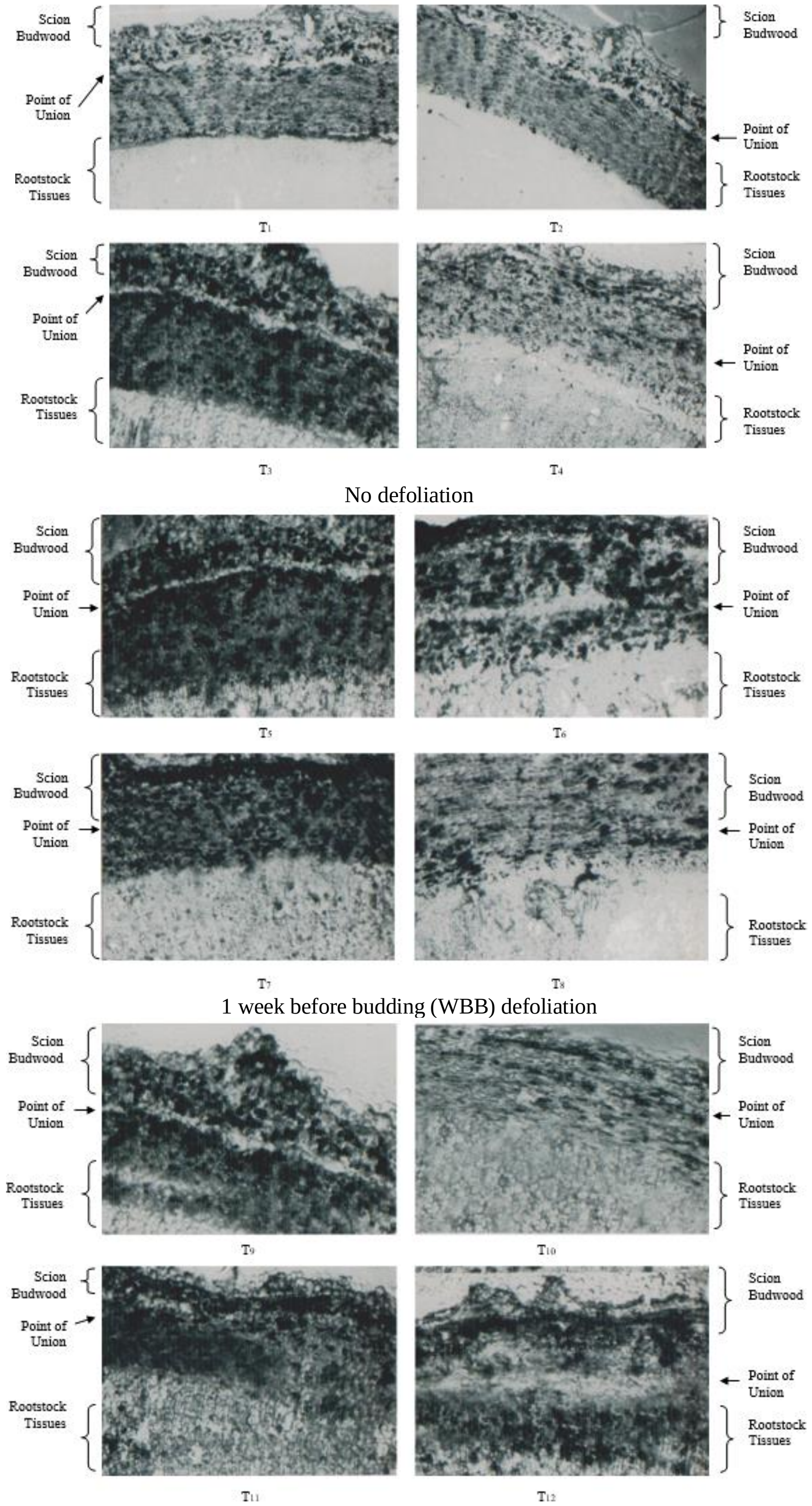
Morpho-anatomical studies of the bud-union in rubber seedlings at 14, 21, and 28 d after budding, in response to budwood defoliation and nutrient application, exhibited varying degrees of bud-union development (Figs. 2–4). At 14 d after budding, cross-sectioned samples showed partial callus formation at both ends of the rootstock's cambial contact, leaving a noticeable gap at the center of the bud-union (Fig. 2). However, for budwood defoliated at 1WBB, the gap at the center of the bud-union was smaller. Budwood defoliated two weeks before budding exhibited more profuse callus formation and a better degree of union, leaving a slight demarcation line at the middle zone of both the scion bud and rootstock, covered by new calli. Cross-sections of budded rootstocks treated with vermicast showed rapid proliferation, followed by those treated with ammonium phosphate. Seedlings fertilized with chicken manure also exhibited more callus formation at the cambial contacts than the control group. A similar trend was observed at 21 d after budding (Fig. 3). The cross-section of the bud-union showed increased callus formation at both sides of the cambial contacts, where cell dovetailing initially occurred. New calli were produced in the middle zones in response to wounding, although slight gaps remained at the center of the bud-union, with cells that had shrunk or died due to the budding operation. At 28 d after budding, cross-sections of the budded rubber seedlings showed nearly complete bud-union and dovetailing of both rootstock and scion tissues (Fig. 4). Scion buds from defoliated (1WBB and 2WBB) budwood, fertilized with vermicast or chicken manure, exhibited more active callus bridging the middle zone gap, with rapid dovetailing at both sides of the cambial contact of the rootstocks.



2 WBB defoliation

T1, T5, T9: No fertilizer applied; T2, T6, T10: 2 g ammonium phosphate; T3, T7, T11: 10 g chicken manure; T4, T8, T12: 10 g vermicast

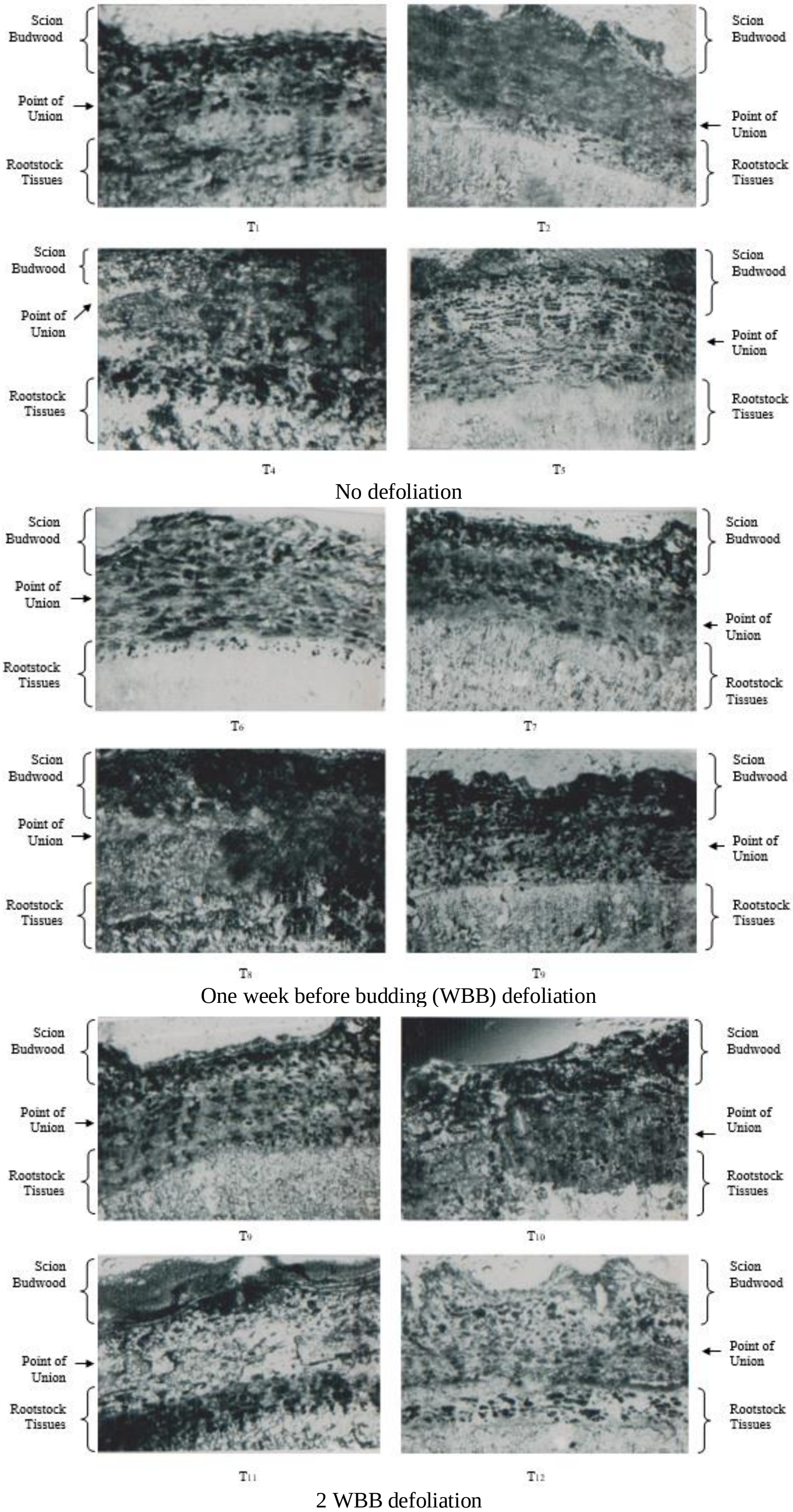
Fig. 2. Cross-section of budded rubber seedlings utilizing scions defoliated at different times showing the degree of union 14 d after budding operation.



2 WBB defoliation

T1, T5, T9: No fertilizer applied; T2, T6, T10: 2 g ammonium phosphate; T3, T7, T11: 10 g chicken manure; T4, T8, T12: 10 g vermicast

Fig. 3. Cross-section of budded rubber seedlings utilizing scions defoliated at different times showing the degree of union 21 d after budding operation.



T1, T5, T9: No fertilizer applied; T2, T6, T10: 2 g ammonium phosphate; T3, T7, T11: 10 g chicken manure; T4, T8, T12: 10 g vermicast

Fig. 4. Cross-section of budded rubber seedlings utilizing scions defoliated at different times showing the degree of union 28 d after budding operation.

Discussion

The use of grafting and budding methods plays a crucial role in the asexual propagation of tree species, particularly for those requiring efficient and reliable means of vegetative multiplication (Ebrahimi et al., 2007; Rezaee et al., 2008; Sadeghi-Majd et al., 2018; Thapa et al., 2021; Mostakhdemi et al., 2022). In addition to these methods, favorable environmental conditions are essential for optimal seedling growth and development, contributing significantly to the overall success of propagation practices (Sadeghi-Majd et al., 2022). In the case of rubber, the high success rates of budding on rootstocks using defoliated budwood can be attributed to several key physiological processes. Defoliation is believed to promote the accumulation of stored food reserves within the budwood, which induces bud dormancy while preparing the scion for active growth upon budding (El-Rouby et al., 2009). The removal of leaves from the budwood not only stimulates the accumulation of nutrients but also accelerates the healing of the budwood scar, thus reducing the risk of injury during subsequent budding operations. As a result, defoliated budwood swells in preparation for budding, fostering an ideal environment for the resumption of growth once the scion is grafted (El-Rouby et al., 2009). This phenomenon highlights the vital role of defoliation in preparing both the scion and bud for successful bud-take. In addition to defoliation, nutrient application plays a significant role in enhancing rootstock growth and ultimately improving the success of budding. Nutrients, particularly organic fertilizers such as chicken manure and vermicast, provide essential compounds that stimulate the vigorous growth of healthy rootstocks, which are foundational for successful propagation. Previous studies have demonstrated that the application of organic nutrients promotes more robust rootstock development by fostering an active growth stage in which the cambium cells are actively dividing, facilitating the easy detachment of the bark without damaging the scion bud (Aguirre et al., 2001). When seedlings are in a state of active growth, the cambial tissue is more pliable, and new tissues are readily formed, making the process of budding more successful (Munjuga et al., 2013). Furthermore, organic supplementation through nutrient sources like chicken manure and vermicast has been shown to promote rapid growth in nursery seedlings, especially in cases where the total replacement of chemical fertilizers with organic materials is employed (Hartman & Kester, 2010). This organic approach may further enhance the development of rootstocks by providing a balanced supply of nutrients conducive to the vigorous establishment of the seedlings. Additionally, defoliation of budwood before budding has been observed to improve budding success in various fruit tree species, such as mango, where defoliation allows for the accumulation of food reserves that support the development of vegetative parts (Akter et al., 2016; Wang et al., 2020). These findings corroborate the results of this study, where budwood defoliation contributed to enhanced scion sprouting and leaf formation, suggesting that such practices are integral to successful rubber tree propagation. Both defoliation and nutrient supplementation are critical to optimizing the success of budding and grafting methods. Defoliation aids in the preparation of budwood by accumulating reserves for bud sprouting, while nutrient application fosters healthy, vigorous rootstocks that facilitate successful bud-take. Collectively, these factors not only improve propagation efficiency but also reduce the time and costs associated with maintaining nursery seedlings, ultimately leading to more effective and sustainable propagation practices.

Conclusions

In this study, scion budwood defoliated two weeks prior to budding resulted in significantly higher budding success and improved growth of budded rubber seedlings. Additionally, rootstocks treated with vermicast or chicken manure demonstrated increased budding success, accelerated sprouting of scion shoots following cutback, and enhanced development of long scion shoots, accompanied by a greater number of leaves in the budded seedlings. The application of vermicast to rootstocks, coupled with scion budwood that underwent defoliation, was particularly effective in promoting rapid and complete bud union, achieving optimal bud integration within 28 d.

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Conflict of Interest
The author indicates no conflict of interest in this work.

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