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Optimization Grafting of *Durio zibethinus* using Various Scion Diameters and Hormone Levels

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ABSTRACT

The expansion of superior durian (*Durio zibethinus*) cultivation, native to Indonesia, is essential to meet the increasing demand in both domestic and international markets. Grafting is widely recognized as the most effective propagation method for large-scale seedling production. However, the availability of scion material from elite local durian trees is limited, as these trees generally produce thin branches located at heights exceeding 15 meters, making collection challenging. This study evaluated the effects of scion diameter and benzylaminopurine (BAP) concentration, as well as their interaction, on grafting success in superior local durian. A completely randomized factorial design was employed, consisting of three scion diameters (2, 3, and 4 mm) and four BAP concentrations (0, 75, 125, and 250 ppm), resulting in 12 treatment combinations, each replicated three times. The observed parameters included the number of live shoots, bud break time, number of leaves, and grafting and acclimatization success rates. Data were analyzed using two-way ANOVA at a significance level of $P < 0.05$, followed by an HSD test, while grafting and acclimatization success were assessed descriptively. The results showed that both scion diameter and BAP concentration had significant independent effects on bud break time and leaf count but not on the number of live shoots. Based on these findings, scion diameters of 3–4 mm combined with BAP concentrations of 75–125 ppm are recommended to optimize grafting outcomes in superior local durian propagation.

Introduction

Durian (*Durio zibethinus* Murr.) is a high-value tropical fruit with substantial consumer demand across Asia (Ali et al., 2020). China is currently the world's largest durian importer, sourcing primarily from Thailand and Malaysia, both of which export fresh whole fruits as well as frozen durian. In 2022, the Malaysian durian industry was valued at USD 23.23 billion, with projections indicating growth to USD 46.35 billion by 2023. Although global market demand continues to increase, durian production remains inconsistent and subject to fluctuations (Ali et al., 2020). The industry faces several persistent challenges, including reliance on traditional cultivation methods, limited utilization of elite germplasm, and the highly perishable nature of the fruit.

Indonesia, despite being one of the leading durian-producing countries, lags behind Thailand and Malaysia in exports to China. Although national production rose to 1.96 million tons in 2024 (BPS, 2025), domestic supply currently meets only about 65% of national demand. Addressing both the domestic shortfall and the need to enhance export competitiveness requires a strategic expansion of cultivation areas using locally adapted superior durian varieties. Such efforts for expansion should be integrated into broader fruit industry development initiatives.

Vegetative propagation through grafting represents a viable strategy for rapidly producing large quantities of high-quality, true-to-type durian seedlings. Grafting offers several agronomic advantages: bypassing the juvenile phase, enabling canopy architecture management, enhancing productivity, and improving tolerance to abiotic stress as well as resistance to pests and diseases. In many horticultural crops, grafting success rates approach 100% (Beshir et al., 2019). Its long-standing application, spanning more than 2,000 years, underscores its central role in the propagation of perennial fruit species, where high establishment and growth rates are crucial. Grafted plants consist of a scion, typically a twig with one or more dormant buds, joined to a compatible rootstock.

A major limitation in fruit crops, however, is the frequent failure of the scion–rootstock union, particularly when physiological or anatomical incompatibilities occur (Adhikari et al., 2022). The success of grafting is influenced by multiple interrelated factors, including scion–rootstock compatibility, choice of grafting technique, timing (season), and prevailing environmental conditions (Bhandari, 2021). Compared with herbaceous plants, grafting in woody perennials such as durian generally requires longer periods for vascular connection establishment and compatibility assessment, which may take several months to years. One crucial factor influencing grafting success is scion–rootstock diameter compatibility. Disparities in diameter may prevent proper alignment of vascular tissues, thereby impairing photoassimilate translocation and reducing lignification, ultimately leading to graft failure (Baron et al., 2019). Numerous studies have reported low grafting success rates when scion and rootstock diameters are mismatched (Rahmatika and Setyawan, 2019).

In durian propagation, scions are ideally collected from semi-mature twigs with a greenish-brown appearance, representing tissues that are neither too young nor senescent. In practice, however, the availability of high-quality scions from superior local durian varieties in Indonesia is limited. Many of these elite genotypes are seed-propagated trees over 50 years old, reaching heights of 25–30 m, with branching typically occurring only above 15 m. As a result, access to suitable scion material is restricted, and available twigs are often narrow, typically 2–3 mm in diameter, further complicating successful grafting. These limitations highlight the need to evaluate grafting performance using scions of varying diameters.

Pembengo et al. (2024) reported that optimal bud growth in durian occurs when scion diameters range from 4 to 6 mm. Under normal conditions, the first bud break in durian grafting is observed between 28 and 35 days after grafting (DAG) (Inderiati and Hambali, 2023). The application of plant growth regulators (PGRs) has been shown to accelerate this process, with cytokinins playing a particularly important role in stimulating cell division, promoting shoot development, and initiating bud break. Benzylaminopurine (BAP), a widely used synthetic cytokinin, has been shown to enhance these responses (Satriawan et al., 2021). Exogenous application of BAP has also been reported to increase expression of the *PsPIN1* gene, which regulates auxin redistribution, thereby promoting cell differentiation and facilitating earlier bud break.

Scion–rootstock incompatibility remains a significant barrier to successful grafting. This incompatibility can arise from factors such as genetic divergence, differences in physiological state, the extent and nature of lignification at the graft union, and metabolic disparities between scion and rootstock tissues. The success of graft union formation is regulated by the complex interplay of multiple plant hormones, including cytokinins, auxins, ethylene, gibberellins, abscisic acid, and jasmonic acid, which collectively coordinate the physiological and biochemical processes of graft healing and vascular reconnection.

Despite the potential importance of these factors, studies investigating the combined effects of scion diameter and BAP concentration on durian grafting success remain limited. Therefore, this study aimed to evaluate the individual and interactive effects of scion diameter and exogenous BAP application on graft performance, using multiple indicators of success. The ultimate goal is to identify optimal scion diameter–BAP concentration combinations that significantly enhance grafting success rates. By enabling the large-scale and accelerated production of superior local durian seedlings, this approach is expected to contribute to the expansion of superior durian populations, thereby supporting domestic supply and strengthening the competitiveness of the Indonesian durian industry in export markets.

Materials and Methods

This experiment was conducted in the Durian Nursery Greenhouse at Semarang State University (7° N latitude, 110° E longitude) from May to December 2023. The site is located approximately 300 m above sea level and is characterized by high humidity (~74%), low light intensity (400 µmol m^{-2} s^{-1}), and an average air temperature of 29.4 °C. Rootstocks were selected from durian seedlings approximately three months old, with heights ranging from 30 to 50 cm. Selection criteria included sturdy epicotyls, healthy and vigorous leaves, and the absence of pests and diseases. Scions were sourced from duplicate trees of the superior local durian cultivar ‘Malika’. To minimize transpiration and maintain freshness, scion collection was performed before 7:30 AM. Only twigs with two dormant buds were selected, and scion diameter was measured at the midpoint using a caliper. The study employed a two-factor Completely Randomized Design (CRD) in a factorial arrangement. The first factor was scion diameter (D) with three levels: 2 mm (D1), 3 mm (D2), and 4 mm (D3). The second factor was BAP (Benzyl Amino Purine) concentration (K) with four levels: 0 ppm (K0), 75 ppm (K1), 125 ppm (K2), and 250 ppm (K3). This resulted in 12 treatment combinations: D1K0, D1K1, D1K2, D1K3, D2K0, D2K1, D2K2, D2K3, D3K0, D3K1, D3K2, and D3K3. Each treatment was replicated three times. Splice grafting was used to unite the scion and rootstock (Fig. 1). The rootstock was cut at the top, leaving approximately 10 cm of stem (epicotyl), which was then vertically split. The scion was prepared by making 1 cm longitudinal cuts on both lower sides, allowing it to be inserted into the split rootstock. The graft union was secured using 0.08 µm polyethylene (PE) plastic tape. Grafted seedlings were enclosed in transparent plastic covers to minimize water loss and maintain high humidity around the graft union. Labels measuring 10 cm × 2.5 cm were prepared using waterproof paper to identify each treatment group.

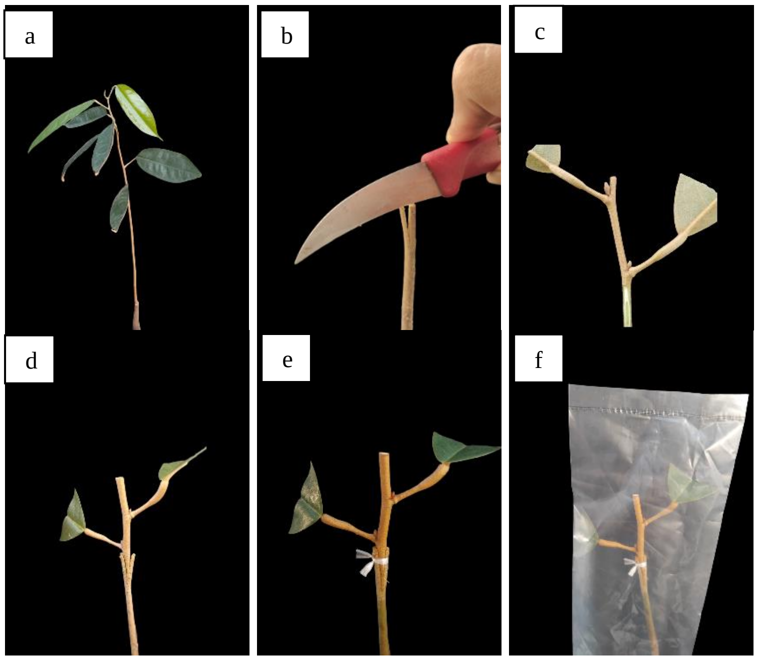

Fig. 1. Stages of the grafting process on durian seedlings. (a) Preparation of rootstock; (b) Making a slit at the middle of the rootstock; (c and d) Inserting scions into the rootstock; (e) Binding the graft union with grafting plastic; (f) scions and rootstocks are covered using plastic.

Statistical analysis

The observed parameters included the number of living buds, bud break time, number of leaves, and the percentage of grafting and acclimatization success. Quantitative data on the number of living buds, bud break time, and number of leaves were analyzed using two-way analysis of variance (ANOVA) at a 5% significance level with SPSS Statistics version 26.0. When significant effects were detected, treatment means were compared using the Honest Significant Difference (HSD) test at $P < 0.05$. The percentages of grafting and acclimatization success were assessed qualitatively and descriptively.

Results

Table 1 summarizes the effects of the treatments on the number of living buds, bud break time, and leaf count, while Figure 2 depicts the corresponding grafting and acclimatization success rates for each treatment combination.

Number of live buds

All treatment groups produced a similar number of living buds. Two-way ANOVA revealed no significant effects of scion diameter (D), BAP concentration (K), or their interaction (D × K) on this parameter, as all significance values exceeded 0.05. Consistently, the HSD test confirmed the absence of significant differences among treatments. These findings indicate that neither scion diameter nor BAP concentration, individually or in combination, influenced bud induction, with all treatment combinations yielding similar outcomes. This suggests that other physiological or environmental factors not assessed in this study may play a more decisive role in determining the number of living buds. The number of live buds was determined as the average count of scions that had broken dormancy (Fig. 3).

Table 1. Average number of live shoots, time of bud break, and number of leaves in the treatment of scion diameter and BAP hormone concentration.

Statistical Result	Parameter		
	Number of Live Buds	**Bud Break Time**	**Number of Leaves**
ANOVA (F/Sig)			
D (Scion Diameter)	3,167/0,060^{ns}	36,810/0,000*	7,000/0,000*
K (BAP Concentration)	0,444/0,723^{ns}	52,080/0,000*	9,540/0,000*
D×K	0,944/0,482^{ns}	2,450/0,050^{ns}	1,460/0,230^{ns}
HSD Test			
D1 (Scion Diameter 2 mm)	(1.58±0,51)	(21,42±6,12)^{a}	(2,50±1,24)^{b}
D2 (Scion Diameter 3 mm)	(1.75±0,45)	(18,67±5,71)^{b}	(3,50±1,51)^{ab}
D3 (Scion Diameter 4 mm)	(2.00±0,00)	(14,00±3,33)^{c}	(4,58±1,83)^{a}
K0 (BAP concentration zero ppm)	(1.78±0,44)	(24,00±5,57)^{a}	(2,55±0,88)^{b}
K1 (BAP concentration 75 ppm)	(1.89±0,33)	(16,11±3,79)^{c}	(3,56±1,33)^{ab}
K2 (BAP concentration 125 ppm)	(1.78±0,44)	(12,00±1,41)^{d}	(5,11±2,26)^{a}
K3 (BAP concentration 250 ppm)	(1.67±0,50)	(20,00±5,93)^{b}	(2,89±1,05)^{b}
D1K0	(1.33±0,57)	(28,33±1,53)	(2,00±0,00)
D1K1	(1.67±0,57)	(19,33±3,05)	(2,67±1,15)
D1K2	(1.67±0,57)	(13,33±1,15)	(3,33±2,31)
D1K3	(1.67±0,57)	(24.67±1,15)	(2,00±0,00)
D2K0	(2.00±0,00)	(26,00±3,60)	(2,00±0,00)
D2K1	(2.00±0,00)	(16.67±2,88)	(4,00±2,00)
D2K2	(1.67±0,57)	(12,00±1,00)	(4,67±1,15)
D2K3	(1.33±0,57)	(20,00±1,00)	(3,33±1,15)
D3K0	(2.00±0,00)	(17,67±3,78)	(3,67±1,15)
D3K1	(2.00±0,00)	(12.33±1,52)	(4,00±0,00)
D3K2	(2.00±0,00)	(10,67±0,57)	(7,33±1,15)
D3K3	(2.00±0,00)	(15,33±0,57)	(3,33±1,15)

Note: ^{ns} = not significant, * = significant.

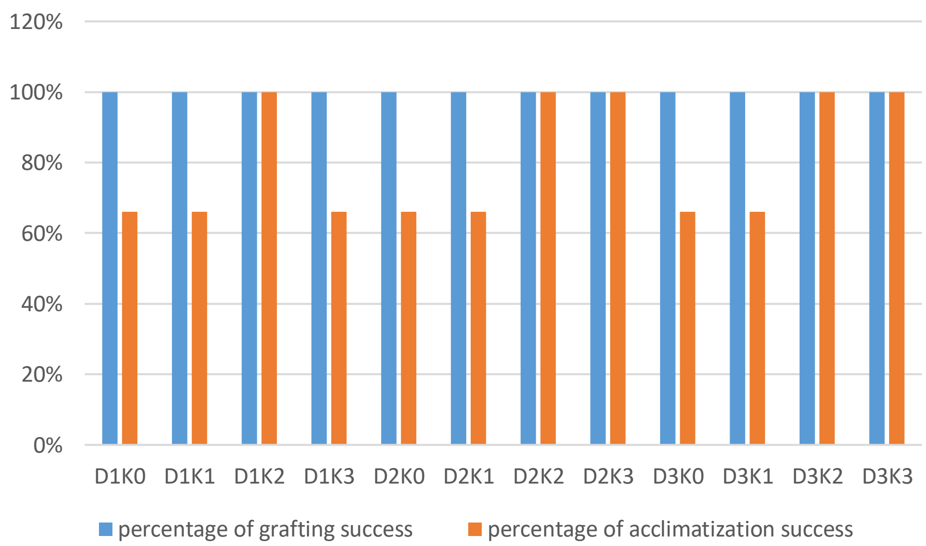

Fig. 2. Percentage of grafting and acclimatization success in the treatment of scion diameter and BAP hormone concentration.

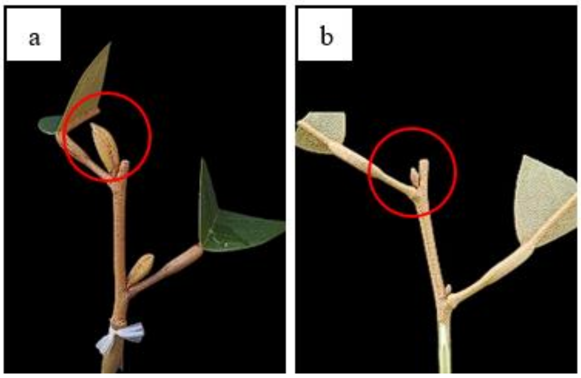

Fig. 3. Durian buds before and after grafting. (a) Buds before dormancy break; (b) Buds still in dormancy before bud break.

Bud break time

Two-way ANOVA indicated that the interaction between scion diameter and BAP concentration (D × K) had no significant effect on bud break time ($P > 0.05$). However, both factors individually exerted significant effects. The fastest bud break was observed in scions with a 4 mm diameter and when BAP was applied at 125 ppm. Scions of 4 mm diameter broke buds earlier than those of smaller diameters, likely due to their greater carbohydrate and nutrient reserves, which support early bud development. Increasing BAP concentration up to 125 ppm accelerated bud break, whereas treatment at 250 ppm delayed bud break to a level comparable with the untreated control (0 ppm). These results suggest that while BAP promotes bud break by stimulating cell division and differentiation, its effects are concentration-dependent, with reduced efficacy or possible inhibitory effects at higher levels. Although the interaction between scion diameter and BAP concentration was not statistically significant, the combination of 4 mm scions with 125 ppm BAP was individually the most effective in promoting early bud emergence.

Number of leaves

Two-way ANOVA revealed that both scion diameter and BAP concentration had significant effects on leaf count ($P < 0.05$), whereas their interaction was not significant ($P > 0.05$). The HSD test indicated that BAP at 125 ppm produced the highest leaf count, although this value did not differ significantly from 75 ppm. Similarly, scions with a diameter of 4 mm yielded the greatest number of leaves, but the difference was not statistically significant compared with 3 mm scions. Considering the importance of cost efficiency in large-scale graft propagation, 75 ppm BAP can be regarded as the most economical treatment, as it provides leaf development comparable to 125 ppm. Although 4 mm scions exhibited the best performance, scions of 2 mm and 3 mm diameters remain viable alternatives, particularly when combined with appropriate post-grafting maintenance to support vegetative growth. This flexibility is advantageous for optimizing the use of available scion material in commercial nurseries.

Percentage of grafting and acclimatization success

The grafting and acclimatization success rates of the grafted durian seedlings are presented in Figure 2. Across all treatments, regardless of scion diameter or BAP concentration, a 100% grafting success rate was achieved. Success was visually confirmed by bud break at 35 days after grafting (DAG). Grafting success was calculated as the number of seedlings exhibiting bud break divided by the total number of grafted seedlings, multiplied by 100%. These results demonstrate that grafting success was not significantly influenced by scion diameter or BAP concentration. Notably, scions of 2 mm diameter performed as effectively as larger scions, confirming their suitability for grafting. This finding is particularly important given the limited availability of superior local durian scion material in Indonesia. The ability to utilize a wider range of scion diameters (2, 3, and 4 mm) provides a practical solution to scion shortages, thereby supporting the intensification of superior durian plantations and facilitating the mass production of high-quality grafted seedlings for both domestic and export markets.

Acclimatization, defined as the initial adaptation of grafted seedlings to ambient environmental conditions following the removal of grafting ties and protective covers, was evaluated one week after cover removal, corresponding to 49 DAG. Acclimatization success was calculated as the number of surviving grafted seedlings divided by the total number of grafted seedlings, multiplied by 100%. Results revealed substantial variation among treatment combinations (Fig. 2). Specifically, the combinations of 2 mm scions with 75 ppm BAP (D1K2), 3 mm scions with 75 ppm BAP (D2K2), 3 mm scions with 125 ppm BAP (D2K3), 4 mm scions with 75 ppm BAP (D3K2), and 4 mm scions with 250 ppm BAP (D3K3) each achieved 100% acclimatization success. By contrast, other scion diameter and BAP concentration combinations yielded survival rates of only 65%.

These findings suggest that scions ranging from 2 to 4 mm in diameter, when combined with BAP concentrations of 75–250 ppm, promote early compatibility by two months after grafting. Moreover, BAP concentrations of 125 and 250 ppm consistently enhanced acclimatization success across all scion sizes, indicating a positive role of the hormone in improving post-graft seedling survival.

Discussion

Number of live buds

The number of live buds did not differ significantly across scion diameters, BAP concentrations, or their interaction. Statistical analysis indicated that all treatments yielded a comparable number of live buds, suggesting that factors other than scion diameter and hormone concentration likely influence bud survival. Bud dormancy represents a key physiological stage in perennial fruit plants, regulated by both internal and external cues. External factors regulate endodormancy through mechanisms such as cold-responsive gene expression and cold accumulation, whereas internal factors govern ecodormancy, which is influenced by heat accumulation and photoperiod (Zhang et al., 2021). In tropical regions such as Indonesia, where day length remains relatively constant throughout the year, photoperiod does not serve as a major environmental signal. Instead, fluctuations in daily solar radiation primarily affect bud survival and development in tropical trees (Borchert et al., 2015). At the study site, bud dormancy release in grafted seedlings was primarily attributed to the successful union between scion and rootstock, as environmental conditions such as temperature (averaging 29.4 °C) and low light intensity remained relatively stable. Notably, low light intensity has been reported to negatively influence the number of live buds in tropical plants, which may explain the uniformity in bud survival across treatments. The unions between scions of 2, 3, and 4 mm diameter and rootstocks were technically successful across all tested concentrations of 6-benzylaminopurine (BAP)—0 ppm (control), 75, 125, and 250 ppm.

The grafter's technical skill is a key determinant of graft success, as it directly affects the survival of viable buds. Another important factor is the degree of genetic relatedness between scion and rootstock (Promsakul et al., 2024). In this study, both scions and rootstocks belonged to the same species, Durio zibethinus, which likely contributed to the consistently high graft success rate. Rootstocks were raised from randomly selected D. zibethinus seeds, while scions were sourced from the cultivar Malika. Incompatibility between scion and rootstock is a major limitation in fruit tree propagation, as it can impair scion growth. Previous studies on the Chanee and Monthong durian cultivars grafted onto local D. zibethinus rootstocks have reported high bud viability, increased stem diameter, enhanced graft union thickness, and greater leaf production (Promsakul et al., 2024), underscoring the importance of genetic compatibility in early graft development.

Water availability is another important factor influencing the survival of grafted buds. In this study, irrigation was applied uniformly and periodically across all treatments, minimizing water as a variable affecting bud viability. Adequate water supply is crucial for bud development and the release of dormancy; mature durian trees require sufficient moisture to effectively break bud dormancy (Eguchi et al., 2025). Future research could investigate the use of controlled water stress techniques to stimulate dormancy release in grafted seedlings, drawing from findings in mature durian trees. Cytokinins are a class of phytohormones that play a significant role in breaking bud dormancy, particularly in tropical species (Bound et al., 2022), by downregulating *TgBRC1* expression to release axillary bud dormancy. In the present study, the lack of significant differences in bud count among the control and BAP-treated groups may be attributed to the presence of sufficient endogenous cytokinins. These hormones are involved in various developmental processes, including cell division, lateral root formation, meristem maintenance, and cambial activity. Low cytokinin levels inhibit cambial cell division, leading to reduced stem thickening and limited bud dormancy release. Conversely, elevated cytokinin biosynthesis enhances cambial cell division (Immanen et al., 2016). Grafting, which involves wounding, stimulates cytokinin biosynthesis and signalling responses (Ikeuchi et al., 2017). Wound-induced callus formation is mediated by the transcription factor *WOUND INDUCED DEDIFFERENTIATION 1* (*WIND1*), which activates cytokinin signalling. Cytokinin deficiency impairs callus formation, suggesting that cytokinin signalling is essential for wound-induced tissue regeneration. This signalling pathway is particularly important in the context of grafting. Together, endogenous and exogenous cytokinins contribute significantly to the formation of a successful scion-rootstock union.

Bud break time

This study demonstrated that both scion diameter and BAP (6-benzylaminopurine) concentration significantly influence the timing of bud break. Scions with a 4 mm diameter combined with a BAP concentration of 125 ppm exhibited the fastest bud break. The rate of bud emergence increased with BAP concentration up to 125 ppm, then declined at 250 ppm, a trend consistent with findings by Ramayana et al. (2022). Bud break can also be promoted by low-temperature conditioning; extended cooling periods have been shown to accelerate bud break (Chen et al., 2023). Consequently, maintaining air temperatures below 28 °C at the grafting site may enhance bud emergence. Grafting propagation sites situated at elevations above 600 m above sea level are recommended to take advantage of naturally cooler conditions that support faster bud break. These results may serve as a foundation for developing grafting schedules under controlled temperature regimes. As buds begin to develop, sugar content in the scion increases (Fermaniuk et al., 2021). Sugars provide essential energy and carbon sources, including precursors for cell wall biosynthesis, thereby supporting bud growth. Moreover, sugars suppress *BRC1* gene expression and indirectly regulate cytokinin biosynthesis, both of which contribute to bud dormancy release.

Scion diameter is positively associated with plant vigor, and larger scions tend to initiate bud break more rapidly (Montesinos et al., 2023). Vigor is typically correlated with stem diameter and the total cross-sectional area of vascular tissues, which together determine hydraulic conductivity. Greater hydraulic conductivity enhances water transport to developing buds, facilitating earlier bud break (Bester, 2020). The effect of BAP on bud break follows a concentration-dependent curve, with stimulatory effects up to an optimal level and inhibitory effects at higher concentrations (Bhattacharya, 2022). Exogenous cytokinins such as BAP promote bud break by upregulating genes related to sugar metabolism and auxin signaling. Cytokinin activity is regulated by the *PpEBB1* gene, which facilitates bud emergence (Zhao et al., 2020), and exogenous BAP enhances *PsPIN1* expression, promoting auxin redistribution and cell differentiation, thus accelerating bud break.

Scion health also plays a crucial role. Scions harvested from healthy source trees possess sufficient nutrients to support callus formation and cambial activity, both of which are essential for rapid bud emergence. In addition, rootstocks contribute by producing long-distance signaling molecules that stimulate bud outgrowth (Yan et al., 2020). Finally, environmental factors such as temperature and relative humidity further modulate bud break timing. Optimal conditions of approximately 25 °C and 75% relative humidity are recommended to promote bud emergence. These findings align with the recommendation by Cheng et al. (2024) to select cooler sites for graft propagation to accelerate bud break.

Number of leaves

Scion diameter and BAP concentration each had a significant effect on leaf count. The application of BAP at 125 ppm resulted in the highest mean leaf count, although this was not significantly different from the 75 ppm treatment. Likewise, scions with a diameter of 4 mm produced the greatest number of leaves, though the difference was not statistically significant when compared to scions with a 2 mm diameter. Figure 4 presents the leaf counts across the 12 treatment combinations. The control treatment (0 ppm BAP) consistently produced the lowest average leaf count, indicating a positive response to exogenous BAP application. The increase in leaf count with BAP application is likely due to enhanced differentiation of meristematic cells into leaf primordia, a process stimulated by cytokinins. Nonetheless, graft tissues inherently contain endogenous cytokinins, which may account for leaf formation even in the absence of BAP. Leaf development is tightly regulated by the hormonal interplay between cytokinins and auxins; therefore, suboptimal auxin levels may limit the full expression of cytokinin activity, even in the presence of exogenous BAP. Environmental conditions also likely played a role in modulating leaf development. The relatively low light intensity (400 µmol m^{-2} s^{-1}) and high humidity during the study may have suppressed photosynthetic activity and reduced transpiration rates, both of which are crucial for vegetative growth and leaf expansion. Although BAP significantly accelerated bud break, its influence on leaf formation was likely modulated by these environmental and hormonal interactions.

Cytokinin plays a central role in initiating leaf growth by regulating the light-dependent photosynthetic pathway. Under low light intensity, cytokinin signaling is diminished, which reduces sugar uptake in developing buds and consequently delays leaf initiation. Light not only modulates cytokinin activity but also influences the levels of other key hormones such as brassinosteroids, auxins, and gibberellins, which collectively regulate chloroplast biogenesis and early leaf development (Cackett et al., 2022). The optimal light intensity for initiating leaf development is reported to be approximately 500 µmol m^{-2} s^{-1} (Feng et al., 2024). In the present study, the average light intensity was 400 µmol m^{-2} s^{-1}, which is below this optimal threshold and likely contributed to the reduced leaf formation observed.

Although statistical analysis showed no significant effect of scion diameter on leaf count, scions with diameters of 2, 3, and 4 mm appeared to contain sufficient stored carbohydrates to support continued meristem activity and cell differentiation. Variability in bud break timing may also have influenced leaf count, as earlier bud emergence generally allows for earlier and potentially greater leaf development. Since new leaves arise from bud expansion, a direct relationship between bud break timing and leaf count is expected (Supriyanto et al., 2022).

Leaf formation is also dependent on nutrient availability, which in turn supports photosynthetic activity. The assimilates produced via photosynthesis are essential for cell proliferation and organogenesis during leaf development (Mehta et al., 2018). However, suboptimal environmental conditions, specifically low light intensity, high relative humidity, and elevated temperatures, can limit these processes. In this study, the relative humidity inside the plastic covers reached 99%, a condition that likely suppressed transpiration. Reduced transpiration can impair nutrient transport and gas exchange, leading to the desiccation of leaf primordia and failure of leaf development.

Fig. 4. Grafting success and number of leaves 42 d after grafting (DAG). (a) D3K3; (b) D3K2; (c) D3K1; (d) D3K0; (e) D2K3; (f) D2K2; (g) D2K1; (h) D2K0; (i) D1K3; (j) D1K3; (k) D1K1; (l) D1K0. Note: D1: Scion diameter 4 mm, D2: Scion diameter 3 mm, D3: Scion diameter 2 mm, K1: BAP concentration zero ppm, K2: BAP concentration 75 ppm, K3: BAP concentration 125 ppm, K4: BAP concentration 250 ppm.

Grafting and acclimatization success percentage

Variations in scion diameter and BAP concentration in this study did not affect the percentage of grafting success, with all treatments exhibiting a 100% success rate by 35 d after grafting (DAG). This finding suggests that durian scions of 2, 3, or 4 mm in diameter are all suitable for use as grafting material. The success of grafting primarily depends on the physiological and genetic compatibility between scion and rootstock. Graft union formation is a complex, multi-stage process that includes the formation of a necrotic layer, cellular adhesion, callus proliferation, and the eventual development of a functional vascular system (Adhikari et al., 2022).

The necrotic layer forms immediately following tissue injury and serves as a protective barrier until the union is established. Cellular adhesion between the scion and rootstock typically occurs within 1 to 7 DAG, often accompanied by latex exudation from the cut rootstock (Loupit and Cookson, 2020). Callus formation, which begins around 14 DAG, results from localized cell proliferation and is one of the earliest indicators of a successful graft (Mo et al., 2018). The subsequent development of vascular connections within the callus tissue is essential for the survival and continued growth of the grafted seedling. Initially, these vascular connections appear as disorganized nodules rather than fully developed xylem and phloem (Fukuda and Ohashi-Ito, 2019). Complete vascular tissue formation, indicative of successful graft union, is typically observed around 30 DAG and is associated with the upregulation of genes involved in lignin biosynthesis (Mo et al., 2018).

Larger scion diameters are generally associated with enhanced grafting performance due to their higher meristematic cell regeneration capacity, which accelerates callus formation and vascular differentiation (Tanuja and Thippesha, 2016). Additionally, increased glucose reserves in larger scions may promote graft success by supporting metabolic activity and vascular tissue development (Melnyk, 2017). Glucose functions not only as a metabolic substrate but also as a signaling molecule that facilitates tissue differentiation at the graft interface.

Water availability also influences vascular tissue development. While low water conditions may reduce overall vascular width, they can increase vascular density, potentially improving stress resilience in grafted plants. For example, Locatelli et al. (2019) found that apple scions under water stress developed narrower but denser vascular tissue, contributing to improved tolerance.

The uniform grafting success observed in this study suggests strong early-stage compatibility between the scion (*D. zibethinus* var. Malika) and the rootstock (seed-derived *D. zibethinus*). Early compatibility is often attributed to close genetic relationships between graft partners (Hannweg et al., 2023). However, graft compatibility must be evaluated not only during the initial stages but also through the reproductive phase, as many grafts may perform well vegetatively but exhibit delayed incompatibility, manifesting in reduced flowering, fruiting, or long-term viability.

Exogenous cytokinin application, such as benzylaminopurine (BAP), can mitigate both biotic and abiotic stresses, thereby increasing the success rate of grafting (Mandal et al., 2022). BAP is commonly applied to facilitate stress recovery and stimulate the formation of new tissues at the graft union. Additionally, BAP has been reported to enhance vascular reconnection between scion and rootstock, a key determinant of graft compatibility and overall success. Interestingly, in this study, even the zero ppm BAP treatment resulted in successful vascular tissue formation, likely due to the presence of sufficient endogenous cytokinins within the grafting tissue (Hayat et al., 2023).

Environmental conditions during the grafting process, particularly temperature, relative humidity (RH), and light intensity, also play a crucial role in influencing grafting outcomes. The average temperature during grafting was maintained at 29.4 °C, within the optimal range of 28–30 °C reported by Tirupathamma et al. (2019). Relative humidity outside the grafting enclosure averaged 74%, while inside it reached 99%. These levels are favorable, as RH between 70 and 80% promotes vegetative growth and minimizes water stress. Although RH below 40% inhibits photosynthesis and overall plant growth, and RH above 85% may impair stomatal function, high humidity (90–100%) is required during early graft stages to promote callus formation and graft union success (Carvalho et al., 2015).

However, light intensity during the experiment averaged 400 µmol m^{-2} s^{-1}, slightly below the optimal range for photosynthetically active radiation. A light intensity of around 414 µmol m^{-2} s^{-1} has been reported to enhance carbohydrate accumulation, which supports cell division, elongation, and vascular tissue development, factors that collectively improve graft success (Bačáková et al., 2014).

Acclimatization success was assessed based on graft survival at 49 DAG. Failures observed in certain treatments were attributed to suboptimal environmental conditions or mechanical damage during transfer to new polybags. In particular, grafted seedlings with 2 mm scions may have had weaker vascular connections or underdeveloped root systems, making them more vulnerable during acclimatization (Baron et al., 2019). Graft incompatibility can be ruled out in this case, as both the scion and rootstock were from the same species, *Durio zibethinus* (Sunaryo et al., 2019). Nonetheless, a temperature increase to 33 °C during the final stages of acclimatization may have introduced thermal stress, thereby reducing the survival rate in some treatments.

Failure during the acclimatization phase was not attributed to initial incompatibility between scion and rootstock. However, for long-term graft performance, comprehensive compatibility assessment remains crucial. Graft incompatibility can manifest in two primary forms: translocation and localized, which may occur independently or simultaneously (Reig et al., 2018). Translocation-type incompatibility is typically characterized by symptoms such as leaf yellowing, premature leaf drop, stunted growth, and poorly developed root systems, all indicative of impaired translocation of water and nutrients (Fig. 5). In contrast, localized incompatibility, unobserved in this study, is defined by anatomical irregularities at the graft union. These include disrupted vascular connectivity, anomalous cambial alignment, and malformed vascular tissues, resulting from defective vascular differentiation and fusion (Li et al., 2021). Although initial compatibility appeared sufficient to support early graft development, ongoing anatomical and physiological assessments are necessary to detect delayed or latent incompatibility symptoms that may emerge during the reproductive phase.

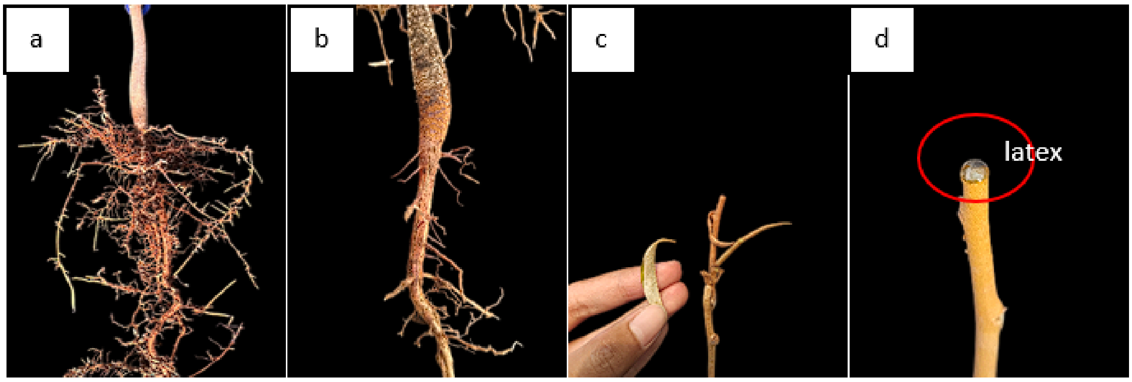

Fig. 5. Incompatibility of scion and rootstock. (a) normal roots; (b) partially developed roots; (c) premature defoliation; (d) Rootstock that exudes latex.

Variations in acclimatization success among grafted plants receiving the same treatment may be attributed to differences in the physiological or anatomical characteristics of individual rootstocks (Primo-Capella et al., 2022). Even within a genetically similar population, scion–rootstock compatibility can vary, leading to inconsistent responses. One key factor contributing to acclimatization failure is *root pressure*, which arises from high water retention and reduced transpiration. Fluctuating temperatures, such as cold nights followed by sunny days, can intensify root pressure (Sadeghi-Majd et al., 2022). Excessive root pressure may cause the rootstock to exude large amounts of latex. When this latex accumulates inside the sealed grafting plastic wrap, it can create a toxic microenvironment around the graft union, ultimately resulting in scion death. Another major cause of acclimatization failure in this study was poorly developed root architecture. Underdeveloped root systems are often associated with low cytokinin biosynthesis and inefficient cytokinin transport (Basedow, 2015). Such rootstocks require higher cytokinin levels to trigger bud break, which can delay or inhibit scion development. A compromised root system also limits water and nutrient uptake, contributing to scion desiccation and shrinkage. This physiological stress reduces glucose availability, thereby disrupting carbon allocation and energy metabolism, i.e., processes crucial for cell division and growth (Zhou and Underhill, 2022).

Acclimatization failure in grafted seedlings can also result from environmental stresses such as suboptimal light intensity, wind exposure, rainfall, and unsuitable soil pH (Hilleary and Gilroy, 2018). Light intensity that is either too high or too low can induce physiological stress in plants, impairing their ability to establish in new conditions (Chen et al., 2018). In this study, the average light intensity was 400 μmol m^{-2} s^{-1}, which is below the optimal threshold for efficient leaf development, potentially contributing to reduced acclimatization success. In addition, the growing medium had a pH of 7.5, which is considered too alkaline for optimal nutrient uptake. This high pH is likely due to elevated carbonate ion concentrations, which can induce iron deficiency in plants. Iron deficiency is typically manifested as interveinal chlorosis and a reduction in photosynthetic pigments, resulting in yellowed or chlorotic leaves (Fig. 6a–b) (Cimen and Yesiloglu, 2016).

A decline in photosynthetic pigment concentration is often associated with impaired chloroplast development. Chlorophyll biosynthesis can be positively influenced by exogenous application of BAP, which has been shown to enhance chlorophyll content in various plant species (Maxiselly et al., 2021). Since chloroplasts are central to photosynthesis and overall plant development, their dysfunction, whether due to nutrient deficiency or water imbalance, can result in growth inhibition during acclimatization (Werner and Schmülling, 2019).

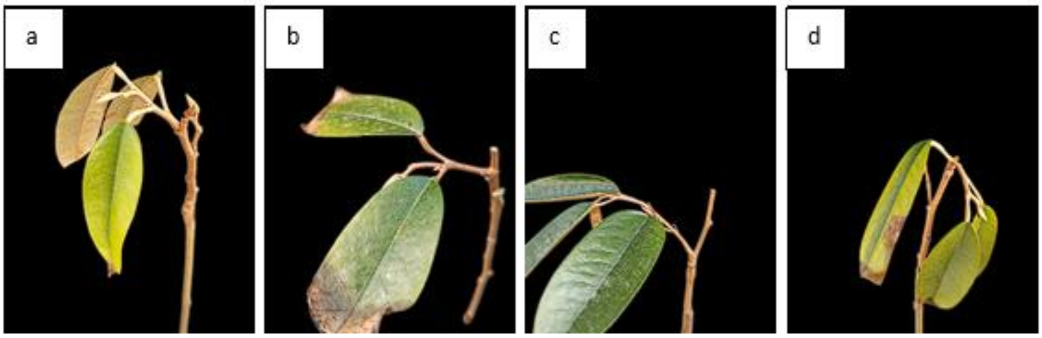

Fig. 6. Abiotic factors affecting graft incompatibility. (a) Grafting results with Fe deficiency; (b) Grafting results with Fe deficiency; (c) Normal grafting results; (d) Grafted plant that has broken.

Conclusion

Grafting is a widely used technique for the mass propagation of fruit seedlings with genotypes identical to their parent plants. In durian cultivation, one of the primary challenges is the limited availability of high-quality scion material from naturally growing, superior local *Pohon Induk Terpilih* (PIT). Investigating the effects of scion diameter and exogenous benzylaminopurine (BAP) application is essential for improving propagation efficiency and meeting the growing demand for superior native Indonesian durian cultivars. This study evaluated key parameters including the number of living shoots, bud break time, and leaf count through statistical analysis, while grafting and acclimatization success were assessed qualitatively and descriptively. A two-factor randomized complete block design (RCBD) was employed, incorporating three scion diameters (2, 3, and 4 mm) and four BAP concentrations (0, 75, 125, and 250 ppm), with three replicates per treatment. Results indicated that both scion diameter and BAP concentration significantly influenced bud break time and leaf count but had no significant effect on the number of living shoots in grafted seedlings of the superior local durian variety, Durian Malika. Based on these findings, the study recommends using scion diameters of 3–4 mm in combination with BAP concentrations of 75–125 ppm to optimize grafting success and promote early compatibility in the propagation of superior local durian cultivars.

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Author Contributions

UM conceived and designed the research, conducted the experiments, performed the data analysis, and prepared the original draft. AR, as the corresponding author, contributed to the research design and critically revised the manuscript. SA contributed to the analysis and interpretation of the experimental data. All authors have read and approved the final version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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