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Anatomical compatibility of avocado grafting at different scion growth stages

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Abstract. *Hidayat R, Da Costa AJS, Pikir JS, Romadhon MR, Talitha O, Fathin TS. 2026. Anatomical compatibility of avocado grafting at different scion growth stages. Asian J Agric 10 (1): g100131. https://doi.org/10.13057/asianjagric/g100131.* This research aimed to evaluate the effectiveness of three grafting methods: cleft, wedge, and splice combined with three scion physiological stages: flushing (10 days after shoot emergence), endodormancy (30 days), and ecodormancy (50 days). The experiment assessed graft success in terms of survival rate, bud break duration, leaf production, flush interval, and vascular tissue compatibility. The splice grafting method using scions at the ecodormancy stage yielded the highest graft survival (72.50%), significantly outperforming other combinations. This treatment also achieved the earliest bud break (6.17 days), produced the most leaves (8.92 leaves per scion), and exhibited the shortest flush interval. Microscopic analysis at 45 and 60 days after grafting (DAG), using an Olympus CX21 binocular microscope equipped with an Optilab camera, revealed superior tissue compatibility in this treatment, characterized by minimal necrosis and successful union of xylem and phloem between the rootstock and scion. It is the first study to integrate physiological scion staging with histological validation of graft compatibility in avocado (*Persea americana*). These findings demonstrate that splice grafting with ecodormant scions significantly enhances morphological performance and vascular integration, providing a practical and scientifically supported protocol for improving avocado propagation in tropical highland conditions.

Keywords: Anatomical, dormance, flushing, phloem, xylem

INTRODUCTION

The increasing demand for high-quality avocado fruit has encouraged farmers to adopt grafted seedlings as a reliable propagation method. Vegetative propagation, particularly grafting, offers several advantages over seed-based propagation, including a shorter juvenile phase, earlier fruit production, and the preservation of desirable parent traits (Zhang et al. 2019). Through grafting, superior characteristics such as fruit quality, yield, and uniformity can be maintained while allowing the combination of beneficial traits from both the rootstock and the scion. The rootstock supplies water, nutrients, hormones, and other essential compounds, while the scion provides photosynthates (Lazare et al. 2020). A successful rootstock–scion association is therefore essential to ensure optimal plant growth, high yields, and good fruit quality.

Multiple factors, including genetic compatibility, environmental conditions, and management practices, influence grafting success. Abiotic factors, such as light intensity, temperature, water availability, and soil fertility, as well as biotic factors, such as pests and diseases, significantly affect plant performance and fruit quality (Lazare et al. 2020). In addition, rootstock–scion interactions can alter plant phenotype, influencing growth, stress tolerance, resistance to pests and pathogens, and fruit quality (Zhou et al. 2022). Studies have demonstrated that these interactions can modify physiological and morphological traits (Wang et al. 2019; Rasool et al. 2020). Furthermore, research on grafted plants indicates that rootstocks often exert a stronger influence than scions, affecting growth, stress resistance, and the synthesis of secondary metabolites (Tedesco et al. 2021; Dong et al. 2022; Habibi et al. 2022; Zhang et al. 2022).

Another important advantage of grafting is the development of a stronger root system. Rootstocks derived from seeds typically form taproots, which enhance plant stability and improve water and nutrient uptake compared to non-grafted plants, which tend to develop weaker, fibrous roots (Gunawan 2014). However, the success of grafting largely depends on the proper healing of the graft union. This process involves the formation of a functional connection between the rootstock and scion, allowing the transport of water, nutrients, hormones, and signaling molecules through vascular tissues, including xylem and phloem (Ahsan et al. 2019). Successful graft unions are characterized by the alignment and integration of cambial layers and vascular tissues (Sunaryo et al. 2019).

Different grafting methods, such as cleft, wedge, and splice grafting, can influence grafting success (Garner

2000). Among these, splice grafting is often reported to produce better results due to its simplicity and effectiveness in aligning cambial tissues (Budi et al. 2016). The physiological stage of the scion also plays a critical role. Scions may be in active growth (flushing) or in dormancy, which includes paradormancy, endodormancy, and ecodormancy (Lang 1987; Rahayu et al. 2020). Research suggests that using scions in the ecodormant stage can yield higher graft survival rates, up to 72.50%, likely due to greater compatibility and reduced metabolic stress (Da Costa et al. 2022). However, most studies rely on morphological observations, and there is still limited microscopic evidence confirming the structural integration of vascular tissues at the graft union.

A common problem in grafted avocado plants is the “elephant’s foot” phenomenon, marked by swelling at the graft union due to weak or incomplete vascular connections, which can reduce long-term productivity (Tedesco et al. 2020). Although vascular reconnection is critical, histological studies in avocado remain limited. This is important because avocados are highly heterozygous, making seed propagation unreliable for fruit quality and yield (Bugudole et al. 2025). Grafting ensures uniformity and preserves superior traits (Cañas-Gutiérrez et al. 2022). However, challenges such as non-standardized rootstocks, environmental variability, and technical limitations persist, requiring further optimization for consistent success (Mauro et al. 2022).

This study aims to address these gaps by investigating the microscopic compatibility of grafted avocado seedlings. Specifically, it focuses on the structural integration of xylem and phloem tissues between the rootstock and scion during both flushing and dormancy stages. Through histological analysis, the research aims to provide direct evidence of vascular connectivity and to identify the optimal combination of grafting method and scion condition for successful, reliable avocado propagation.

MATERIALS AND METHODS

Research time and location

The research was carried out at the Nursery Garden, Self-Help Agricultural and Rural Training Center (SARTC) Agro Utama Mandiri, Mangunrejo Village, Ngadiluwih Sub-district, Kediri District, East Java, Indonesia (7°52'02.0" S-7°52'01.0" S and 112°01'05.8" E-112°01'05.1" E). The altitude is 600 m above sea level, with air temperatures ranging from 25 to 27 °C and air humidity of 67-82%. Microscopic observations of the connecting areas were conducted in the Plant BioScience and Technology Laboratory, Department of Biology, at the Institut Teknologi Sepuluh Nopember (ITS), Surabaya, East Java.

Planting materials and grafting procedures

Rootstocks were 6-month-old local avocado seedlings from generative propagation, with uniform height (40 cm) and stem diameter (7 mm). Rootstocks were cut to 15 cm above the soil, and grafting was performed using three methods (Figure 1):

Cleft grafting: A vertical incision (1.5 cm deep) was made in the center of the rootstock.

Wedge grafting: The rootstock was split into a V-shaped cleft.

Splice grafting: A diagonal cut was made on the rootstock.

Scions were collected from a 9-year-old, fruit-bearing 'Alligator' avocado tree selected for its health and consistent productivity. Scions were 10 cm long, with a diameter matching that of the rootstock (7 mm), and were taken from vertical shoots at the top of the canopy. Three scion physiological stages were tested:

Flushing: 10 days after shoot emergence

Endodormancy: 30 days after shoot emergence

Ecodormancy: 50 days after shoot emergence

Scions were cut to match the rootstock incision: tapered for cleft and wedge grafts, and diagonal for splice grafts. The scion was aligned with the rootstock to ensure cambial contact and then secured with plastic tape. The graft union was covered with a transparent plastic sleeve to maintain humidity.

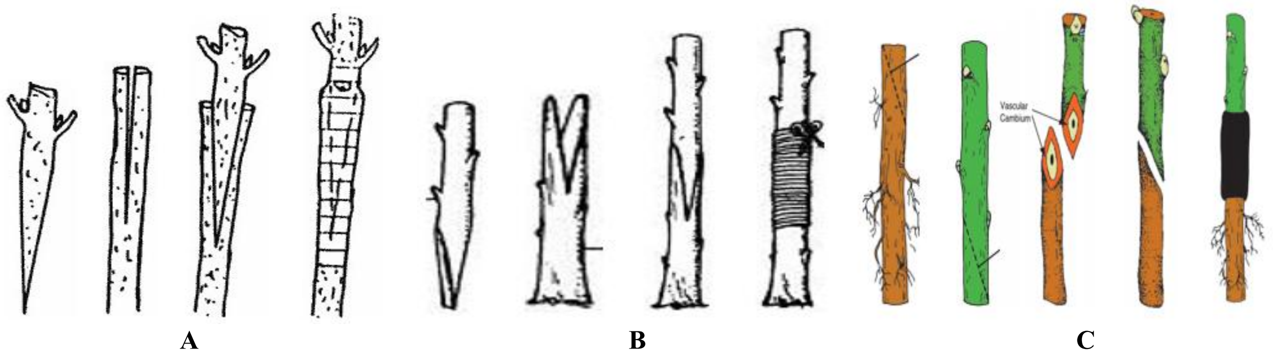


Figure 1. Grafting procedures A. Cleft grafting, B. Wedge grafting, C. Splice grafting

Data analysis

The experiment was arranged factorially with two factors in a Completely Randomized Design (CRD) and repeated with 5 blocks of multiple plants. The first factor is the grafting method treatment, which consists of Cleft Graft, Wedge Graft, and Splice Graft. The second factor is the growth stage of the scion, which consists of three growth stages of the scion, namely: flushing growth stage (scions aged 10 days after the first shoot), endodormancy stage (scions aged 30 days after the first shoot), and ecodormancy stage (scions aged 50 days after the first shoot). The data from observations at the first times bud burst and the number of leaves were analyzed statistically using Analysis of Variance (ANOVA). If the observation results showed a natural effect, the test would continue with the Honestly Significant Difference (HSD).

The microscopic analysis of graft union compatibility was conducted at 45 and 60 DAG, when grafts showed signs of successful union (Sarker and Gomasta 2024). Cross-sections of the graft interface were prepared using a microtome and observed under an Olympus CX21 binocular microscope (4×, 10×, 40×, 100× objectives), equipped with an Optilab camera. Observations focused on the continuity of vascular tissue (xylem and phloem) and the presence of necrosis at the rootstock-scion interface. Images were captured at 10× magnification.

RESULTS AND DISCUSSION

The influence of the growth stages of scion on the growth of grafted seedlings

The success of grafting is also determined by the growth stage of the scion that will be used. The growth stages of the scion include the active growth stage (flushing) and the dormancy stage. During the active growth stage, several stages occur, including: the early bud stage, the rapid bud stage, the full bud stage, and the mature bud stage. The plant also experiences a dormant period, meaning it is in a resting phase while still performing metabolism, albeit at a slower rate.

Dormancy stages

Dormancy is a state in which a plant is resting. In other words, the plant is still metabolising, but the process is slow. Dormancy can occur in seeds, buds, or underground storage organs. Dormancy occurs due to internal conditions within the plant, even when external conditions are ideal. However, external conditions indirectly influence dormancy. A common form of plant dormancy is leaf shedding (Campbell and Reece 2002). Dormancy is a condition in which metabolic processes slow down (Usher 1966). Plant dormancy has three phases: paradormancy, endodormancy, and ecodormancy. Paradormancy refers to the dormancy of the bud induced by factors other than the bud itself, primarily related to apical dominance.

During the subsequent period, development and growth are controlled by internal signals within the bud in response to environmental cues. In this state, the structure cannot grow or develop even if external physiological signals are removed, and the plant is returned to conditions conducive to growth (Lang 1987). The dormancy in seeds and buds is almost identical. However, the function of this dormancy is not only to eliminate the barriers to bud formation but also to protect the plant from extreme temperatures. Depending on the plant type, most plants experience dormancy at low temperatures. Conversely, in deciduous plants, dormancy occurs at high temperatures (Kimball 1983).

Paradormancy is the period of bud dormancy caused by factors other than the bud itself, particularly linked to apical dominance. On the other hand, endodormancy is a period of dormancy in the bud, controlled by internal signals in response to unfavourable environmental factors (such as drought, high or low temperatures). If environmental factors are ideal, the dormant bud cannot break into growth (bud break). Ecodormancy is a period of dormancy that is susceptible to favorable environmental conditions and is marked by bud break or sprouting (Lang 1987).

Endodormancy

Endodormancy plays a critical role in the life cycle of perennial plants by regulating bud break timing and ensuring survival through winter. This phase serves as a protective mechanism, preventing premature bud break in response to temporary warming during late autumn, which could otherwise expose vulnerable plant tissues to frost damage. Such a response is crucial because frost at this period can cause irreversible harm to the plant, affecting its reproductive success and overall health. Endodormancy is not merely a passive dormancy state but an active adaptation to winter conditions, allowing plants to acquire full cold hardiness (Penfield et al. 2021). This process begins in early autumn, even before leaf senescence, marking a physiological shift that prepares the plant for harsh environmental conditions.

As the season progresses, once endodormancy is released, the plant transitions into ecodormancy. In this state, growth is arrested by external environmental factors, such as temperature or day length, rather than by internal physiological processes. This progression from endodormancy to ecodormancy is crucial for synchronizing plant growth with seasonal changes, ensuring survival during the coldest winter months, and facilitating a transition to spring growth. Powell (1987) highlighted the complex interaction between internal and external factors in regulating dormancy and subsequent bud development, underscoring the dynamic nature of plant responses to environmental cues.

Ecodormancy

Ecodormancy represents a critical phase in the seasonal growth cycle of plants, occurring just before the first bud break. During this period, growth is suppressed due to unfavorable environmental conditions, such as low temperatures, insufficient light, or drought, which are not conducive to optimal plant development. Unlike endodormancy, where internal physiological factors drive growth inhibition, ecodormancy is primarily governed by external factors that limit the plant's ability to resume growth, despite its physiological capacity to do so. The duration and severity of ecodormancy are closely tied to prevailing environmental conditions, and the plant remains in a state of dormancy until these conditions improve, signaling the onset of favorable environmental cues, such as warmer temperatures or longer photoperiods. Upon alleviating these stressors, growth resumes, allowing the plant to continue developing and transition into the active growing season. Powell (1987) provided a comprehensive understanding of ecodormancy, emphasizing its role as a dynamic adaptation to seasonal changes. This transition between dormancy and growth, influenced by environmental factors, is critical for ensuring that plants synchronize their life cycles with the most favorable conditions for survival and reproduction (Figure 2).

The rootstock preparation

The criteria for avocado plants to be used as rootstock are: the root system is strong enough and resistant to attacks by pests and diseases, as well as unfavorable conditions such as drought, has broad adaptability, the growth speed matches that of the scion so that they can live together, and the trunk is strong and sturdy. It does not, in an unfavorable way, affect the quality or quantity of the plants that result from the connection.

The rootstock used is a local avocado seedling from a 6-month-old generative nursery, approximately 40 cm high, with a stem diameter of 7 mm. Avocado seeds used as rootstock are cut to 15 cm and split according to the method used. In the gap grafting method, the rootstock is divided in the middle to a depth of 1.5 cm; in wedge grafting, it is divided into a V shape; and in diagonal grafting, it is cut at an angle (Figure 3). The statistical analysis results of the influence of scion growth stage and grafting method on bud burst of grafted avocado seedlings showed a significant interaction (Table 1).

The entrees preparation

The criteria for selecting twigs or branches of avocado plants to be used as scions are that the trees are healthy and have already produced dense, high-quality fruit. Entres are taken from branches or twigs that have vertical growth, the diameter of the twigs is almost the same as the diameter of the rootstock, selected (superior) characters, and are healthy, strong, and free of pests and diseases; taken from straight stems and from healthy and thriving branches; taken from a parent tree that has already produced fruit.

The scion comes from an alligator avocado tree that is already bearing fruit (Figure 4). Entrees are used at 10, 30, and 50 days after the first trubus or flush. Entres are taken from the tops of tree branches, then the entres are cut to 10 cm, and the base is slashed according to the method used. The incision for the scion in the gap joint and V-joint methods has the same shape, namely, it is tapered at the base. In contrast, the incision for the diagonal splice is made at an angle (Figure 5).

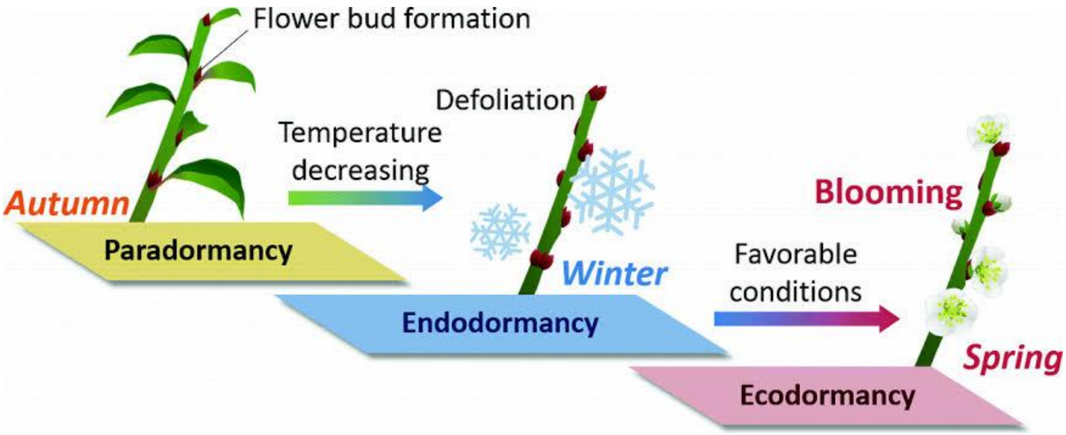


Figure 2. Shoot dormancy period

Table 1. Effect of combination treatment of scion growth stage and grafting methods on the time of bud burst of grafted avocado seedlings (days)

Treatments of growth stage scion	Grafting methods		
	Cleft graft	Wedge graft	Splice graft
Flushing	7.92 d	6.54 b	6.50 b
Endodormancy	6.59 b	6.25 ab	6.42 b
Ecodormancy	6.92 c	6.42 b	6.17 a
HSD		0.24	

Note: Numbers followed by the same letter in grafting methods indicate that they are not significantly different in the 5% HSD test

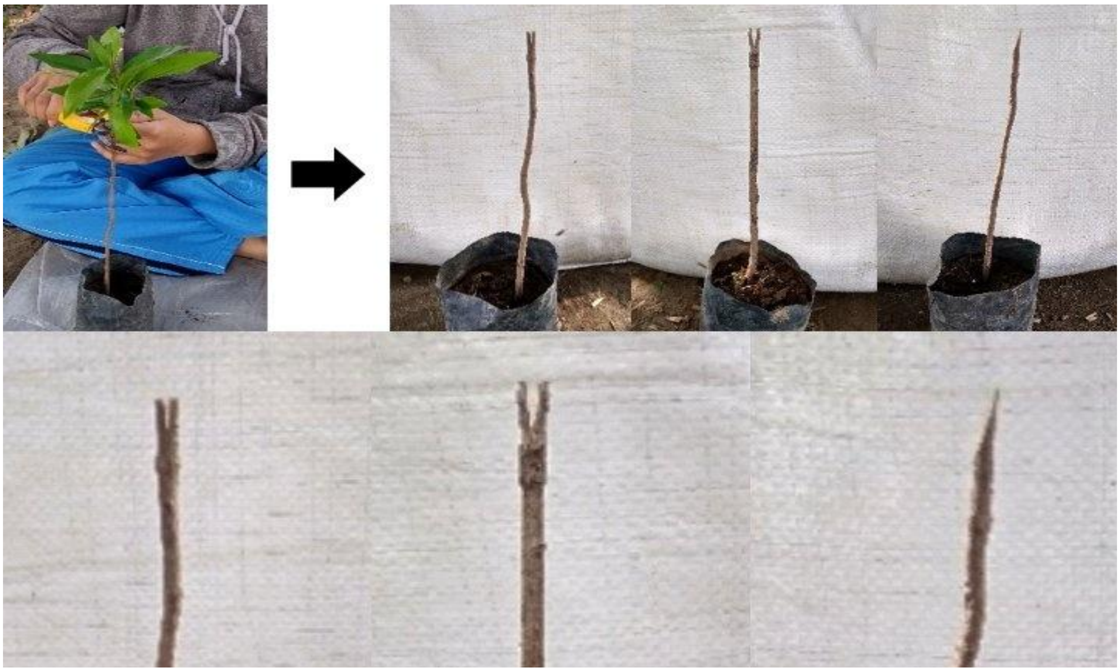


Figure 3. Rootstock preparation

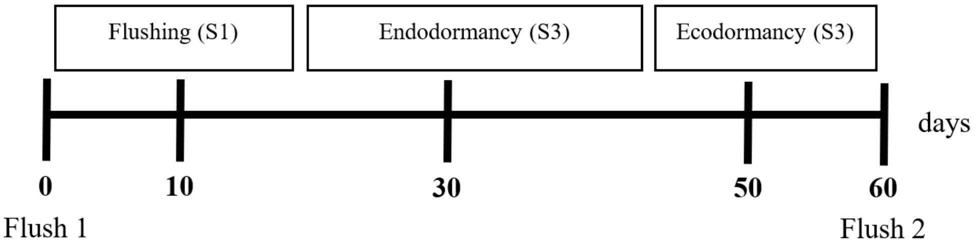


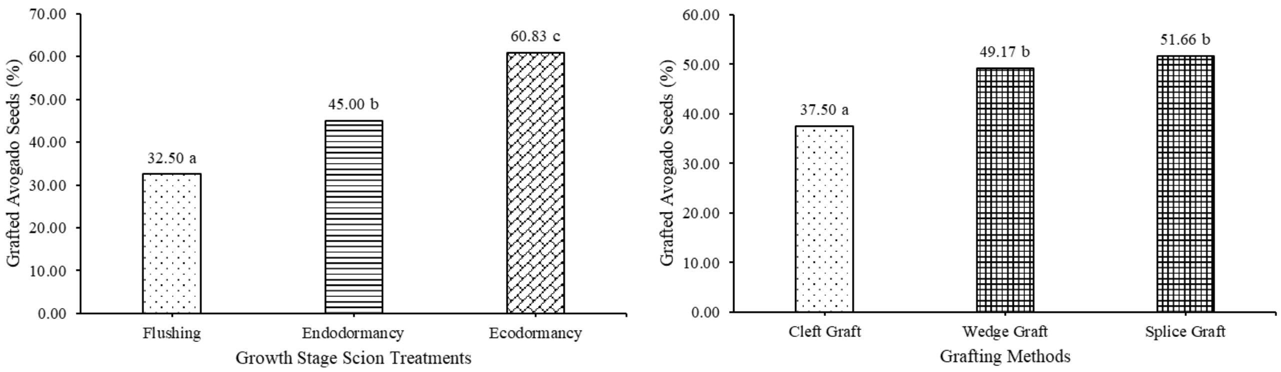
Figure 4. Illustration of entres growth stage age

Percentage of grafted avocado seeds

The success of grafting propagation can be assessed by the percentage of seedlings that survive and the compatibility between the scion and rootstock used (Saman et al. 2022). The selection of the scion influences the success of grafting. The physiological conditions at each scion phase (flushing, endodormancy, and ecodormancy) have different physiological characteristics. These characteristics will affect the scion's ability to integrate with the rootstock and adapt to survive. The use of scions during the ecodormancy phase results in the highest survival rate compared to other phases. During the ecodormancy phase, the scion is in an optimal physiological condition for adaptation. Scions in the ecodormancy phase have optimal energy and nutrient reserves, especially in the form of starch and carbohydrates. These reserves support the growth of callus and buds after grafting. During ecodormancy, the scion's tissues remain ready to regenerate when conditions are favourable. Once grafting is performed, the callus activity can proceed optimally because energy and nutrients are sufficient. In the ecodormancy phase, the scion has tougher, more mature tissues, making it more resistant to pathogens that may attack the graft junction. Furthermore, scions in the ecodormancy phase exhibit low metabolic activity and fewer or no active leaves, thereby minimizing transpiration (Figure 6.A).

Several grafting methods, such as the splice, cleft, and wedge, have been tested. The splice method yields the highest survival percentage for avocado seedlings compared to other methods. The splice method is a more straightforward grafting method than wedge and cleft, making it easier to perform and more likely to succeed. In splice grafting, both stem sections (scion and rootstock) are cut with simple slanted cuts. The splice method allows easier alignment of the cambial layers between the scion and rootstock, enabling optimal union and formation of new tissues. The formation of connecting tissues is crucial as it determines the transport of nutrients in the plant (Rasool et al. 2020). Using local avocado as the rootstock and alligator avocado as the scion yields a reasonable compatibility rate of 51.66% (Figure 6.B), achieved through the splice method. The choice of grafting method and materials used will affect the success rate of vegetative propagation (Nguyen et al. 2020).

Figure 5. The incision for the diagonal splice is made at an angle



A

B

Figure 6. Effect of treatment of scion growth stage and grafting methods on the percentage of grafted avocado seeds. Note: Numbers followed by the same letterof the same treatment indicate that they are not significantly different in the 5% HSD test

Additionally, the splice grafting allows for tighter binding with tape or other materials, as both sections typically have similar shapes and sizes. A proper seal prevents air or pathogens from entering, which could affect the success of the graft. The splice method is better suited to plants with small stem diameters, such as the avocado seedlings used in this study.

The scion stage of ecodormancy, using the splice graft method (Table 1), produces the fastest first-time budburst. It is significantly different from other treatment combinations, with an accelerated time to first shoot breaking of 2 days compared to flushing scion using the Cleft Graft method. The earliest bud sprouting observed in splice grafting in the present study may be attributed to the earlier and more extensive contact between the rootstock and scion cambial layers, resulting in earlier callus formation than in cleft and wedge grafting.

The success of grafting depends on rootstock/scion compatibility, where a combination can form a solid and durable graft union, resulting in better growth and development of grafted plants (Cano-Gallego et al. 2023). Graft compatibility is generally defined as a sufficiently close genetic (taxonomic) relationship between scion and rootstock to allow the formation of a successful graft union (Loupit and Cookson 2020). There is rootstock/scion compatibility when a given combination can form a solid and durable graft union, resulting in the development of a successful graft (Gainza et al. 2015). The formation of the graft union depends on several events, including molecular and physiological/biochemical responses and anatomical components in scions and rootstocks (Rasool et al. 2020).

Five biological steps have been described to occur during graft union formation: (i) alignment of vascular cambium tissue between the scion and rootstock; (ii) response to the wound; (iii) formation of the callus bridge; (iv) repair of the xylem by the differentiation of the vascular cambium tissue through the callus bridge; and (v) production of the secondary xylem and phloem from the new vascular cambium tissues of the callus bridge (Hartmann et al. 2011). Initial tissue cohesion between the scion and the rootstock is produced due to the deposition and subsequent polymerization of cell wall materials in response to the wound. The effects of phenolic compounds on the re-establishment of vascular tissues have been studied (Canas et al. 2015; Pina et al. 2017). More specifically, several studies have shown that phenolic compounds play a role in lignification. Such compounds are essential during the early growth stages of connections between scion–rootstock combinations, as the cell walls of xylem tissues are dynamic structures composed of polysaccharides, phenolic compounds (for example, lignins), minerals, and proteins (Herrero et al. 2014). Moreover, the presence of phenolic compounds has been identified as an essential marker for evaluating graft compatibility between scions and rootstocks (Prabpreea et al. 2018).

The growth stage of the scion and the grafting method interacted significantly with the number of leaves of grafted avocado seedlings at the ages of 45 and 60 DAG (Table 2). At 45 DAG and 60 DAG, the growth stage of ecodormancy scion using the splice graft method produced the highest number of leaves and significantly differed from other combination treatments, except for the wedge graft method. There was an increase in the number of leaves by the combination treatment of the growth stage of the ecodormancy scion in the 60 DAG splice graft method by 67% compared to the combination treatment of the growth stage of the flushing scion in the cleft graft method. Quick and proper contact between the scion and rootstock during grafting may facilitate the easy translocation of soil nutrients from the rootstock to the growing tip, ultimately increasing the number of leaves in grafted saplings (Tripathi and Karunakaran 2019).

Grafting using the splice graft method produces around 9 avocado leaves (Sarker and Gomasta 2024). It has been identified that the affinity between the rootstock and the scion is a key factor in ensuring successful compatibility between these two tissues (Cano-Gallego et al. 2023). Since it has been proven that the rootstock favors the absorption of nutrients and a good connection between the rootstock and the scion is vital for the movement of solutes through the vascular system, there must be no interruptions between them at their point of connection (graft scar) (Lazare et al. 2020).

Scions provide essential nutrients, such as sugars, hormones, and nucleic acids, to rootstocks, which significantly affect carbohydrate storage and root growth. Nevertheless, the rootstock biomass was significantly affected by the scion, varying by species (Ferlito et al. 2020; Zhang et al. 2024). It is related to the predominant role of the rootstock-scion interaction, rather than the independent additive effects of each genotype, with combined effects primarily on the transport of water and nutrients, as well as the large-scale movement of hormones, proteins, and messenger RNAs (Wang et al. 2017).

Microscopic observation of the connection area between the rootstock (R) and scion (S) of grafted avocado seedlings was conducted to analyze the effectiveness and compatibility of different grafting techniques across various growth stages of the scion (Rasool et al. 2020). This study examined three scion growth stages: Flushing, characterized by active growth and leaf development (Luo et al. 2022); Endodormancy, a phase where growth is temporarily halted due to internal plant conditions (Fadón et al. 2020); and ecodormancy, where growth cessation is due to external environmental factors (Chen et al. 2023). These stages were analyzed using three grafting methods: Cleft, Wedge, and Splice (Akhilraj and Suresh 2023). Observations were made at two critical time points, 45 and 60 DAG, to assess the progression and quality of vascular connection formation. The microscopic examination focused on the anatomical and physiological integration of the scion with the rootstock (Huang et al. 2024), which is crucial for the transport of nutrients and water (Morales-Alfaro et al. 2023). By comparing these variables, the study aimed to identify the optimal grafting conditions that promote successful graft union and enhance the survival and growth of avocado seedlings. The results of this investigation could provide valuable insights into best practices for avocado grafting, with implications for improving commercial propagation techniques and ensuring the sustainability of avocado production.

The cleft graft (Figure 7), wedge graft (Figure 8), and splice graft (Figure 9) methods show that at the age of 45 DAG, the grafted avocado seedlings, both at the flushing, endodormancy, and ecodormancy stages, are flushed and compatible (the vascular tissue of xylem and phloem between the rootstock and scion is already connected). However, microscopic examination of the connection area still reveal wounds. They are not yet fully connected in the vascular tissue at the connection area. Meanwhile, in microscopic observation of the connection area at 60 DAP, the joint wound was more unified between the rootstock and scion, as shown by the thinning of the wound and the merging of the vascular tissue between the rootstock and the scion. The compatibility rate of the wedge graft method is higher and faster than that of the cleft graft method. The faster fusion of the vascular tissue (xylem and phloem) between the rootstock and the scion is shown by fewer cavities in the connection area. The compatibility rate of the splice graft method is the highest among the three graft methods. This condition is shown by the fusion of the vascular tissue (xylem and phloem) between the rootstock and the scion, which is better when compared to the cleft graft method (Figure 2) and the wedge graft method (Figure 3), where there are still many areas of connection that have not yet united (there are cavities in the connection area). Connections marked by incision marks on the joint are already disguised, and the xylem between the rootstock and scion combines to form a combined xylem, which is a perfect connection. In contrast, the connection area still appears necrotic, and the incision marks indicate it has not been wholly connected (Handayani et al. 2013). A lengthier diagonal cut surface on both sides of the scion, corresponding to the vertical flip of the rootstock, encourages the precise intermingling and interlocking of vascular bundles, enabling a quick union of the grafts, as noted previously by Tripathi and Karunakaran (2019).

The joining of the connection area using the cleft graft method at the flushing and endodormancy stages of the scion showed several weaknesses, including that at 45 DAG, cavities remained in the connection areas, and the joint wounds had not completely healed. The cleft graft method is not precise when connecting the rootstock and scion. There are a few uneven parts during the grafting process, where the rootstock is stretched while the scion is flushing and establishing the graft connection area. Endodormancy treatment in the wedge grafting method showed more precise results than the cleft graft method. The cleft-grafted plants show better growth because the scion used in grafting had more buds. Buds will grow to form shoots after the scion and rootstock are successfully grafted (Sunaryo et al. 2019).

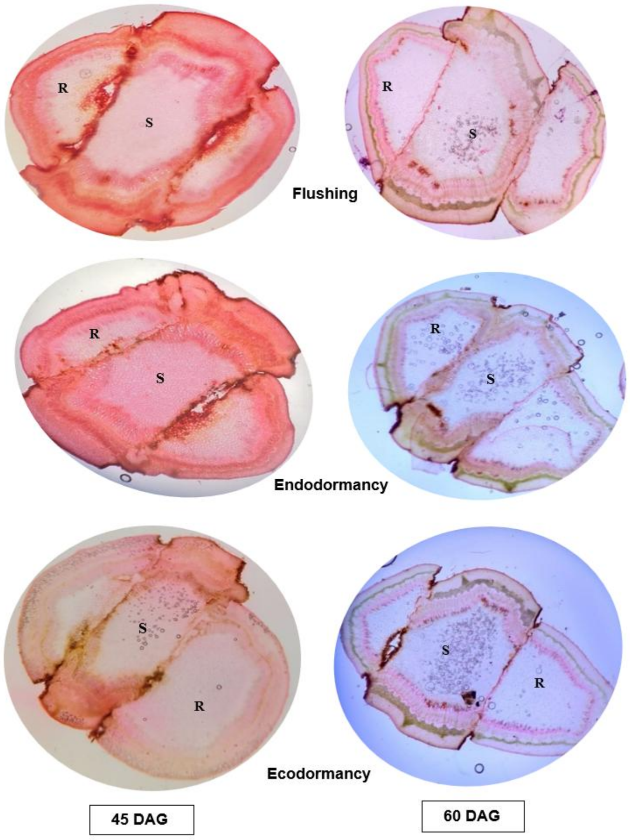


Figure 7. Cross-section of grafted avocado seedlings in the combination of cleft graft methods at growth stages of scion flushing, endodormancy, and ecodormancy aged 45 and 60 DAG. Note: R: Rootstock; S: Scion; DAG: Day After Grafting

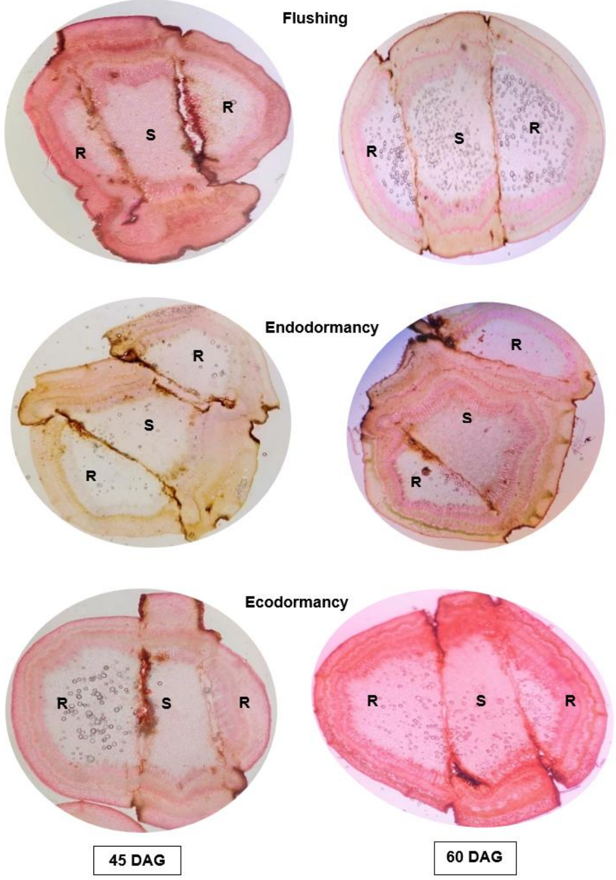


Figure 8. Cross-section of grafted avocado seedlings in the combination of wedge graft methods at growth stages of scion flushing, endodormancy, and ecodormancy aged 45 and 60 DAG. Note: R: Rootstock; S: Scion; DAG: Day after grafting

Table 2. Effect of combination treatment of scion stage and grafting methods on the number of leaves (strands) of grafted avocado seedlings, age 45 and 60 DAG

Time observation	**Treatments**	**Grafting methods**		
	Growth stage scion	**Cleft graft**	**Wedge graft**	**Splice graft**
45 DAG	Flushing	4.42 a	6.42 b	6.92 bc
	Endodormancy	6.50 b	7.00 bc	7.25 c
	Ecodormancy	6.67 bc	7.08 bc	8.17 d
	HSD 5%		0.63	
60 DAG	Flushing	5.33 a	7.17 b	7.25 b
	Endodormancy	7.84 bc	8.00 c	8.00 c
	Ecodormancy	7.34 bc	8.00 c	8.92 d
	HSD 5%		0.68	

Note: Numbers followed by the same letter indicate that they are not significantly different in the 5% HSD test, DAG: Day After Grafting

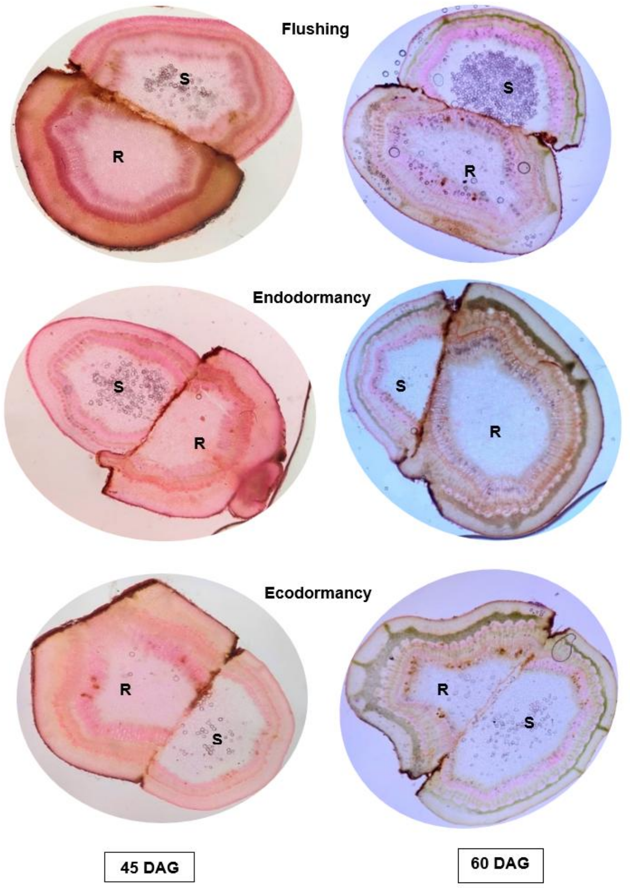


Figure 9. Cross-section of grafted avocado seedlings in the combination of splice graft methods at growth stages of scion flushing, endodormancy, and ecodormancy aged 45 and 60 DAG. Note: R: Rootstock; S: Scion; DAG: Day after grafting

The incision wound in the cleft graft and wedge graft methods is more comprehensive, namely on both sides of the upper trunk, which has a tapered shape (the wound surface measures around 3 cm², whereas in the splice graft, the incision wound is only 1.5 cm²). When using scion flushing (10 days after sprouting), the shoot's endogenous compounds (metabolites) are fully translocated to other plant organs. In endodormancy or deep dormancy, even though the environment is favorable, the bud wood still does not respond because its metabolic activity is prolonged at this stage, so the healing of the wound is prolonged. This condition causes the growth of grafted avocado seedlings from the combination treatment of the wedge graft method at the flushing and endodormancy growth stages of the scion to proceed more slowly than the shoots originating from the ecodormancy growth stage. In ecodormancy shoots, this is a period during which cells are resting but remain sensitive to environmental changes. If the climate is favorable, the shoots will quickly sprout and grow into new shoots.

Figure 4 shows that the performance of grafted avocado seedlings using the splice graft method at 45 DAG has grown well. Microscopic observations of the cross-section of the joint area show that although there are still scars from being connected to the area using the splice graft method, the compatibility rate is faster, which is demonstrated by the fusion of the vascular tissue (xylem and phloem) between the rootstock and the scion, when compared with Figure 7 (cleft graft method) and Figure 8 (edge graft method) shows that there are still many areas of connection that have not yet united (there are cavities in the connection area). Graft incompatibility generally occurs at the early stages of graft development when vascular connections are forming; however, symptoms may manifest at later growth stages, such as low plant development related to physiological differences in stem diameter, which impairs the normal flow of photoassimilates and the lignification of grafted tissues (Souza et al. 2018). Scion-rootstock incompatibility limits grafting. It can be caused by various factors, including insufficient genetic proximity, physiological or biochemical factors, lignification at the graft union, poor graft architecture, inadequate cell recognition between the union tissues, and metabolic differences between the scion and rootstock (Habibi et al. 2022).

The linkage of the connection area by flushing scion growth-stage treatment in the microscopic splice graft method shows the most precise results compared to the cleft graft and wedge graft methods. It is because only one connection wound covers an area of 1.5 cm^2 on the scion and rootstock, which are then connected. In flushing shoots past the shoot period, endogenous compounds (metabolites) have been translocated to the plant shoots. Although the environment is favorable in endodormancy or deep dormancy, the scion still does not respond because its metabolic activity is prolonged at this stage. In scion, ecodormancy occurs when cells rest but remain highly sensitive to environmental changes. The scion will quickly form new shoots if the climate is favorable. It facilitates the smooth translocation of water and nutrients, allowing this treatment's growth to be faster than that of other treatments.

The grafting treatment with splice grafts at the ecodormancy stage is the most effective method for producing grafted avocado seedlings with faster graft wound healing, as it achieves a more complete vascular tissue linkage than other combination treatments. This condition is indicated by a 2-day acceleration of first bud break (Table 1) and a 67% increase in the number of leaves at 60 DAG compared to the cleft graft with scion combination treatment at the flushing growth stage (Table 2). The same results were reported by Budi et al. (2016), who found that the splice graft produces the best growth in coffee graft seedlings. The splice graft method has a narrower incision at the connection area than the incision in the cleft graft or wedge graft. The narrower wound tends to heal more quickly, thus allowing for a connection area between the rootstock and scion. Grafting can be done at any time during the scion's dormant period. The chances for successful healing of the graft union are best if the work is done in early spring, just when the scion buds are beginning to swell (ecodormancy), but before active growth has started (Beshir et al. 2019). The success of a grafting operation depends on the strength of the union formed. Graft union formation depends on several events, including molecular and physiological/biochemical responses and anatomical components in scions and rootstocks (Rasool et al. 2020).

During callus formation, the density of components in the connection area is significant. Incision marks can indicate perfect compatibility at the connection area, which is not visible, and the xylem between the rootstock and scion to form a combined xylem. In contrast, the connection area is not perfectly connected. Stems still appear necrotic, and the incision marks are as described by Handayani et al. (2013). Grafting technology in the field should be carried out based on the fundamentals of grafting, including the condition that the plant must have sufficient food reserves, the optimal grafting time and site conditions, and the freshness of the material, all of which influence grafting success (Sunaryo et al. 2019).

In conclusion, the combination of the splice grafting method and scions at the ecodormancy stage provides the most effective protocol for avocado propagation. This phase ensures that scion tissues are metabolically active enough to resume growth quickly but not stressed by full metabolic demand, leading to faster bud break and more uniform leaf emergence. The physiological balance during ecodormancy enhances callus formation and reduces desiccation stress at the graft interface, making this timing particularly favorable for callus formation. Anatomical evidence further confirms the superiority of this combination. Minimal necrosis at the union site indicates reduced oxidative stress and improved alignment between the cambial layers of the rootstock and scion. Complete fusion of xylem and phloem tissues demonstrates that water, nutrients, and photoassimilates can move freely across the graft junction, ensuring long-term functionality. The splice method itself provides a large, smooth contact surface, maximizing cambial overlap and enhancing the efficiency of cell-to-cell signaling, callus bridge formation, and vascular differentiation. For nursery practice, these findings present a scientifically validated strategy for achieving rapid and reliable avocado propagation. By optimizing both the grafting method and the physiological stage of scions, nurseries can secure higher success rates, uniform plant development, and shorter production cycles—critical factors for establishing high-density orchard systems. Moreover, understanding the underlying mechanisms highlights opportunities for further refinement, such as integrating hormonal treatments or molecular markers to predict compatibility, and supports sustainable large-scale avocado production.

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