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The Effect of Shoots Number on Upper Stem and Length of Lower Stem on The Success of Mango Plants Grafting

Pengaruh Jumlah Mata Tunas Batang Atas dan Panjang Batang Bawah Terhadap Keberhasilan Grafting Tanaman Mangga

Aulia Ayu Fitrisia*, Rinie Pratiwi Puspitawati, Ahmad Bashri
Study Program of Biologi, Faculty of Mathematics and Natural Sciences,
Universitas Negeri Surabaya
*e-mail: aulia.20015@mhs.unesa.ac.id

Abstract. Mango is one of the horticultural crops that has the potential to be developed in Indonesia. Fulfillment of the increasing availability of mango is done by grafting. This study aims to determine the growth of the connection results which include stem length, stem diameter, length of shoots break, number of leaves, anatomy of the connection union, and percentage of success. The experimental research used a two-factorial group randomized design with treatment combinations consisting of E1B1, E1B2, E1B3, E2B1, E2B2, E2B3, E3B1, E3B2, E3B3. Data were analyzed using descriptive statistics. The results showed that there was no interaction between the number of scion shoots and rootstock length on all parameters observed. The treatment combination with the number of scion shoots 4-5 and rootstock length 30-35 cm produced the best percentage of grafting success. The anatomical structure of the connection treatment combination influenced the tissue attachment. A less than optimal connection is characterized by differences in the shape and size of the vessel network on the rootstock which is larger than the scion. Optimal connectioning is characterized by the same shape and size of the vessel network circles on the scion and rootstock.
Keywords: V grafting; plant anatomy; mangos; agriculture

Abstrak. *Mangga merupakan tanaman hortikultura yang berpotensi untuk dikembangkan di Indonesia. Pemenuhan ketersediaan mangga meningkat sehingga produktivitas mangga dilakukan dengan cara sambung pucuk (grafting). Penelitian ini bertujuan untuk mengetahui pengaruh jumlah mata tunas batang atas dan panjang batang bawah terhadap keberhasilan grafting tanaman mangga dengan mengetahui pertumbuhan hasil sambungan yang meliputi panjang batang, diameter batang, umur pecah tunas, jumlah daun, anatomi penyatuan sambungan, serta persentase keberhasilan. Penelitian eksperimental menggunakan Rancangan Acak Kelompok (RAK) dua faktorial dengan kombinasi perlakuan terdiri dari E1B1, E1B2, E1B3, E2B1, E2B2, E2B3, E3B1, E3B2, E3B3. Data yang diperoleh akan dianalisis secara statistika deskriptif. Hasil penelitian menunjukkan bahwa kombinasi antara jumlah mata tunas batang atas dan panjang batang bawah tidak terdapat interaksi di semua parameter pengamatan. Kombinasi perlakuan 4-5 mata tunas batang atas dan panjang batang bawah 30-35 cm menghasilkan persentase keberhasilan grafting terbaik. Struktur anatomi penyatuan sambungan kombinasi perlakuan memberikan pengaruh terhadap kelekatan jaringan. Sambungan yang kurang optimal terdapat celah pada bagian sambungan dan luka sambungan belum sepenuhnya sembuh, ditandai dengan bentuk dan ukuran lingkaran pembuluh yang berbeda pada batang bawah lebih besar dibandingkan pada batang atas. Penyatuan sambungan yang optimal ditandai dengan bentuk dan ukuran lingkaran jaringan pembuluh yang sama pada batang atas dan batang bawah.*
Kata kunci*: grafting V; anatomi tumbuhan; mangga; agrikultur*

INTRODUCTIONS

Horticultural crops are one of the leading crop cultivations in Indonesia with broad market potential and high economic value. Horticultural crop commodities have become one of the leading products in Indonesia. One of the best horticultural crops in Indonesia is mango. According to FAO (2019), Indonesia produces an average of 2 million tons of mangoes per year, making it the fourth largest producer in the world and the seventh largest exporter of mangoes in Asia. Mango (Mangifera indica L.) is a plant that contains a source of vitamins and minerals. Among the most important horticultural crops to be developed in Indonesia are mangoes, along with oranges and bananas (Widjaja, *et al.* 2014). Indonesia's mango production in 2021 reached 2.84 million tons, down 2.07% from

2.9 million tons in the previous year (BPS, 2021). If this decline in mango production is allowed to continue, it could threaten the availability of mangoes in Indonesia. In addition, the problem of low aromatic mango exports is influenced by several factors, such as small mango production and business scale, limited land, and climate (Kementerian Republik Indonesia, 2018). To increase mango productivity, the availability of superior and quality seeds is required. However, this is still an obstacle in increasing the productivity and quality of mango fruit. Therefore, the solution to overcome this problem is to propagate vegetatively. It is more effective and efficient than generative propagation (seeds) because it produces fruit quickly and is similar to the parent fruit, so it is necessary to propagate vegetatively by grafting (Maulana, *et al.* 2020).

Grafting has several advantages, such as maintaining clonal traits; strengthening plants with rootstocks that are resistant to low temperatures and poor soil conditions or other soil disturbances; improving new plant varieties for the better; and faster plant growth (Suwandi, 2014). According to Astutik (2008), the wedge or cleft graft method is the best for mango. This is because it has the largest number of leaves, the fastest shoot growth, and has a hundred percent grafting success percentage. In line with the opinion of Santoso *et al.*, (2013) stated that the V-shaped shoot grafting method has a higher connection success than other grafting methods. This is because the xylem, phloem, and cambium tissues between the upper and lower stems are easily fused in V-shaped shoot grafting where the cambium of the upper and lower stems is very sturdy. Therefore, this study used the V or wedge grafting method to measure the best rootstock length and number of scion shoots eyes in mango seedlings. The ability of each variety of mango plant to produce connection links correlates with the amount and speed of callus formation (Rukmana, 1999). Honey mango has a strong root system among existing varieties and supports the scion to grow. The rootstock is also compatible with other scion types (Supriati, *et al.*, 2016). Rootstock length is also a factor that can affect the success of seed production using the grafting method. According to Kartika (2019), the height of the rootstock affects the formation of callus and the union of the connections from the inside. This is influenced by hormones, food reserves, proteins, and root strength of the rootstock. According to Riady and Ashari (2017), shoot grafting with stem heights of 10 cm, 20 cm, and 30 cm is required. The number of scion shoots eyes and rootstock length are other factors that determine the success rate of the grafting method. The more shoots eyes used, the bigger the shoots will grow and produce more leaves. Pratama's research (2020) showed that the treatment of cuttings with four shoots eyes was most effective, this treatment affected all factors, including the percentage of survival, time of shoots emergence, shoots length, number of leaves at the age of 4, 5, 6, 7 and 10 weeks after planting, root length, and root wet weight. The best connection results can be obtained by conducting anatomical studies on the union of mango stem connections. According to Essau (1964), the anatomical structure of the mango stem can be used as an identification key because the results obtained are very different for each plant type.

Based on the description that has been discussed, it is necessary to carry out this research with the aim of knowing the effect of the number of shoots of the upper stem and the length of the lower stem on the success of grafting mango plants by knowing the growth of the grafting of mango plants which includes stem length, stem diameter, length of shoots break, number of leaves, and the anatomy of connection fusion as well as the percentage of success in grafting mango plants. The hope of this research is that we can apply this method to all types of mango plants, so that Indonesia can produce mangoes of high quality and quantity.

MATERIALS AND METHODS

This experimental research used a two-factorial Randomized Group Design (RAK), namely the first factor, rootstock length (25 cm, 30 cm, and 35 cm) and the second factor, the number of shoots eyes (3, 4, and 5). Thus, 9 treatment combinations with 3 replications were obtained, resulting in 27 experimental units. The treatment combinations consisted of 3 shoots eyes + 25 cm rootstock (E1B1), 3 shoots eyes + 30 cm rootstock (E1B2), 3 shoots eyes + 35 cm rootstock (E1B3), 4 shoots eyes + 25 cm rootstock (E2B1), 4 shoots + 30 cm rootstock (E2B2), 4 shoots + 35 cm rootstock (E2B3), 5 shoots + 25 cm rootstock (E3B1), 5 shoots + 30 cm rootstock (E3B2), 5 shoots + 35 cm rootstock (E3B3). This research was conducted at the Horticultural Seed Garden Pohjentrek, Pasuruan in April 2023-December 2023.

The rootstock used was honey mango seed from a mango hatchery located in Pasuruan City. Compatibility of honey mango is very suitable for grafting because it has uniform growth at the age of three months, strong root system and stem, resistance to pests and diseases, and water shortage. The scion used was mango arumanis-143. The fruit has a sweet flavor, the flesh is soft and fluffy, and the seeds are thin. The Horticultural Seed Garden (KBH) in Pohjentrek, the Horticultural Seed Development Unit of East Java Province, produced the scion used. To obtain the scion, the branch tips of the parent tree with a diameter of 1 cm were cut. Grafting was carried out in the morning from 10:00-12:00 WIB and lasted for two hours to ensure the grafting time between treatments and the moisture content of the scion (entres) did not change. Grafting was carried out using the V grafting method by cutting the rootstock according to the length of the treatment and splitting it until a gap was formed using a grafting knife. The number of shoots eyes of the entres used were E1 (3 shoots eyes), E2 (4 shoots eyes) and E3 (5 shoots eyes). Each entry was first cleaned of any moss or dirt attached to the entry before grafting. The grafting method was carried out by cutting the entry according to the treatment and slicing obliquely on both sides of the base to form a wedge or blunt axe blade measuring 3 cm. Subsequently, the rootstocks were cut according to the treatment, namely B1 (25 cm), B2 (30 cm), and B3 (35 cm). Each rootstock base was split into two parts 3 cm deep. Then the entres were inserted into the rootstock and all parts of the entres were wrapped by means of a talkup using plastic, so that water could not enter the connection or entres. After that, they were placed in a place that was shaded with paranet. Sample plants were observed weekly, starting two weeks after grafting, i.e. in the third week, and every other week for eight weeks of observation.

Anatomical preparations of mango stem grafting results were made using the paraffin method. The stem samples were cut into 1-2 cm lengths, then fixed by putting them into FAA solution and kept for 1x24 hours. After that, samples were washed and processed in graded alcohol in vacuum desiccator for 30-60 minutes per stage. Samples were then cleared in three steps of alcohol : xylol (3:1, 1:1, 1:3), each for 30 minutes before being soaked in xylol. After that, at the infiltration stage, xylol was replaced with xylol : paraffin (1:9), the samples were kept in an oven at 60°C for 1x24 hours. Next, samples were embedded in paraffin mold. After that, paraffin blocks were affixed to holder and trimmed. Samples in paraffin block were cut into section of 15 μm thickness, fixed in object glass with albumin and heated in oven. Sections were stained with safranin and counterstain fast green, then mounted cover glass using entellan. Results of stem grafting were evaluated using light microscope.

The data for this study included quantitative and qualitative data. Qualitative data include the anatomical characteristics of the connection union structure between the number of scion shoots eyes and the length of the rootstock, which were described descriptively. While the quantitative data in this study was the morphological characteristics of vegetative growth of mango seed grafting results such as stem length, stem diameter, length of shoots break, number of leaves, and percentage of grafting success analyzed statistically using Variance Analysis method (ANOVA), at 5% level (Utomo, 2018).

RESULTS

The different rootstock length treatments aimed to determine the most effective rootstock length for increasing shoot length. While the treatment of different number of scion shoots eyes is used to determine the most effective number of shoots eyes for *grafting*. This is done to increase the chance of shoot growth and increase the success rate of *grafting*. The success of *grafting* can be known from the growth of mango plants after *grafting* which consists of stem length (cm), stem diameter (cm), length of shoots break (days), number of leaves (strands), and percentage of *grafting* success (%). The average value of stem length growth is presented in Figure 1.

Based on Figure 1, the highest average stem length was produced by the treatment combination of 4 shoots eyes and 35 cm rootstock length (E2B3) at the age of 8 WAG which was 58 cm and the lowest average stem length was produced by the treatment combination of 3 shoots eyes and 25 cm rootstock length (E1B1) at the age of 3 WAG which was 15.67 cm. The average value of stem diameter growth is presented in Figure 2.

The observation of the largest stem diameter was produced by the treatment combination of 5 shoots eyes and 30 cm rootstock length (E3B2) at the age of 8 WAG, namely 1.47 cm and the lowest mean stem diameter was produced by the treatment combination of 3 shoots eyes and 25 cm rootstock length (E1B1) at the age of 3 WAG, namely 0.43 cm (Figure 2). The average growth value of the number of leaves is presented in Figure 3.

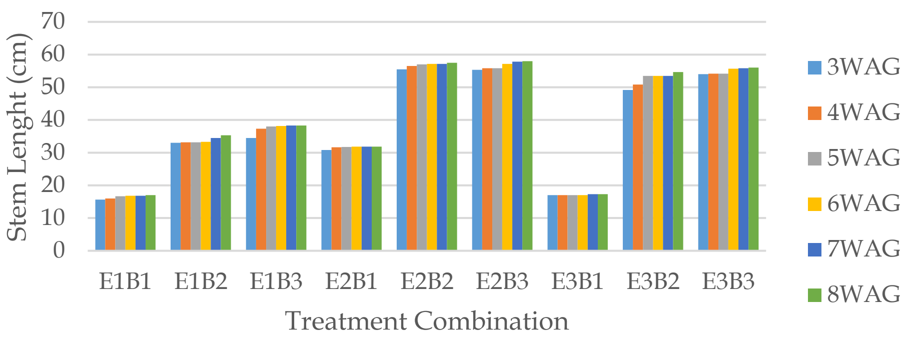


Figure 1. Graph of stem length growth (cm) due to the treatment combination between the number of scion shoots and rootstock length. WAG= weeks after grafting; E1B1= 3.25; E1B2= 3.30; E1B3= 3.35; E2B1= 4.25; E2B2= 4.30; E2B3= 4.35; E3B1= 5.25; E3B2= 5.30; and E3B3= 5.35.

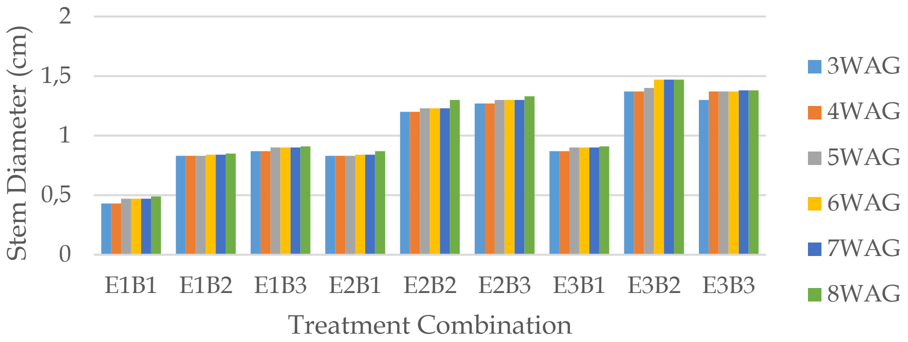


Figure 2. Graph of stem diameter growth (cm) due to the treatment combination between the number of scion shoots eyes and rootstock length. WAG= weeks after grafting; E1B1= 3.25; E1B2= 3.30; E1B3= 3.35; E2B1= 4.25; E2B2= 4.30; E2B3= 4.35; E3B1= 5.25; E3B2= 5.30; and E3B3= 5.35.

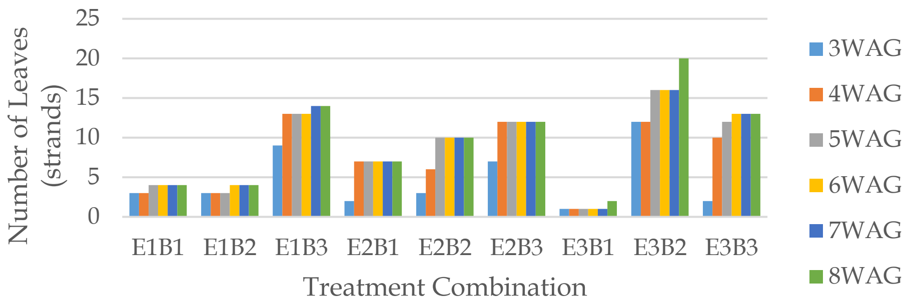


Figure 3. Graph of growth in the number of leaves (strands) due to the treatment combination between the number of scion shoots and rootstock length. WAG= weeks after connection; E1B1= 3.25; E1B2= 3.30; E1B3= 3.35; E2B1= 4.25; E2B2= 4.30; E2B3= 4.35; E3B1= 5.25; E3B2= 5.30; and E3B3= 5.35.

The observation of the number of leaves in Figure 3 shows that there are mango seeds that have grown the most leaves produced by the treatment combination of 5 shootsding shoots and rootstock length of 30 cm (E3B2) at the age of 8 WAG, namely 20 leaves and the lowest average number of leaves produced by the treatment combination of 5 shootsding shoots and rootstock length of 25 cm (E3B1) from the age of 4 WAG to 3 WAG, namely 1 leaf. The combination of the number of scion shoots and rootstock length can affect the growth of the number of leaves on mango seeds after *grafting*, this happens because the length of the rootstock serves as a place for the absorption of nutrients from the soil by the roots as a source of photosynthetic raw materials so that its role is very important. The results of the finished and perfectly connected mango connection seeds will grow and develop optimally, as shown by the addition of an increasing number of leaves (Figure 4). The average value of shoot rupture age growth is presented in Figure 4.

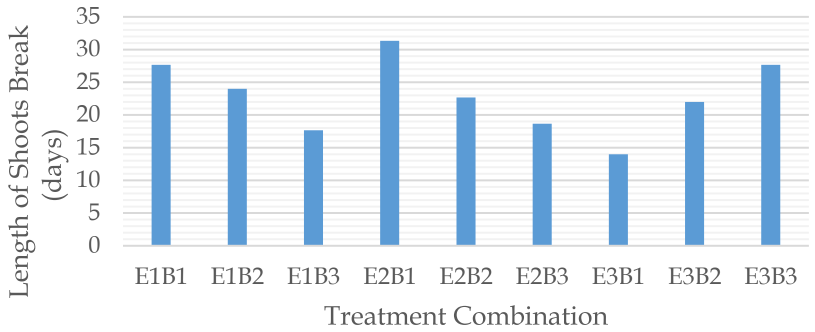


Figure 4. Graph of length of shoots break (days) due to the treatment combination between the number of scion shoots eyes and rootstock length. WAG= weeks after grafting; E1B1= 3.25; E1B2= 3.30; E1B3= 3.35; E2B1= 4.25; E2B2= 4.30; E2B3= 4.35; E3B1= 5.25; E3B2= 5.30; and E3B3= 5.35.

Based on the data in Figure 4, it shows that the treatment combination of 4 shoots eyes and 25 cm rootstock length (E2B1) gives the best shoot growth results at 14 days after *grafting* compared to other treatments. The longest shoot growth was produced by the treatment combination of 5 shoots eyes and 25 cm rootstock length (E3B1), which was 31.33 days after *grafting*. From this data, the difference in the number of scion shoots eyes and the length of the rootstock is directly proportional to the speed of shoots breakage. The average value of the percentage of *grafting* success at 8 WAG is presented in Figure 5.

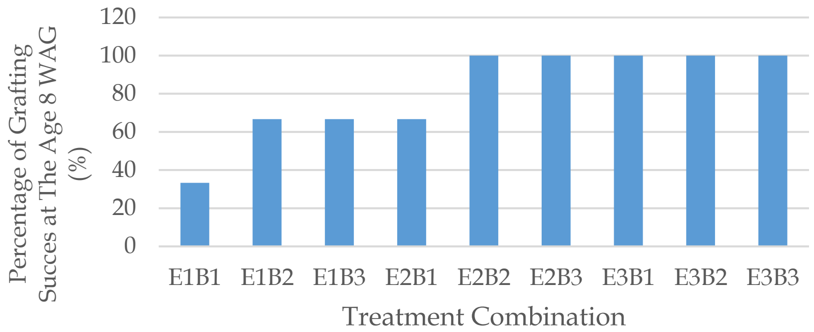


Figure 5. Graph of the percentage of grafting success at the age of 8 WAG (%) due to the treatment combination between the number of scion shoots eyes and rootstock length. WAG= weeks after grafting; E1B1= 3.25; E1B2= 3.30; E1B3= 3.35; E2B1= 4.25; E2B2= 4.30; E2B3= 4.35; E3B1= 5.25; E3B2= 5.30; and E3B3= 5.35.

Data on the percentage of success is obtained from the calculation of the number of live grafts divided by the number of grafts planted times 100% in each experimental unit. Based on the data in Figure 5, the lowest percentage of *grafting* success is found in the treatment combination of 3 shoots of the entres and rootstock length of 25 cm (E1B1) which is 33.33%, while the highest is found in the treatment combination of E2B2, E2B3, E3B1, E3B2, and E3B3 which is 100%.

The results of the data that have been processed with the F test using two-way ANOVA analysis, it is known that the resulting data is not significant, therefore the data is not subjected to further BNT test at the 5% level. This is because F count < F table, namely F count < 2.51 so that the treatment combination between the number of scion shoots eyes and rootstock length has no effect on all observation parameters (Table 1).

Table 1. Analysis of variance of the effect of the number of scion shoots and rootstock length of mango plants (*Mangifera indica* L.)

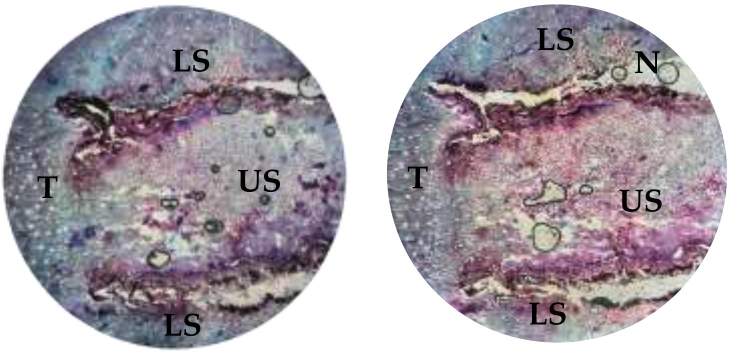
Observation Variables	F Test	
	F count	F table
Stem length (cm)	1.87	2.51
Stem diameter (cm)	1.19	2.51
Length of shoots break (days)	1.11	2.51
Number of leaves (sheet)	1.52	2.51

Percentage of *grafting* success (%)	0.90	2.51

Description: F value > F table indicates there is an effect of treatment, otherwise if F value < F table indicates there is no effect of treatment.

Microscopic observations of the success of *grafting* (shoot *grafting*) between the number of upper stem shoots (US) and the length of the lower stem (LS) of mango seeds were carried out when the graft had experienced growth as indicated by an increase in stem length, stem diameter and number of leaves. The results of microscopic analysis of *grafting* tissue fusion show that the method used to improve tissue attachment has an effect. The results of observations of the anatomy of mango seeds are presented in Figure 6.

Based on anatomical observations on the union after 24 weeks of treatment, the results are shown in Figure 6. This observation shows that the union (Figure 6-A) is the most precise compared to other treatment combinations because it has more shoots eyes, namely as many as 5 shoots eyes on the scion and rootstock which are then connected. The length of the rootstock used is 35 cm so that the available food reserves can be translocated to the scion (entres) more easily to grow into new trubus. The connection (Figure 6-B) showed a weakness, namely that there was a gap in the connection and the connection wound had not fully healed.



(A) (B)

Figure 6. Anatomical connectioning results in treatment combination E3B3 (A), and treatment combination E1B1 (B); Upper Stem (US); Lower Stem (LS); Trachea (T); Necrotic (N) (*Personal document*).

DISCUSSION

The results showed that all parameters observed did not interact due to the treatment combination between the number of shoots of the scion (entres) and the length of the rootstock. This occurred due to the non-uniformity of the mango seeds used. Based on the analysis of variance, the treatment combination of E2B2, E2B3, E3B1, E3B2, and E3B3 had a higher percentage of grafting success than the treatment combination of E1B1, E1B2, E1B3, and E2B1. This supports the research by Supriyono *et al.* (2020), which showed that the length of shading did not affect the success of grafting water guava shoots at the age of 30 Days After Splicing (DAG). In addition, there was no significant correlation between rootstock height and the number of scion shoots.

Grafting success of mango (Mangifera indica L.) plants in this study was recognized by characteristics including that the scion (entres) was still green, fresh, and there were shoots and leaves growing. However, it can also be seen that the mango seeds resulting from grafting fail if they have characteristics such as brownish, dry, shriveled, hard, and there are no shoots or leaves growing. The number of shoots that grow depends on the number of shoots eyes on the scion (entres). A greater number of shoots eyes on the entry resulted in an increase in the length of the entry. Longer entries affect stem length growth. Mariyati *et al.* (2020) investigated avocado and found that longer entries produced more leaves, shoots, and length of growing shoots.

It is possible that differences in plant stem length are influenced by the ability of the scion and rootstock to channel nutrient uptake from the roots to the leaves. This allows the photosynthesis process to run smoothly and nutrients can be transferred back to the rest of the rootstock. One sign that the grafting process is going well is the huge difference in plant growth speed (Sudjijo, 2009). Pruning the length of the scion shoots also affects the grafting process in increasing the activity of the shoots eyes. Sleeping shoots eyes receive the food reserves needed to grow. This causes the shoots eye to turn into a growing one. As a result, the amount of food available in the shoots eye increases, which allows for rapid shoots break (Popene, 1974). The success of the growth of new shoots is influenced by the carbohydrate component because it is able to spur the initial growth of shoots (Suliswati *et al.*, 2020). Harjadi (2009) states that the metabolism of food reserves in the scion (entres) consisting of carbohydrates will produce energy, which will encourage cell division and the formation of new cells.

Several factors, such as temperature and humidity, as well as the dormant period of the shoots eye, can also affect the timing of shoots emergence. High temperatures lead to low humidity, which causes the connection to fail. For successful grafting, the environment should have sufficiently high humidity. In addition, light greatly affects the timing of grafting, so grafting should be done in the late afternoon or early morning (Dastama *et al.*, 2022). Different types of growth will result from different shoot scions. In a connection with three scion shoots eyes, at shoots break, only the apical shoots break, and the apical shoots develops later on. After the apical shoots breaks and leaves appear, the internode length increases. Then lateral shoots appear, which take longer to form new branches than connections with 4-5 scion shoots eyes.

Based on the results of stem length growth, it is related to the right temperature when grafting affects the increase in length, so that various processes in the growth and development of plant cells, as well as internal plant factors can adapt to the extreme environment. As stated by Dastama *et al.* (2022), grafting should be done during the dry season and at temperatures and humidity that are not too high or too low. The ambient temperature should range from 25-32°C, if it is lower or higher than that, callus formation will be inhibited and will damage cells in the grafting area. Putri *et al.* (2016), sufficient food or energy reserves to repair cells damaged by wounding is a component that affects stem length. In general, the longer the stem, the more energy reserves. It is expected that energy reserves are greater along with the length of the entry, along with the length of the entry and the number of shoots eyes.

The more the number of scion shoots eyes also affects the increase in the number of leaves. This is in accordance with the explanation of Dastama *et al.*, (2022) that the process of leaf formation is caused by elongation, division of meristem cells from terminal shoots and lateral shoots to form new tillers. The increase in the number of leaves is influenced by the results of good connections and fuses quickly. The formation of new leaves will increase the rate of photosynthesis and the faster the rate of photosynthesis, the more leaves are formed (Lesilolo et.al., 2023). This is reinforced based on the results of research by Fitriyanto *et al.*, (2019) which states that, a perfect connection will process a good connection will deliver nutrients absorbed by the roots to the leaves and vice versa channeled to all parts of the plant. The process of cell division requires high energy which is absorbed by the roots, metabolic processes in plants can be disrupted due to poor plant tissue, resulting in hormone biosynthesis does not work optimally, causing the growth and development of leaves being inhibited. Studies show that rootstocks and scions that take place perfectly and quality will produce more leaves (Arlianzy *et al.* 2022).

Based on the data on the results of stem diameter growth, it occurs because the mango seeds after grafting have fused optimally so that the links between cambium cells in the scion and rootstock can differentiate actively divide quickly. This is reinforced based on the results of research by Suharjo (2019), explaining that stem diameter is a secondary growth that occurs due to the division of cambium cells inward to form xylem and outward to form phloem. When the cambium divides actively, the formation of cells runs quickly so that the growth of stem diameter is also faster. Secondary growth is when the cambium cells divide inwards to form xylem and outwards to form phloem. The formation of cells occurs quickly, so the diameter of the stem also increases quickly. The success of grafting (shoot grafting) is influenced by the skill of the grafter. One of the things to note is the V-cutting procedure. There is no need to cut many times; instead, the cut should be made evenly and the scion fused precisely into the cleft of the rootstock. This is a significant success in grafting. To obtain high-quality seeds, the scion and rootstock must be fused and form a good connection plane (Hartmann *et al.*, 2014).

Fathan *et al.* (2017) found that rootstock length treatments affected shoot length and number of leaves, but did not affect the percentage of top working success, shoot growth time, or durian shoot diameter. Callus formation and union of connections from the inside are affected by rootstock length (Kartika, 2019). Rootstock length is influenced by food reserves, hormones, and proteins in the stem as well as the root strength of the rootstock. For a perfect union process, the cut area must be connected for meristem tissue activity. This will happen if the two plants are compatible with each other, the cut is even, and the connection is not too strong or weak.

According to Ballesta *et al.* (2010), a strong linkage between scion and rootstock is essential for ideal connection growth. It is also important for nutrient transfer and water uptake in the plant. During the early stages, high growth, indicated by the growth of meristematic tissue from terminal shoots cells, will be more noticeable. The hormone auxin controls apical dominance in the terminal shoots portion. Auxin aids stem elongation and cell elongation. By pumping ions into the cell wall, auxin triggers protein compounds on the cell plasma membrane. This allows enzymes to work and partially break hydrogen chain bonds through the cellulose that makes up the cell membrane. In addition, water entering through the process of osmosis can help plant cells grow longer (Lestari, 2011). As a result, height growth will usually occur faster than diameter growth (Wilson, 2000).

Anatomical studies of the connection union show that suboptimal connections have gaps in the connection and the connection wound has not fully healed, which is characterized by the shape and size of the different vessel rings on the rootstock which are larger than on the scion so that a necrotic layer is still visible between the connection incision scars. On the other hand, optimal connection is characterized by the same shape and size of the vessel rings on the scion and rootstock due to the process of cambium cells of the two stems coming into contact with each other. The splice network of the best treatment combination was seen to develop over 24 weeks of grafting (Figure 6-A), with the appearance of secondary reticulum walls indicating an increase in the tracheal component made of callus.

Callus bridge formation at very slow rate can inhibit the formation of new cambium and vascular tissue. This is indicated by the width of the gap formed. In mango (Mangifera indica L.) grafting, the process of callus differentiation into cambium was not completed in most of the connections after 24 weeks of grafting. According to Hartmann *et al.* (2014), the mechanism of union begins when the scion and rootstock are injured during the grafting process, resulting in damage and death of a number of parenchyma cells. The living cells carried by the necrotic cells will divide profusely and expand past normal size to form tissue that covers the wound or callus. Necrotic tissue serves as an insulating layer that prevents contamination or infection of microorganisms.

According to Ridwan *et al.* (2015), the 100% grafting percentage in all clones tried was caused by a good link between the scion and rootstock due to strong binding during grafting and a good ability between the scion and rootstock to form new plants. This is in accordance with the observation of the most optimal treatment (Figure 4.6-A), which is characterized by an appropriate interlocking of the vascular tissue between the scion and rootstock. The treatment combination E3B3 (Figure 6-A) showed the most optimal attachment compared to treatment E1B1 (Figure 6-B). The most optimal tissue adhesion is characterized by the same shape and size of the vessel network circle on the scion and rootstock due to the process of unification of the cambium cells of the two stems in contact with each other. According to Elsheery *et al.* (2020), a connection area that shows fainter and more unified incision scars indicates that the linking process is happening well and the wound has healed.

In line with the results of research by Gusriani *et al.*, (2019) splicing will be more successful if the cambium links of the rootstock and scion are more numerous and the callus tissue forms faster. This is due to the compatibility between the scion and rootstock is good enough so that both can adjust each other to grow into new plant candidates. With the rapid compatibility process that occurs, the photosynthate from the photosynthesis process can be flowed quickly to all parts of the plant. Nutrients are utilized for the process of plant growth such as stem length, age of shoots break, number of leaves, and the percentage of success of grafting mango plants. When the grafted plants have connected perfectly and the cells between the rootstock and the scion can differentiate properly, the increase in the diameter of the rootstock occurs, because the cambium cells that have differentiated are actively dividing rapidly. This is reinforced based on the results of research by Suharjo (2019), showing that the growth of stem diameter occurs due to the division of cambium cells inward to form xylem and outward to form phloem. When the cambium divides actively, the formation of cells runs very quickly so that the growth of stem diameter is also faster.

The connection (Figure 6-B) shows a weakness in that there is a gap at the connection and the connection wound has not fully healed. This is because the grafting between the scion and rootstock was not precise and there were a few uneven parts during grafting. In addition, there is still a large gap, where the process of tissue fusion takes place relatively slower, characterized by the shape and size of the different vessel circles on the rootstock which are larger than on the scion so that a necrotic layer is still visible between the connection incision scars. The compatibility between scion and rootstock needs to be considered. The plant chosen as the rootstock must be compatible with the scion so that the rootstock can link and support the growth of the scion, and the condition of the plant must have food reserves (energy) to accelerate the grafting process (Hartmann *et al.*, 2014).

To obtain high quality mango seeds, the rootstock and scion must be able to form a perfect connection plane. This result is in line with the research of Sari (2012) who found that the unique compatibility of each type of scion and rootstock can cause differences in the connection. Plant connections will die due to imperfections in the cambium cells between the scion and rootstock. Research by Handayani *et al.* (2013) found that when the vessel network environment of the scion and rootstock is not precise, the growth becomes slower and the union of the cells of the two stems is inhibited. As a result, the grafting process may fail. Although incompatibility is not a quantitative trait that can be measured, varying degrees of incompatibility can be distinguished, from mild interference with normal plant development to death of the rootstock, scion or both (Gautier *et al.* 2021). It is possible that this incompatibility is due to trait differences that exist between the genotypes used. Anatomical structures, physiological, and biochemical traits differ due to the distance of relatedness of rootstocks used in vegetative propagation (Prawoto *et al.* 2004).

Therefore, in mango (Mangifera indica L.), the better combination of the number of scion shoots eyes and rootstock length is the combination of 4-5 scion shoots eyes and rootstock length of 30-35 cm. The high percentage of grafting success in this study is due to the combination of treatments where the process of connecting can be accelerated because the young tissue is still dividing. According to Rahmi (2018), the union process is influenced by the availability of food reserves and hormones in the rootstock. Food reserves will be converted into energy during the grafting process. The success rate of the connection increases if the scion and rootstock used are compatible. This is based on the experimental results and histology analysis, which showed that the combination of 3 scion shoots eyes and 25 cm rootstock length had a lower percentage of live seeds and a lower rate of tissue connection union.

CONCLUSION

The combination of the number of scion shoots eyes and rootstock length has no interaction in all observation parameters consisting of stem length, stem diameter, length of shoots break, number of leaves, and percentage of *grafting* success. V-*grafting* method with 4-5 scion shoots eyes and 30-35 cm rootstock length gave the best *grafting* success percentage on mango (*Mangifera indica* L.) seedling growth. The anatomical structure of the connection union from the treatment combination between the number of scion shoots eyes and rootstock length influences the tissue adhesion. A less than optimal connection has a gap in the connection and the connection wound has not fully healed, which is characterized by the shape and size of the different vessel circles on the rootstock which are larger than on the scion so that a necrotic layer is still visible between the connection incision scars. Optimal connectioning is characterized by the same shape and size of the vessel network circles on the scion and rootstock due to the process of unification of the cambium cells of the two stems that come into contact with each other.

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Authors:
Aulia Ayu Fitrisia, Wahyu Safira Wulandari, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Surabaya, Jl. Ketintang Gedung C3 Lt.2 Surabaya 60231, Indonesia, e-mail: aulia.20015@mhs.unesa.ac.id
Rinie Pratiwi Puspitawati, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Surabaya, Jl. Ketintang Gedung C3 Lt.2 Surabaya 60231, Indonesia, e-mail: riniepratiwi@unesa.ac.id
Ahmad Bashri, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Surabaya, Jl. Ketintang Gedung C3 Lt.2 Surabaya 60231, Indonesia, e-mail: ahmadbashri@unesa.ac.id

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