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Influence of rootstock age and gibberellin on the survival rate of clove (*Syzygium aromaticum* L. Merr. & Perr.) grafting

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Abstract: Clove (*Syzygium aromaticum*) is a cross-pollinated crop with significant genetic variation in planting materials derived from seeds. Vegetative propagation through grafting is a valuable strategy to achieve genetically identical characteristics in progeny. This study investigates the influence of rootstock age and hormone (gibberellin) treatments on the grafting union and survival rate of top clove grafting under greenhouse conditions. The experiments followed a randomized block design with three replications, each containing 10 samples, and involving two factors: rootstock age (4, 6, and 8 weeks old) and gibberellin (GA_3) concentrations (0 and 1 mg L^{-1}). The variables observed include success percentage, survival percentage, number of leaves, chlorophyll content, and examining graft unions through a Scanning Electron Microscope (SEM). The results reveal that 4-week-old rootstocks treated with GA_3 exhibited better-grafting plants, with a success rate of 81.33%, survival rate of 56.67%, an average leaf count of 4.56, chlorophyll content of 32.69%, and the absence of cavities in the graft union. This study highlights the importance of rootstock age and GA_3 treatment in optimizing clove grafting for enhanced production. GA_3 treatment and SEM analysis on the graft union of clove grafting is the novelty of this research.

Key words: Vegetative propagation, plant growth regulator, chlorophyll content, success rate, scanning electron microscope

1. Introduction

Clove (*Syzygium aromaticum* L. Merr. & Perr.), native to Indonesia's Moluccas islands, has been grown for its spices throughout the country, as well as in other tropical countries. High clove production could be achieved through intensification, rehabilitation, and rejuvenation using high-quality planting materials (Pratama and Darwanto, 2019).

Clove trees face 80% cross-pollination (Pool and Bermawie, 1986), causing genetic variation. Asexual propagation, although rare due to low success rates, maintains the genetic identity of the mother tree in a high yield and produces identical plants. However, low meristematic activity limits vegetative propagation (Thangaselvabai et al., 2010), and techniques like cuttings, marcotting, budding, and top grafting have been attempted with limited success (Darwati et al., 1988; Darwati et al.,1993; Suryadi et al., 2020).

Grafting success in woody plants is influenced by factors like rootstock compatibility, weather, grafting technique, rootstock types, and scion health, with season affecting softwood grafting success (Haldankar and Jadhav, 2001). *Allanblackia stuhlmanni* (Munjuga et al., 2013) and *Annacardium occidentale* L (Chipojola et al., 2013) show high success rates in grafting, which includes top-cleft grafting with coppiced scions and tea grafting using lignified and green shoots with one leaf and one internode (Korkutal et al., 2011; Ranjith and Ilango, 2017). Grafting is a technique for enhancing plant tolerance to abiotic stresses and pests and involves the cutting and uniting of 2 separate plants (Nanda and Melnyk, 2018), regulated by phytohormones and phloem connections (Melnyk et al., 2015).

Hormones and plant growth regulators influence graft unification (Gainza et al., 2015), while auxins cause vascular tissue differentiation (Koepke and Dhingra, 2013; Aloni et al., 2021). Incompatible grafting can occur due to polyphenols at the top of the joint (Lattanzio et al., 2006), preventing auxin transport essential for the growth and development processes. The low indole-3-acetic acid concentration in imperfect grafting unions affects xylem, phloem differentiation, and lignification (Koepke and Dhingra,

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2013; Aloni et al., 2021). Auxin promotes vascular development, wound healing, and graft response, and regulates gene expression during grafting. Exogenous auxin initiates grafting (Melnyk et al., 2015; Yin et al., 2012). Cotyledons are essential for auxin in plant growth and graft formation (Procko et al., 2014), and their removal or inhibition can hinder hypocotyl graft reunion in Arabidopsis (Matsuoka et al., 2016).

In addition, cytokines regulate plant cell division, lateral root development, meristem maintenance, and cambium activity (Matsumoto-Kitano et al., 2008; Immanen et al., 2016). Gibberellins, a diterpene, promote plant growth and development by fostering cell expansion, differentiation, and proliferation. They also stimulate xylogenesis in cambium tissue (Claeys et al., 2014; Davière and Achard 2013). Gibberellin treatment improves graft success in Okra by enhancing survival rates of GA-deficient mutant seeds (Aminu et al., 2019). Inhibiting GA production or signaling in grafted Arabidopsis hypocotyls inhibited cortical cell spread, while vascular tissue proliferation remained unaffected (Matsuoka et al., 2016). Balancing GA and cytokinin levels in scion grafts can enhance vascular bundle formation, influencing cell differentiation signaling and the mitogen-activated protein kinase (MAPK) pathway, crucial for cell proliferation and differentiation (Feng et al., 2017).

The success of grafting depends on callus tissue proliferation and vascular tissue union, influenced by plant species, graft type, and environmental variables like temperature and moisture (Kiran, 2019). This study investigates the impact of rootstock age and hormone treatment on clove top grafting, focusing on the survival rate of top clove grafting under greenhouse conditions.

2. Materials and methods

This study used clove rootstocks of different ages (4, 6, and 8 weeks) with a 1.5–2 mm diameter along with specific growth regulators: Ascorbic acid (AA), 1-Naphthaleneacetic acid (NAA), 6-benzylaminopurine (BAP), and Gibberellic acid (GA_3) (Sigma, USA). The experiment followed a randomized block design with two factors, resulting in nine treatment combinations, and each was replicated three times with 10 samples per replication. The first factor examined rootstock age, while the second factor involved various treatments, including (1) immersion of shoots for 2 h in a solution containing 25 mg L^{-1} AA as control, (2) 25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 0 mg L^{-1} GA3, and (3) 25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA3. Application of NAA and BAP was adapted from Prakash et al. (1999) for tea grafting, but the concentrations were adjusted for clove micrografting.

2.1. Rootstock preparation

The clove seeds were collected from the high-yielding trees in a farmer's clove plantation in West Java, Indonesia. The seeds were germinated in plastic trays, and afterward, the seedlings were transplanted into polybags measuring 15 × 20 cm filled with soil. Before grafting, the rootstocks were examined to calculate plant height, stem diameter, and the number of leaves. Phenol content in the stem and leaves was quantified using a modified method adapted from Marinova et al. (2005). The content of plant hormones, i.e., IAA, GA_3, kinetin, zeatin, and abscisic acid (ABA), was measured on leaves using a TLC scanner, according to Unyayar et al. (1996).

2.2. Scion preparation

Scions were collected from the clove trees of the Zanzibar variety grown in an experimental garden in West Java, Indonesia. Juvenile clove shoots were cut 10 cm long. All leaves were removed prior to grafting, aside from the first pair of fully developed leaves which was left intact, then immersed in the treatment solutions described above.

2.3. Grafting

Grafting was performed on 4-, 6-, and 8-week-old rootstocks grown in polybags with intact cotyledons. The top grafting method was applied, including decapitating rootstock shoots, splitting the middle of the stems, and creating a V-shaped scion base; then, the scions were carefully inserted into the cut stem. A 2-3 mm diameter silicone hose and plastic tape were used to hold the rootstock and scion union, then covered with plastic bags. Fungicide was added to the watering of the growing media, and then the grafted plants were maintained in a controlled environment at 25–26 °C with 70%–80% humidity. Success rates were monitored at 2, 4, 6, and 8 weeks after grafting before acclimatization.

2.4. Acclimatization

Eight weeks after grafting, the plants were acclimatized in a 60% shaded screen house, initially covered with perforated plastic bags for a week. For evaluation, survival rate and leaf count were recorded at 1, 2, and 3 months after acclimatization, with leaf chlorophyll content measured at the 3-month mark using a portable digital chlorophyll meter (Konica Minolta SPAD 502 type). Graft union examination via Scanning Electron Microscope (SEM) occurred 3 months after acclimatization, following the method by Talbot and White (2013) adapted for cloves. Morphological observations on plant growth were made at 1 and 1.5 years after grafting.

2.5. Data analysis

Parameters of plant characteristics and hormone content were analyzed with one-way ANOVA, while success rate, survival rate, number of leaves, and chlorophyll content parameters underwent two-way ANOVA. If significant differences were found, Duncan's multiple range tests (DMRT) were applied at a 5% significance level.

3. Results

3.1. Phenol, plant hormones, and characteristics of rootstock before grafting.

The plant height, stem diameter, leave number, and total phenol content increased with seedling age (Table 1). The phenolic content at rootstock aged 4 weeks was the lowest and differed significantly from 8-week-old rootstocks. Regarding the plant growth regulators content of clove rootstocks such as IAA, GA_3, zeatin, kinetin, and ABA (Figure 1), the contents at the youngest rootstock age were the lowest. The content increased with the age of the rootstock. These plant hormones play essential roles in plant growth. Kinetin was the highest content among all plant growth regulators. Kinetin content increased to 1.4-fold at 6 weeks, and 4.3-fold at 8 weeks. Gibberellin content increased to 1.7-fold at 6 weeks, and then to 3.8-fold at 8 weeks. IAA content in the rootstock at 4 weeks was reasonably high, compared to the other plant growth regulators, but still lower than kinetin. It increased to 3.4-fold at 8 weeks. Unlike kinetin, zeatin content was the lowest among all plant growth regulators. The content of zeatin remained stagnant for up to 6 weeks but increased to 4.3-fold at 8 weeks. ABA content has increased to 1.8-fold at 6 weeks and 4-fold at 8 weeks. At 8 weeks, the highest plant growth regulators found in clove rootstock were kinetin, followed by ABA, IAA, GA_3, and zeatin.

3.2. The success and survival rate, the growth of acclimatized, as well as SEM of clove grafting

The graft success was affected by rootstock ages (Table 2). The grafting success rate was similar at 4 and 6 weeks after treating the rootstock with NAA, BAP, and GA_3. However, at 8 weeks, and especially 2 weeks after grafting, there were differences with all treatments. Only the control treatment and without GA_3 showed a difference between 4 and 8

Table 1. Plant characteristics and phenol content on clove rootstock before grafting. The same letters are not significantly different from each other at DMRT. Data are expressed as *p<0.05 (One-way ANOVA).

Rootstock ages (weeks)	Plant height (cm)	Stem diameter (mm)	Number of leaves	Phenol (mg/100 g)
4	4.51 a	0.96 a	2.61 a	7310 a
6	7.41 ab	1.30 b	4.31 ab	9455 ab
8	8.39 b	1.51 c	5.01 b	11,756 b
CV (%)	13.97	4.72	11.89	19.72

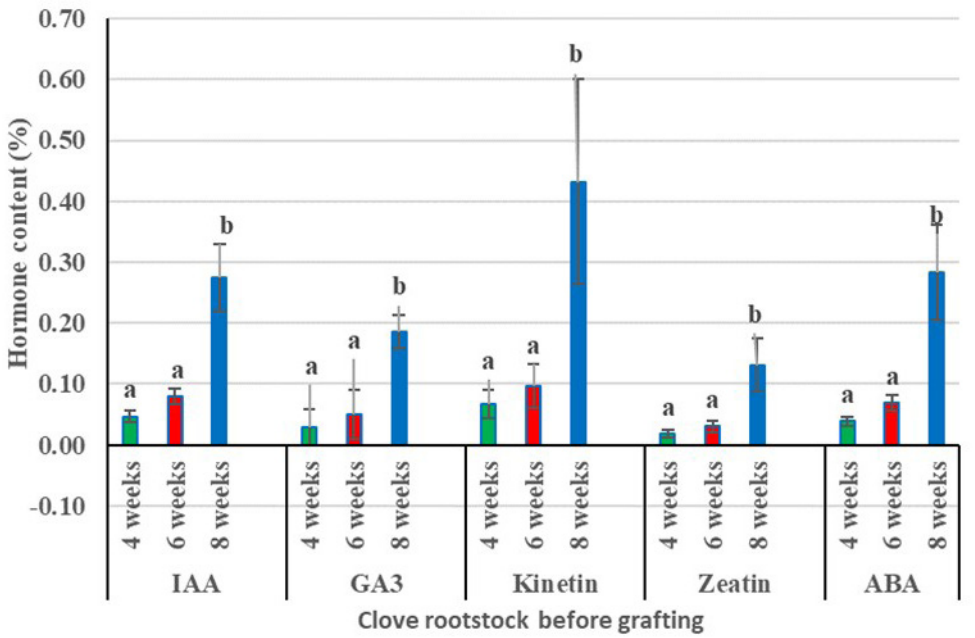

Figure 1. Contents of plant growth hormones on clove rootstock before grafting at 4, 6, and 8 weeks. Values are means ± SE (n = 2). Significant differences (*p < 0.05) are based on Student's t-tests (one-way ANOVA).

Table 2. The success rate (%) of clove grafting in a controlled room. The same letters in each column are not significantly different at DMRT. Data are expressed as *p < 0.05 (two-way ANOVA).

Treatment		Weeks after grafting			
Rootstock age (weeks)	AA and Plant Growth Regulators	2	4	6	8
4	25 mg L^{-1} AA (Control)	86.33 ab	79.33 a	75.33 a	77.67 a
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	93.67 a	83.33 a	79.00 a	77.00 a
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	96.67 a	88.67 a	84.33 a	81.33 a
6	25 mg L^{-1} AA (Control)	89.33 ab	81.33 a	77.00 a	71.00 a
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1}BAP	89.33 ab	76.67 a	70.33 a	64.00 a
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1}BAP + 1 mg L^{-1} GA_3	84.33 ab	78.67 a	75.00 a	71.33 a
8	25 mg L^{-1} AA (Control)	73.00 bc	57.33 b	43.00 b	39.00 b
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	66.33 c	59.00 b	51.33 b	45.33 b
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	89.33 b	82.33 a	74.33 a	67.67 a
Significances:					
1st Factor: Rootstock ages		*	*	*	*
2nd Factor: Doses of GA_3		*	*	*	*
Interaction: Rootstock age × Doses of GA_3		*	*	*	*
CV (%)		11.29	12.39	13.59	15.73

weeks after grafting. The percentages of graft survivability were influenced by different age rootstocks (Table 3).

The number of leaves on the grafted plants showed no significant differences among treatments throughout the observation period (Table 4). However, after acclimatization, leaf count was slightly higher at 1, 2, and 3 months in plants grafted with 4-week-old rootstock. In contrast, older rootstocks showed minimal leaf growth, except for an 8-week-old rootstock treated with GA_3 1 mg L^{-1}, which exhibited a twofold increase in leaves after 1 month of acclimatization, with no further development from 1 to 3 months. The number of leaves per graft varied with rootstock age, with the lowest count at 8 weeks. A higher number of leaves was detected in 4 and 6 weeks. Additionally, chlorophyll levels were significantly higher in 4-week-old rootstocks than in 6- and 8-week-old ones (Table 5).

The scanning electron micrograph revealed the condition of the graft union. Grafting of cloves using 4-, 6-, and 8-week-old rootstocks showed the presence of big cavities in all control treatments, indicating less unification between rootstocks and scions. The cavity was also still present in all treatments without GA_3, but the size of the cavity was much larger than in treatment with GA_3. The grafted plants using 4 weeks of rootstocks showed that cell fusion between rootstock and scions was formed, and callus formation was much better in the graft using 2 mg L^{-1} NAA + 10 mg L^{-1} BAP with 1 mg L^{-1} GA_3 (Figure 2). On the older rootstocks, with and without GA_3 treatment, the callus was less formed, and unification between rootstock and scion cells was also reduced, hence the development of the cavity (Figures 3 and 4). The size of the cavity is even more prominent in the older rootstock. The success of graft union at 4-week-old rootstocks, treated with 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3, showed good union at the graft joint up to 1.5 years after grafting. However, 8-week-old started to show an excessive overgrowth at 1 year after grafting, and at 1.5 years after grafting, and it also displayed more prominent callus growth (Figure 5).

4. Discussion

4.1. Phenol, plant hormones, and characteristics of rootstocks before grafting.

There have been very few reports on the topic of clove grafting. Study on hormone content and rootstock aged on clove grafting has not been reported. Additionally, there is little information available on phenol content, which is significant considering that the phenol content in the rootstock could affect the success of grafting. Several phenolic compounds regulate cell division, development, and differentiation at the graft union (Gainza et al., 2015). Plant graft incompatibility is partly determined by phenolic compounds (Mahmoud et al., 2017). Phenolic compounds hinder grafting success by oxidizing to phenoxy radicals,

Table 3. Survival rate (%) of clove grafting after acclimatization in the screen house. The same letters in each column are not significantly different at DMRT. Data are expressed as *p < 0.05 (two-way ANOVA)

Treatments		Months after acclimatization		
Rootstock ages (weeks)	AA and Plant Growth Regulators	1	2	3
4	25 mg L^{-1} AA (Control)	44.87 bc	44.67 b	44.67 b
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	56.67 a	56.67 a	56.67 a
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	56.67 a	56.67 a	56.67 a
6	25 mg L^{-1} AA (Control)	13.33 e	13.33 e	13.33 d
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	40.00 c	33.33 c	33.33 c
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	46.67 b	46.67 b	46.67 b
8	25 mg L^{-1} AA (Control)	11.33 e	11.33 e	11.33 d
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	22.33 d	22.33 d	11.33 d
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	56.33 a	33.33 c	33.33 c
Significance:				
1st Factor: Rootstock ages		*	*	*
2nd Factor: Doses of gibberellin		*	*	*
Interaction: Rootstock × Doses of GA_3		*	*	*
CV (%)		7.09	7.48	7.50

Table 4. Numbers of leaves of clove grafting after acclimatization in the screen house. The same letters in each column are not significantly different at DMRT. Data are expressed as *p < 0.05 (two-way ANOVA)

Treatments		Months after acclimatization		
Rootstock ages (weeks)	AA and Plant Growth Regulators	1	2	3
4	25 mg L^{-1} AA (Control)	3.58 ab	4.06	4.72
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	4.00 a	4.50	5.50
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	4.11 a	4.22	4.56
6	25 mg L^{-1} AA (Control)	3.89 a	4.11	4.78
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	3.00 ab	4.00	4.67
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	4.00 a	4.00	4.00
8	25 mg L^{-1} AA (Control)	4.00 a	4.00	4.00
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	4.00 a	4.00	4.00
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	2.00 b	4.00	4.00
Significances:				
1st Factor: Rootstock ages		*	*	*
2nd Factor: Doses of gibberellin		*	*	*
Interaction: Rootstock × Doses of GA_3		*	*	*
CV (%)		24.81	20.86	18.89

negatively affecting scion-rootstock connection and cell processes in various plant species (Leng et al., 2010), such as araticum plants (Usenik and Stampar, 2002), and *Vitis* sp (de Almeida et al., 2014).

This is why the phenol content in clove rootstocks was analyzed before grafting (Table 1). The data showed that 4-week-old rootstock has the lowest phenolic content. As the rootstocks age, their phenolic content increases. Specifically, it increases to 1.3 times in 6-week-old rootstocks and 1.61 times in 8-week-old rootstocks. Phenolic content of 14.68% was identified in the scions from mature trees, which is quite a high rate (Suryadi et al., 2020), while in the rootstocks it was 11.76%. The high phenolic content in the scions could affect grafting success on older rootstocks.

Table 5. Chlorophyll content (%) of clove grafting 3 months after acclimatization. The same letters are in each column not significantly different at DMRT. Data are expressed as *p < 0.05 (two-way ANOVA).

Rootstock ages (weeks)	AA and Plant Growth Regulators	Chlorophyll contents (SPAD)
4	25 mg L^{-1} AA (Control)	31.30 ab
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	31.72 a
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	32.69 a
6	25 mg L^{-1} AA (Control)	28.90 c
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	29.15 c
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	29.47 bc
8	25 mg L^{-1} AA (Control)	27.96 c
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP	28.35 c
	25 mg L^{-1} AA + 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3	29.09 c
Significances: 1st Factor: Rootstock ages 2nd Factor: Doses of gibberellin Interaction: Rootstock × Doses of GA_3		 * * *
CV (%)		3.87

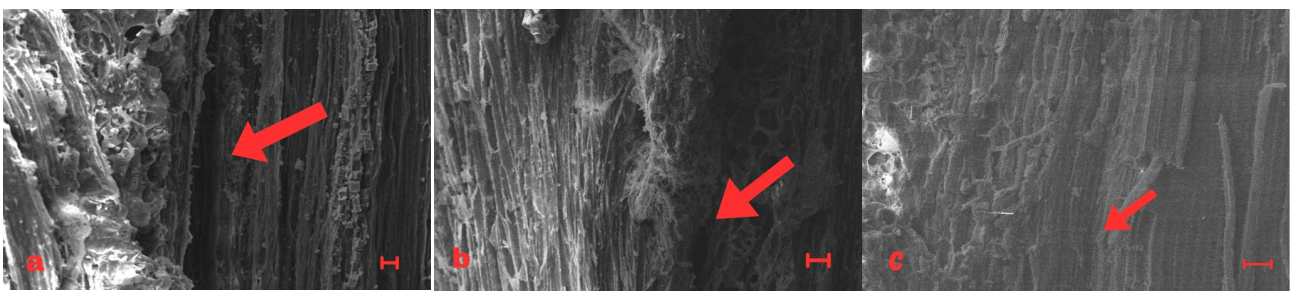

Figure 2. Scanning electron micrographs of the grafting union on 4-week-old rootstock: (a) control, (b) without GA_3, and (c) with GA_3. The red arrows indicated the cavity between rootstock and scion cells. (Magnification 250×, scale bar 20 µm)

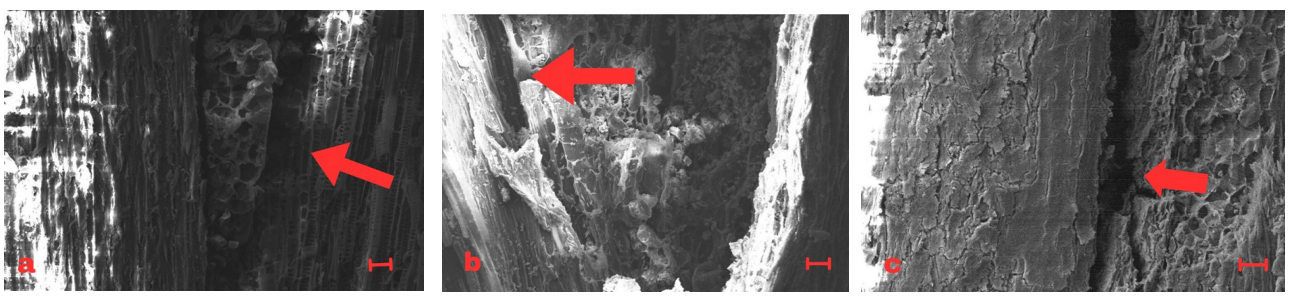

Figure 3. Scanning electron micrographs of grafting rootstock on 6-week-old: (a) control, (b) without GA_3, and (c) with GA_3. The red arrows indicated the cavity between rootstock and scion cells. (Magnification 250×, scale bar 20 µm)

Overall, this data implies that the age of rootstocks and the phenolic content in both scions and rootstocks should be considered when planning grafting procedures.

However, phenolic oxidation would be inhibited when AA is in the cell walls (Takahama, 1993). In this study, 25 mg L^{-1} AA was applied in all scions to inhibit the oxidation of phenolic compounds. The data in Table 2 indicates that AA treatment had a similar success rate in all control treatments, except at 8-week-old rootstock. The treatment of 25 mg L^{-1} of AA effectively neutralizes the detrimental ef-

Figure 4. Scanning electron micrographs of grafting rootstock on 8-week-old rootstock: (a) control (magnification 50×), (b) without GA_3, and (c) with GA_3. The cavity between rootstock and scion cells (red arrows). (Magnification 250×, scale bar 20 µm)

Figure 5. Grafting with rootstock treated 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3 aged: (a) 4 weeks at 1 year after grafting, (b) 8 weeks at 1 year after grafting, (c) 4 weeks at 1.5 years after grafting showed no excessive callus growth, and (d) 8 weeks showed excessive callus growth at 1.5 years after grafting. The yellow circle is grafting connection part.

fect of higher phenolic compounds in the older rootstock. Ascorbic acid is a cofactor of GA enzyme, i.e., gibberellin 3-β-dioxygenase enzyme, and AA can boost GA activity. Ascorbic acid is also a cofactor for many major enzymes, mitosis, and plant cell development (Pastori et al., 2003). External application of AA in scions before grafting can increase AA in scions. That implies it can increase the activity of GA_3, which plays a role in cell elongation and promotes growth (Smirnoff and Wheeler, 2000). Plants that have low AA levels cause GA deficiency, which could inhibit cell elongation and plant growth (Mazid et al., 2011). Treatment that employs a higher concentration of AA could give better results, but further studies are needed to confirm this.

Suryadi et al. (2020) utilized ascorbic acid at a concentration of 75 mg L^{-1} on a 4-week-old rootstock with a high survival rate. In our research, 25 mg L^{-1} treatment obtained a lower survival rate of 11.33%. Even with the addition of NAA (2 mg L^{-1}) and GA_3 (1 mg L^{-1}), the survival rate could not reach those reported by Suryadi et al. (2020). This study underscores the significance of ascorbic acid (AA) in achieving higher survival rates. However, it is worth noting that no specific investigation was carried out by Suryadi et al. (2020) regarding the quality of the grafted union on further graft development.

The low endogenous GA_3 in clove rootstock affects cell division activity in the wounded area. Thus, the application of exogenous GA_3 gives better plant growth. There is a significant rate of difference when the rootstock is 8 weeks old compared to the control and GA_3-free treatment groups at 4, 6, and 8 weeks after grafting. Notably, applying GA_3 to 8-week-old rootstock substantially enhances the success rate compared to the control and GA_3-free treatment groups, particularly benefiting older rootstocks (Table 2).

Gibberellin regulates vascular plant growth by affecting cambium activity, xylem fiber differentiation, expansion, and secondary plant growth. GA_3 is essential for successful grafting, influencing vascular protein gene expression, and stimulating cell elongation and expansion. Grafting success depends on the healing ability of rootstock and scion after mechanical injuries, with auxins from vascular

strands being critical regulators of vascular tissue development (Gainza et al., 2015; Koepke and Dhingra, 2013).

Yin et al. (2012) found that auxin accumulating in graft unions is linked to graft union formation. The survival rate of grafted seedlings is also affected by ABA. In this study, auxin levels were measured in the rootstocks before grafting, which were low in young rootstocks but increased in older ones. Although older rootstock had higher IAA and ABA levels, their success rate was lower. Increasing auxin with synthetic auxin (NAA) immersion did not significantly change success rates compared to the control (without NAA), except when combined with GA_3. This study shows the importance of GA_3 for successful grafting.

4.2. The success and survival rate, the growth of acclimatized, as well as SEM of clove grafting

Based on the success and survival rate, the grafting growth condition and SEM results revealed that grafting in younger rootstocks (4 weeks) was more successful than in 6- and 8-week-old rootstocks. The same results were also found in *Syzygium cumini* (Devi et al., 2018). In papaya, the success of grafting was significantly influenced by the rootstock age (Nguyen and Yen 2018). In this study, the percentage of survived grafted plants decreased 2 weeks after grafting due to the inability to form callus in the union of the scion with the rootstock. Callus formation did not develop well; as a result, the vascular network, which functions to transport nutrients, water, minerals, and photosynthetic products, is hampered.

The reduced survival rate may be due to a lack of nutritional and water transport from rootstock to the scion part, which may be attributed to the low unification of the grafts.

These results indicated that young rootstocks are more likely to be successful than older ones. Older plants generally possess more prominent vacuoles, and the phenolic compounds accumulate in them. The age of the rootstock is related to its ability to regenerate meristematic activity, causing the faster formation of calli and healing on the graft union. The grafted plants may require longer recovery after wounding due to grafting.

Significant differences existed among the treatment's graft survivability at 1, 2, and 3 months after acclimatization. After the grafting was transferred to the screen house for acclimatization, the survival rate decreased, presumably due to changes in temperature and humidity. Even though the survival rate decreased, this study showed that applying NAA, BAP, and GA_3 gave significantly different results from the control.

The success rate in this study means the percentage of surviving grafts at the age of 1 month in the treatment room (preacclimatization) under ambient conditions of approximately 23–25 °C and 80%–85% humidity. The survival rate refers to the percentage of grafts that remain alive after acclimatization to natural environmental conditions with temperatures of 30–35 °C and humidity around 60%–65%, indicating their readiness for transplantation to the field. Clove, a tropical plant, thrives in an environmental condition with a temperature range 30–35 °C. Since acclimatization at this temperature closely corresponds to the clove's natural growth conditions, the temperature during the acclimatization process is not regulated.

Chlorophyll is a solar energy absorber that transforms the sun's radiation into chemical energy via photosynthesis. The amount of chlorophyll of clove grafting after 3 months of acclimatization peaked in 4 weeks of the rootstock with 1 mg L^{-1} GA_3 (32.69%) (Table 5). Chlorophyll content measurement is not conducted at 1 and 2 months of age since the leaves have yet to develop fully, making them insufficient for measurement. High chlorophyll content may enhance plant growth and the development of grafted plants. Better chlorophyll content at 4-week-old grafting may be associated with the condition of the grafted union. An increase in chlorophyll concentration will result in a higher rate of photosynthesis capacity (Mahulette et al., 2020). In this study, the total chlorophyll content of the grafted seedlings was measured 3 months after acclimatization. The chlorophyll content is significantly higher in younger rootstock than older ones. The lower chlorophyll content on rootstock older than 4 weeks is caused by imperfect union. The lower chlorophyll content in the older rootstock may be due to the lower uptake of nutrients, especially nitrogen, by the roots of grafted seedlings with older rootstocks (Souza et al., 2019). Chlorophyll content has been used as a predictive method to assess grafting success (Neves et al., 2016).

Examining the quality of the grafting union is crucial to ensure robust growth once the transplanted grafts are placed in the field, particularly given the numerous grafting failures, which may occur 1, 2, or many years after the graft is made. Overgrowth often occurs above, at, or below the graft union, resulting in a visible difference in the trunk diameters (Neves et al., 2016). Therefore, to support the experimental data on the grafting success and survival, we analyzed the graft union using SEM, and this is the first report in clove.

Based on scanning electron microscopy, the graph union on a rootstock that was 4 weeks old also demonstrated improved connection. While the content of plant growth regulators was insufficient for callus formation, the cavity between the graph connections was still produced. However, the callus developed after 1 mg L^{-1} GA_3 treatment, and cell unification took place, eliminating the cavity (Figure 2). Gibberellin encourages cell proliferation, differentiation, and expansion, which leads to the formation of a callus and the fusion of rootstock and scion cells (Claeys et al., 2014).

The cavity is still present in all treatments with GA_3 but much smaller than in the control (without NAA + BAP + GA_3). This cavity, present in all grafts except those with 4-week-old rootstocks + 1 mg L^{-1} GA_3, might explain the slower plant growth in older rootstocks, as indicated by fewer new leaves, but did not differ much from 1, 2, and 3 months after acclimatization. However, compared to rootstock characteristics before grafting, the grafted plants had fewer leaves after grafting, possibly because they needed time to recover from the grafting process. Grafting wounds used endogenous auxins for recovery instead of leaf development. The cavity formation in grafted plants also blocked the transport of water, nutrients, and photosynthate, hindering plant growth. In grafting with a good union, water, nutrient movement from the rootstock, and photosynthate distribution were efficient, leading to robust plant growth (Rasool et al., 2020). In this study, the number of leaves in grafting using 4-week-old rootstocks increased with the seedling age (1, 2, 3 months old), but the increase was not statistically different. The number of leaves in older grafting remains unchanged from 1, 2, and 3 months. Grafting success is significantly influenced by the rapid formation of a graft union between scion and rootstock, which fuse to form a compact plant. The healing of vascular tissues following grafting causes the exchange of nutrients and mineral elements to become more intense. It improves the signal transduction between the shoot and root (Li et al., 2017), thereby catalyzing plant growth and development.

The initial stages of successful grafting involve the adhesion of the two partners, callus formation, and the development of a functional vascular system. The rootstock/scion graft must be compatible and closely united to allow the intake and transfer of nutrients, minerals, water, assimilates, and hormones. The biochemical and functional changes at the graft interface due to incompatibility can result in a blockage of carbohydrates at the scion, which is above the graft union. This lack of understanding makes it challenging to identify the mechanisms that govern the formation of the rootstock/scion union, which limits the development of novel grafting techniques that can overcome the problem of incompatibility (Gainza et al., 2015).

A compatible and intricate interaction between the two components of the graft, the scion and rootstock, is necessary for successful grafting. It comprises a complex network of molecular processes at the graft junction linked to the physiology and growth of the scion. It requires the coordinated action of nutrient hormonal, metabolic transcriptional, and epigenetic pathways. Figure 2 indicates that cavities were not found in the graft union at 4-week-old rootstock with gibberellin. Grafting of rootstock at 6- and 8-week-old showed cavities in all treatments (Figures 2 and 3). This condition might be due to hormones such as gibberellin, which play a crucial role in the interaction between scion and rootstock. Applying GA_3 develops the xylem and induces xylem formation, cambium activity, and cellulose production. The graft success was also indicated by no cavities as visualized by SEM. This research shows that using 4-week-old rootstock treated with 2 mg L^{-1} NAA + 10 mg L^{-1} BAP + 1 mg L^{-1} GA_3 significantly affects perfect grafting union up to 1.5 years after grafting (Figure 5). This study indicated that the optimal approach for clove grafting involves 4-week-old rootstocks treated with 2 mg L^{-1} NAA + 10 mg L^{-1} BAP with 1 mg L^{-1} GA_3.

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Conflict of interest

There are no conflicts of interest related to this publication. As the corresponding author, I affirm that the manuscript has been read, reviewed, and approved for submission by all authors.

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