The effect of ascorbic acid on clove (Syzygium aromaticum) grafting

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Abstract. Clove plants can be generatively propagated by seeds and vegetatively by grafting. However, the success of clove grafting is still low (<50%), presumably due to the high content of phenol in the stem which inhibits callus formation and causes connection failure. The aim of the study was to obtain the best ascorbic acid concentration to reduce phenol content in the clove stem. The study was conducted at the Ecophysiology Laboratory, ISMCRI from January to June 2019. The study was arranged in randomized block design, 9 treatments with 4 replications. The treatments were ascorbic acid concentrations: 0 ppm (control), 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm, 175 ppm, and 200 ppm. The parameters observed were percentage of grafting survival rate, shoot length and number of leaves.

The results showed that ascorbic acid had significant effect on the percentage of grafting survival rate, shoot length and number of leaves. The highest percentage of grafting survival rate (75%) was obtained at an ascorbic acid concentration of 128.93 ppm while the lowest was control (47.50%).

Keywords: Syzygium aromaticum, grafting, ascorbic acid, phenol

1. Introduction

Domestic Clove needs to support the cigarette industry reach 120,000 tons/year or absorb up to 90% of national Clove production and amount to 70.99% of world clove needs [1]. In 2017, the total imports are 13,572 tons with a value of US $ 113.47 billions [2]. The area of clove plantation in 2017 was 559,566 ha with production of 113,178 tons and productivity of 345 kg / ha. The productivity of cloves still has the potential to be increased through the use of superior clove plant material, such as Karo varieties with the potential to produce dry cloves 2.3 tons/ha.

Clove plants are reproduced generatively and vegetatively. In general, clove plants are propagated generatively, vegetative propagation is still not done much and the success rate is still low (<50%). One reason is the large amount of phenol found in plant kingdoms [3]. Phenol in plants tends to accumulate in high carbon conditions which exceeds the need for growth and also the accumulation of phenylalanine which is a phenylpropanoid synthesis substrate due to the lack of protein synthesis. The internal balance causes the accumulation of phenol and nitrogen deficiency which can suppress plant primary metabolism [4]. Phenol compounds are the result of plant secondary metabolism which plays an important role in plant resistance [5] and is formed in response to stress and injury [6]. Phenol compounds oxidized by Polyphenol Oxidase (PPO) produce quinones. Highly reactive quinone reacts spontaneously to polymerize proteins and other cellular components that produce melanin black pigments amor [7] that interfere with metabolism. The accumulation of phenol compounds oxidized by PPO forms necrotic and poor callus levels on the part of the rootstock and scion result in imperfect
grafting union [6;8;9]. The stem of olive plant of the Ayvalik and Domat varieties has high phenol compounds such as 4-Hydroxyphenylacetic acid and ferulic acid causing disruption in the differentiation of callus tissue in the cambium [10].

One method to increase the success of grafting in plants that have high phenol content is to use antioxidants such as polyvinylpyrrolidone (PVP), ascorbic acid (AA), or citric acid [11]. Ascorbic acid as an antioxidant and contributes to detoxification so that it can suppress the formation of toxic compounds such as quinone, H₂O₂ etc which are harmful to plant metabolism and can damage plant cells [12]. Ascorbic acid not only functions as the main antioxidant in plant cells but also supports membrane-bound essential antioxidants in its role as cellular protectors [13]. In clove plants, efforts to suppress oxidation of phenol content can be done by using AA. Currently there have been no reports of the ascorbic acid application effect as an antioxidant to increase the success of clove grafting. This study aims to obtain ascorbic acid concentrations that can increase the success and growth of clove grafting.

2. Materials and method

The research was conducted from January to June 2019 at the Ecophysiology Laboratory, ISMCR1 Bogor, West Java. The experiment was arranged in a randomized block design with 9 treatments and 4 replications. The single treatment of ascorbic acid concentration level was tested at 0, 25, 50, 75, 100, 125, 150, and 200 ppm. The percentage of grafting survival rate, plant height, leaf number, stem diameter at 2 mm above grafting connection, chlorophyll content, and phenol content at 4 months after grafting was observed.

The media for rootstock were prepared using a 15 x 20 cm polybag filled with soil. The clove Zanzibar seeds that have germinated are transferred to the polybag, grafting was done after the rootstock was 4 weeks old. Scions are prepared using Zanzibar clove plants (Syar no. 17), 10 cm long, soaked in ascorbic acid according to the treatment for 2 hours. The grafting used the top graft method. Rootstock and scion are combined with a 2 mm silicone hose diameter and then wrapped with tape. The planting media is watered with 0.2% fungicide as much as 100 ml so that it is not attacked by fungi, then grafting is covered with transparent plastic. The grafted plants were kept at a temperature of 24-26° C and 70-80% of humidity. After 30 days old grafted plants were acclimatized at a 60% shade condition. The grafted plants were covered with a holed plastic bag during a week. The phenol content in clove scion was analyzed after soaking AA for 2 hours using the Spectro method [14]. Leaf chlorophyll content was measured using the portable digital chlorophyll meter (Konica Minolta SPAD 502 type). The data were analyzed using ANOVA and tested further with Duncan multiple range tests (DMRT) at a 15% level if there were any significant differences because many factors influence the success of grafting in Myrtaceae. Previous studies' success on Myrtaceae only 20 % - 87.5%, [15]. The phenol content was analyzed by the descriptive statistics.

3. Results and discussion

3.1. Percentage of grafting survival rate and phenol content

The treatment of clove scion soaking in an ascorbic acid solution can reduce the phenol content (Figure 1). Scion that was soaked with 75 ppm ascorbic acid can reduce phenol content 12.24% compared to control that is without immersion treatment which produces the highest phenol content (14.68%).
The content of phenol in scion or rootstock can affect the success rate of the grafting. The treatment scion in to 75 ppm ascorbic acid gave the lowest phenol content (12.24%) and the highest percentage of grafting success (75%) whereas the control treatment had the highest phenol content (14.68%) but the lowest percentage of clove grafting success (47.50%). The regression analysis of the percentage of clove grafting success showed that the optimal ascorbic acid concentration was 128.93 ppm ($r^2 = 74.3\%$) (Figure 2). The high phenol compounds will inhibit the growth and regulator of IAA activity and increase oxidative decarboxylation of IAA [15]. Phenol compounds are found in parenchymatic cells and are a limiting factor for the success of grafting in araticum plants [16]. Phenol compounds in *Vitis* sp. plant identified as benzoic acid (gallic acid), three cinnamic acids (caffeic acid, ferulic acid and sinapic acid) and two flavonols (catechin and epicatechin) which have the potential to cause incompatible grafting [17]. Phenol compounds influence cell division, development and differentiation of the scion and rootstock connection because phenol accumulation will occur in the injured part such as the scion and rootstock connection [7] which is a major problem in graft compatibility [18].

Phenol compounds, especially r-coumaric acid and flavonoids, cause inhibited callus formation so that the cause of the connection is incompatible [9;19]. Phenol will be oxidized by PPO which causes necrosis and poor callus [6]. Poor callus influences new cambial cells which differentiate from new callus formation and continuing to form cambial connections between scions and rootstock. Furthermore, prior binding of vascular cambium across the callus bridge, initial xylem and phloem begin to differentiate. At the first repair xylem wound differentiated from the tissue to form a union bridge, followed by the wound-repair phloem [20]. After grafting, the concentration of Flavonols (catechins and proanthocyanidins) also increases due to stress induced during wound healing. The occurrence of vacuolar membrane disruption which results in the release of phenol from the vacuole into the cytoplasm causing dysfunction in the growth of certain tissues (xylem and phloem), disrupting lignin synthesis and hormonal imbalance, resulting in incompatible joints [21;22;23].
Figure 2. The relationship between ascorbic acid and percentage of grafting survival rate. The value means ± SE of 40 plants.

3.2. Growth of grafting plant

The scion treatment soaked in ascorbic acid solution showed a significant different on shoot length and number of leaves compared to the control, whereas the stem diameter was not significantly (Table 1). The highest of shoot length (13.25 cm), leaves number (7.75 sheets) and stem diameter (1.52 mm) were obtained in AA treatment with concentrations of 75 ppm, 125 ppm and 125 ppm, respectively. Whereas the lowest of shoot length (9.30 cm), leaves number (4.5 sheets) and stem diameter (0.98 mm) were resulted in the control treatment. The application of AA to clove grafting increased growth in shoot length, number of leaves and stem diameter compared to without AA. [24] reported that a lack of AA in the vtc1 arabidopsis mutant resulted in late plant growth and flowering. The function of ascorbic acid is not only an antioxidant but also a cofactor in a large number of key enzymes, mitosis and cell growth in plant [25]. External application of AA causes an increase AA in plants. The functions of AA as a cofactor of the Gibberellin 3-β-dioxygenase enzyme which can increase its activity. The Gibberellin 3-β-dioxygenase enzyme plays a role in Gibberellin (GA) biosynthesis which functions as cell elongation and promotes growth [26]. Plants that have low AA levels cause GA deficiency which has the function of cell elongation and induces plant growth [12].

| Treatments | Ascorbic acid (ppm) | Shoot length (cm) | Stem diameter (mm) |
|------------|---------------------|-------------------|-------------------|
|            |                     |                   |                   |
|       | Leaves number (average) | CV (%) |
|-------|-------------------------|--------|
| 0 (control) | 9.30 a                  | 0.98 a |
| 25    | 12.75 b                 | 1.16 a |
| 50    | 12.60 b                 | 1.17 a |
| 75    | 13.25 bc                | 1.18 a |
| 100   | 12.50 bc                | 1.17 a |
| 125   | 12.45 bc                | 1.52 a |
| 150   | 13.08 b                 | 1.24 a |
| 175   | 11.25 c                 | 1.24 a |
| 200   | 11.03 c                 | 1.34 a |
| CV (%)| 8.56                    | 19.92  |

Numbers followed by the same letter in the same column were not significantly at DMRT 15%.

The correlation between AA dosage with leaves number formed quadratic regression function with correlation coefficient 85.4%, the optimal dose of ascorbic acid was 124.46 ppm (Figures 3).

Figure 3. The relationship between ascorbic acid and leaves number. The value means ± of 40 plants.

The average temperature and humidity in the room is lower (24.80 °C and 71.46%) than in transparent plastic shells (24.94 °C and 71.52%) (Figures 4). Changes in temperature and relative humidity, especially during grafting affected the process of grafting union formation [27].
3.3. Chlorophyll content

The soaking treatment of scion into ascorbic acid solution has an effect on the chlorophyll content of clove leaves, although statistically there is no significant difference. The AA treatment of 200 ppm produced the highest chlorophyll content in clove leaves (40 SPAD), while the lowest was control (31.58 SPAD) (Figure 5). Ascorbic acid solution significantly affected the chlorophyll content, and 3 g • dm⁻³ ascorbic acid produced the highest chlorophyll content in the leaves of the stem [28].
Figure 5. The relationship between ascorbic acid and chlorophyll content. The value means ± SE of 40 plants.

4. Conclusion

The application of AA in clove scion reduced phenol content and increased the success of grafting, and growth of clove grafting. The concentration of AA between 124.46 ppm to 128.98 ppm gave the highest grafting result to grafting success and number of leaves in the clove grafting plant. To optimize clove grafting the addition of growth regulators is needed.

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