Compatibility of Rimau Gerga Lebong (RGL) mandarin grafted onto three genotypes of citrus rootstock

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Abstract. Introduction of Citrumelo and Kanci, an alternative citrus rootstock, into citrus cultivation system in Indonesia as complement of Japansche citroen (JC) is very appreciated. Compatibility of Citrumelo and Fortunella to commercial citrus in Indonesia have not been evaluated. The aim of the study is to assess the compatibility of Rimau Gerga Lebong (RGL) mandarin citrus grafted onto JC, Citrumelo, and Kanci based on anatomical, morphological and physiological parameters. The results showed that 100% of RGL mandarin shoot bud grew on Citrumelo and JC rootstocks, but only 88.75% on Kanci rootstock. Anatomically, RGL mandarin at 60 days after budding (DAB) had perfectly adhered on Citrumelo rootstock and not yet fully adhered on JC and Kanci rootstock. Morphologically, RGL-Citrumelo and RGL-JC combinations at three years old plant resulted in the same plant growth parameters, better than RGL-Kanci combination. Physiologically, RGL-Citrumelo and RGL-JC combinations had the same chlorophyll index, better than RGL-Kanci combination. RGL-Citrumelo and RGL-JC combinations showed graft compatible and RGL-Kanci combination showed late incompatibility.

1. Introduction

Grafting is a traditional method of asexual propagation in fruit crops that is widely used to produce seedling which does not reproduce true-to-type from seed [1]. This method creates a single plant by surgically attaching the shoot bud from one plant (the scion) to the stocks, joining their vascular and cambial systems [2]. Plant grafting originated during the ‘Warring States’ period of China’s history, and is thus more than two thousand years old [3]. Today, grafting is commonly used for seedling production of many types of plant, including fruit trees, vegetables, and flowers.

Citrus is one of the most economically important fruit crops in the world. Because of the long juvenile period of citrus trees (often more than six years), citrus propagated by grafting commercial citrus onto a rootstock to shorten the juvenile phase [4]. A specific cultivar for the rootstock influences the size, fruit quality, yield, tolerance/resistance to biotic and abiotic stresses [5–9]. Active interaction of rootstock and scion resulted an impact to scion. The most important interactions are compatibility or incompatibility between rootstocks and scion. There is no precise definition of ‘graft compatibility’; it generally means the establishment of a successful graft union as well as prolonged...
survival and proper functioning of the single plant, grafted plant [10]. Nevertheless, there was not rootstock compatible to all citrus scion and grew well at all biotic and abiotic conditions.

Citrus fruit production areas in Indonesia generally use Rough lemon (RL) and Japansche citroen (JC) (Citrus limonia Osbeck). Both of the rootstocks are not native of Indonesian citrus rootstock but they are adaptive and popular citrus rootstocks used in Indonesia. These rootstock types cause scions to grow rapidly, lead to broad canopy, and produce high yield, produce fruits with large size, low total soluble solid (TSS) and high acidity [11]. Nonetheless, it’s high susceptibility to citrus sudden death [12]. Citrus sudden death (CSD) is a highly destructive disease and has caused the eradication of millions of trees in southern Brazil within the last 15 years [13]. CSD can be transmitted by grafting [14], and the successful transmission by unknown aerial biotic vectors [13]. Introduction of other rootstock genotype into citrus cultivation systems in Indonesia necessary to be done.

Citrumelo (Citrus paradisi Macf. cv. Duncan x Poncirus trifoliata (L.) Raf) and Kanci (Fortunella sp.)) are the potential citrus rootstock to be used in citrus cultivation system in Indonesia. Citrumelo (Citrus paradisi Macf. cv. Duncan x Poncirus trifoliata (L.) Raf) is characterised by its tolerance to Citrus tristeza Virus (CTV) [15], nematodes, Phytophthora spp., citrus sudden death [12], and the superior fruit quality it confers on the scion, when compared to Rangpur lime rootstock. The use of Fortunella as rootstock caused tree size and canopy volume of citrus scion smaller than other rootstock [16,17]. However, compatibility of Citrumelo and Fortunella to commercial citrus in Indonesia have not been evaluated.

The aim of the study is to assess the compatibility of Rimau Gerga Lebong (RGL) mandarin citrus grafted onto JC, Citrumelo, and Kanci based on anatomical, morphological and physiological parameters. RGL mandarin one of the important mandarin citrus varieties in Indonesia that has good characteristics, such as big size, good taste, and high productivity.

2. Material and Methods

2.1. Plant Materials

The shoot bud of mandarin citrus trees cv. Rimau Gerga Lebong (RGL) grafted onto six months old of three citrus rootstocks genotypes (Japansche citroen/JC (Citrus limonia Osbeck), Citrumelo (Citrus paradisi Macf. cv. Duncan x Poncirus trifoliata (L.) Raf) and Kanci (Fortunella sp.)) were used for this study. All materials were grafted by using chip budding method [5]. Plant materials were planted in the experimental field of Indonesian Citrus and Subtropical Fruits Research Institute (ICISFRI). There were two experimental groups, anatomical observation on seedling phase and morphological observation on three years old plant. More than 100 seedlings per graft combination and five adult plants per graft combination were used for this study.

2.2. Anatomical Observation

Anatomical observation of graft union observed was the adherence of scion to rootstock. The observation was conducted on samples of 60 days after budding (DAB) seedling of RGL mandarin grafted onto Citrumelo, JC, and Kanci rootstocks. 0.5-cm long fresh samples obtained from 60 DAB seedling were cut transversely. Microscopic observation of the grafting union was conducted by Scanning Electron Microscopy (SEM) (Merk FEI-Inspect-S50) at 250 x magnification.

2.3. Morphological Observation

Morphological observations of graft union were conducted on three years old RGL mandarin grafted onto Citrumelo, JC, and Kanci rootstocks. The scion/rootstock compatibility was evaluated by making bark sections removed from the grafting line. One to three score used to decide the compatibility degree (score 1 for low compatibility, 2 for medium compatibility, and 3 for high compatibility) adapted from (Figure 1.) [18].

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**References**

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The following vegetative growth parameters were evaluated from each tree: trunk diameter (in cm, -measured at 5 cm below and above the grafting line), plant height (in m, -measured from the soil to the top of the plant), and the canopy diameter (in m, -measured perpendicular to the planting line). The average canopy volume (in m$^3$) was determined using the diameter and canopy height measurements using the formula $V=\frac{2}{3}\pi \times (D/2)^2 \times H$, where $V$ is the canopy volume (m$^3$), $D$ is the diameter of canopy (m), $\pi = 3.14$ and $H$ is the plant height (m) [19].

2.4. Physiological Observation

The physiological parameter evaluated was the leaf chlorophyll index. The leaf chlorophyll index was estimated using a portable Chlorophyll Content Meter (CCM-200 plus, Opti-Sciences). Measurements were taken in the fully expanded leaves.

2.5. Statistical analysis

The experiment was conducted with a randomized block design with combination grafting factors (RGL-JC, RGL-Citrumelo, RGL-Kanci), five replications. Each data of the observation variable was evaluated by one-way ANOVA with the LSD advanced test at 5% level of probability. Data processing and analysis were carried out using the GENSTAT 19th edition software program.

3. Result and Discussion

The result of the observations on RGL mandarins grafted onto three types of rootstocks (JC, Citrumelo and Kanci) showed that shoots bud grew up in the fourth week after grafting. All of shoots bud of RGL mandarins grew on JC and Citrumelo rootstocks, while on the Kanci rootstock only 88.75 percent and 11.25 percent of shoot buds observed was dormant, turned brown and then death. Graft success was indicated by grew and graft failure was indicated by dormant, turned brown, and then death of the shoot bud. Graft failure can be caused by anatomical mismatching, poor craftsmanship, adverse environmental conditions, disease, graft incompatibility [20], and failure of adherence and differentiation of the vascular cambium tissue. The attachment between shoot buds and rootstocks in the early phase is associated with cell deposition and polymerization in response to injury [21]. When the cambium of the scion joins fully with that of the rootstock, intact cells divide and proliferate into calli, which eventually differentiate into vasculature and plasmodesmata forms [22].

Observation on 60 DAG graft union of RGL mandarins grafted onto Citrumelo, JC and Kanci rootstocks show that graft union of RGL mandarin on JC and Kanci rootstocks still have more callus tissue than on Citrumelo rootstock. Based on anatomical observation, RGL mandarin grafted onto Citrumelo rootstocks adhered better than that of JC and Kanci rootstocks. Graft union of RGL mandarin on JC and Kanci rootstocks show some parts that were not yet fully adhered (Figure 2). The success or failure of grafting primarily depends on the ability of the graft union to enable rapid development of vascular connections between the rootstock and the scion [23–25]. Vascular regeneration is a complex process that includes structural differentiation of the parenchymatous tissue.
from both sides of the graft union into xylem and phloem [23,26] and varies according to the scion/rootstock combination [18].

**Figure 2.** Morphological and anatomical observation of 60 DAG RGL mandarin grafted onto Citrumelo, JC and Kanci rootstocks (arrow: graft union).

Based on morphological observation of graft union of RGL mandarin on JC, Citrumelo and Kanci show that graft union of RGL mandarins on all of three type of rootstocks were no visible connection line and showed high compatibility (Figure 3). However, trunk diameter of scion and rootstock in all combination varied. RGL-Citrumelo combination had a bigger diameter than RGL-JC and RGL-Kanci combinations, respectively. Ratio of scion to rootstock diameter in RGL-JC, RGL-Citrumelo, and RGL-Kanci combinations were same (Table 1). Trunk diameters in scion and rootstock combinations of several species is mostly related to genetic traits of plant growth, rather than to true incompatibility between both materials [27]. Moreover, the similarity between scion and rootstock trunk diameters at the grafting union does not always correlate with graft compatibility. Functional vascular tissue connection is more necessary for the graft. Signaling molecules, such as polypeptides in the phloem, could be transported through the functional vascular, which is significant in cell recognition and compatibility between the graft union [28].

**Figure 3.** Graft morphology of three years old RGL mandarin citrus grafted onto Citrumelo, JC and Kanci (arrow: graft union).
Table 1. Scion stem diameter, rootstock stem diameter and ratio of scion to rootstock stem diameter of three years old RGL mandarin citrus grafted onto Citrumelo, JC and Kanci.

| Combination | Scion stem diameter (cm) | Rootstock trunk diameter (cm) | Ratio of scion to rootstock trunk diameter |
|-------------|--------------------------|-------------------------------|-------------------------------------------|
| RGL-Cit     | 49.12 c                  | 53.70 c                       | 0.915 ns                                  |
| RGL-JC      | 41.80 b                  | 45.20 b                       | 0.925 ns                                  |
| RGL-K       | 34.00 a                  | 38.80 a                       | 0.876 ns                                  |

Means in each column followed by the different letter are significantly different. ns = not significant.

Compatibilities between scion and rootstock affected plant physiology and plant growth as a result of water, nutrient and photo-assimilate flow [29]. In this study, RGL mandarin grafted onto Kanci rootstock had trunk diameter, plant height, canopy volume, number of leaves, and chlorophyll index less than that of JC and Citrumelo rootstocks (Figure 3, Table 2). Several researchers also reported that citrus rootstocks from the *Fortunella* group produced lower canopy volumes and plant heights than other rootstock types [17,30]. In this study, RGL mandarin grafted onto Citrumelo and JC rootstock had the same value in all parameters observed (Figure 4, Table 2). The same condition also occurred in “Jaffa” sweet orange, that “Jaffa” sweet orange grafted onto Citrumelo had the same canopy volume and plant height [31].

Figure 4. Performance of three years old RGL mandarin citrus grafted onto Citrumelo, JC and Kanci.
Table 2. Plant height, canopy volume, number of leaves, leaves area and Chlorophyl index of RGL mandarin citrus grafted onto Citrumelo, JC and Kanci

| Combination     | Plant height (m) | Canopy volume (m³) | Number of leaves | Leaves area (cm²) | Chlorophyl index |
|-----------------|------------------|--------------------|------------------|-------------------|------------------|
| RGL-Cit         | 1.96 b           | 2.32 b             | 6373 b           | 24.17 ns          | 82.25 b          |
| RGL-JC          | 1.85 b           | 1.77 b             | 6061 b           | 20.97 ns          | 78.51 b          |
| RGL-K           | 1.52 a           | 0.85 a             | 4000 a           | 25.20 ns          | 51.34 a          |

Means in each column followed by the different letters are significantly different. ns = not significant

Plant growth is influenced by several factors (environmental conditions, plant nutritional status, and hormone activities) related to different physiological processes. In grafted fruit trees, the physiological processes influenced by vascular connections that also affected to uptake and translocation of mineral nutrients in plants. The compatible combinations had higher chlorophyll content and net photosynthetic rate, presenting stronger field growth potential [28]. Plants with partial phytohormone, nutrients, and carbohydrate translocations will be a dwarf plant and maybe have late incompatibility.

4. Conclusion

All of RGL mandarin shoot buds grew on Citrumelo and JC rootstock, but only a part of them grew on Kanci rootstock. Anatomically, RGL mandarin grafted onto Citrumelo showed better adherence at early phase of grafting than that of JC and Kanci. At three years old plant, RGL-Citrumelo and RGL-JC combinations resulted the same plant growth parameters and chlorophyll index and better than RGL-Kanci combination. RGL-Citrumelo and RGL-JC combinations showed graft compatible and RGL-Kanci combination showed late incompatibility.

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