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Research Article
Techniques for Accelerating of Scion Growth in Pummelo Grafting (*Citrus maxima* (Burm.) Merr.)

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Abstract

Background and Objective: Pummelo can be propagated by using many methods including seedling, cutting, air layering, grafting and culturing tissue. One of them is driven by the technique of accelerating scion growth. This study aimed to find out the proper scion growth acceleration techniques for growing the grafted scion. **Materials and Methods:** The research was conducted from March-June, 2017. This study used Randomized Block Design (RBD) with 6 treatments and 4 replications including B_0 (no treatment), BAL (bending the rootstock above the inserted scion), BAP (cutting off the rootstock 2 cm above the inserted scion), BAK (cutting half the rootstock 4 cm above the inserted scion), BAR (cutting half of the rootstock 4 cm above the inserted scion and bending it) and BAPL (cutting the rootstock 20 cm above the inserted scion and bending it). The obtained observation data were analyzed by using F-test (5%). If there was a significant effect, then LSD test (5%) was conducted. **Results:** The results showed that cutting the rootstock above the inserted scion and bending the rootstock above the inserted scion had significantly accelerated the growth of the scion of grafted Pummelo plants compared to other treatments. Bending the rootstock above the inserted scion resulted in the longest scion length at the end of the observation (90 days) by 15.2 cm, compared to cutting the rootstock above the inserted scion which resulted smallest growth of 98%, less than bending the rootstock for length of 8.96 cm. **Conclusion:** The treatment of cutting and bending the rootstock above the grafting area accelerates the rupture of the buds of the pummelo plants resulted from the grafting compared to other treatments.

Key words: Acceleration, *Citrus maxima* (Burm.) Merr., grafting, pummelo, scion

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pummelo is a type of plants from the citrus genus which is popular among people. Pummelo or large oranges have a special characteristic of having a larger size than ordinary oranges with the shape of fruit flesh similar to ordinary oranges. In Indonesia, Pummelo is a plant that has potential development because it provides a high economic value both in fresh and in processed products. It has a lot of nutritional contents which are effective for human health. Pummelo fruit skin contains β-sitosterol for the treatment of stomachaches, diarrhea, headaches and eye problems. Its flowers are used as tranquilizers and the raw fruits containing limonin and geraniol can be used for the treatment of asthma, cough, hiccups and epilepsy[1-3].

Even though Pummelo is a potential commodity, its development in Indonesia is still limited. Pummelo production is relatively low at approximately 5% of total orange production. The total national commodity of Pummelo in 2012-2016 fluctuated with the downward trend from 5.608-5000 ha with the total production of 113.375-124.252 t and its productivity[2] reached 20.22-27.88 t ha^{-1}. This productivity is still categorized as low compared to its potential which can reach[3] up to 40 t ha^{-1}.

The measures to meet the increasing demand on Pummelo can be conducted by developing high quality Pummelo seeds to ensure high productivity. One way to obtain quality seeds is from grafting. Pummelo plants are generally cultivated using seedlings resulting from grafting. Grafting is one of the methods of plant propagation, in which one plant part (upper stem) is attached to another plant part (rootstock), so that there are two plant parts growing together into a new plant[4]. The purpose of grafting is to combine two different cultivars into a composite plant consisting of the upper stem, rootstock and interstock parts each of which has special characteristics. It also rehabilitates the physically damaged plants[5]. In grafting, a problem that may arise is dormancy or inability of the plant to start growing from meristems, organs and other cells which possess the ability to perform growth in favorable conditions[6]. The problem of bud dormancy results in not only economic losses but also waste of time for regrafting[7].

Basically, the technique of accelerating shoot growth is used to inhibit or break the apical dominance of the shoots of the lower stem, so that it can spur the growth of grafting buds. The growth of the grafting buds starts after the new combined rootstock was given accelerated growth treatment such as bending, cutting and bending. The grafting buds are expected to grow faster with better quality. Based on this, the study was carried out to examine the shoot growth acceleration technique on different grafting methods to find out the most effective technique to grow grafted shoots in Pummelo plants.

MATERIALS AND METHODS

Location and plant materials: The study was conducted from March-June, 2017 in Banaran Village, Geger Sub-district, Madiun, east Java. The rootstocks used were taken from *Japansche citroen* (JC) citrus variety and the upper stems were from the Nambangan Pummelo cultivar using Urea fertilizer at the dose of 0.182 g $polibag^{-1}$.

Experimental design: The study used a randomized block design (RBD) with 6 treatments and 4 repetitions. When the inoculated Pummelo citrus plants were 25 days old, the rootstocks of the Pummelo citrus plants above the grafting part were treated as follows, B0 (no treatment applied), B1 (bending the rootstock above the inserted scion) in which the lower stems were bent in the opposite direction to the location of attached scion and then tied to the base of the rootstock to keep the plant curved, B2 (cutting the rootstock part at 2 cm above the inserted scions) in which cutting was done at 2 cm above the grafting in the scion, B3 (cutting the rootstock part at 4 cm above the inserted scions) in which the lower parts of the upper stems at 4 cm above grafting were wounded in such a circular crease but the Pummelo orange plant was not completely cut at once, B4 (cutting the rootstock part at 4 cm above the inserted scions and bending it) in which the lower stems were scraped at 4 cm above the grafting then bent to the ground and B5 (cutting 20 cm above grafting and bending it) in which the rootstocks were cut at 20 cm above the grafting and bent.

Parameter observation: The observations were conducted on the components of growth including the periods of bud sprout, periods of first leaf appearance, living seedling percentages, grafting shoot lengths, grafting shoot diameters, number of leaves on the grafting shoots, grafting shoot leaf areas and percentage of living seeds.

Statistical analysis: All data were analyzed by one-way analysis of variance (ANOVA). Then the data were analyzed using F-test (5%). Furthermore, LSD test at the level of 5% was performed if there was a real influence of the treatment[8].

RESULTS

Bud sprouting time: According to Table 1, it is shown that the treatment of scion growth acceleration affected the sprouting time of the buds in the grafted plants. The sprouting time in B0 treatment was significantly different from those in B1, B2, B3, B4 and B5 treatments.

First leave growth time: Based on Table 1, it can be seen that the acceleration treatment of scion growth had a significant effect on the time of first leaf appearance on the grafted shoots. The treatment of cutting the rootstocks at 2 cm above the grafting area resulted in a significantly different effect from the treatment of bending the rootstocks above the grafting area.

Living seedling percentage: According to Table 1, it can be seen that the acceleration treatments of scion growth in the Pummelo citrus plants had significantly affected the living seedling percentages after the grafting plastics were opened and they were able to live until the end of observations.

Grafting shoot length: According to Table 2, it can be seen that the acceleration treatment of scion growth on the scions of Pummelo citrus plants had significantly affected the length of grafting shoots. The length of the grafting shoots resulted from B0 treatment was significantly different from the grafting shoot length in the B1, B2, B3, B4 and B5 treatments. The lengths of the grafting shoots at 45, 60, 75 and 90 days after grafting showed the same results.

Number of grafting shoot leaves: According to Table 3, it can be seen that the acceleration treatments of scion growth on the Pummelo citrus plants had significantly affected the number of grafting shoot leaves. The number of leaves in B0 treatment was significantly different from those in the treatment of B1, B2, B3, B4 and B5.

Grafting shoot diameter: According to Table 4, it can be seen that the acceleration treatments of scion growth on the Pummelo citrus plants had significantly affected the grafting shoot diameter on the 45-90 DAG. The bud diameter of treatment B0 was significantly different from those of the B1, B2, B3, B4 and B5 treatments. The observation on the 45-90th DAG showed that four grafting scion growth acceleration treatments resulted in longer diameters.

Table 1: Sprout growth in different treatments

Treatments	Sprouting time (day)	First leaf appearance (day)	Living seedling (%)
B0	0.00^{a}	0.00^{a}	0.00^{a}
B1	10.21^{c}	13.33^{d}	77.50^{b}
B2	7.17^{b}	10.21^{b}	100.00^{d}
B3	9.48^{c}	12.50^{cd}	85.00^{bc}
B4	7.21^{b}	10.29^{bc}	92.50^{cd}
B5	8.16^{bc}	11.33^{bcd}	80.00^{b}

A value of 0 indicates that there was no growth, the numbers followed by the same letters in the same column indicates that there was no significant difference in the result of LSD test at 5%, the notation was obtained from the transformation result of $\sqrt{x+1}$

Table 2: Grafting shoot length of pummelo plants in different treatments

Treatments	Length of grafting shoot (cm) in days (DAG)			
	45	60	75	90
B0	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
B1	10.25^{c}	14.36^{c}	14.91^{d}	15.20^{c}
B2	11.28^{c}	12.10^{c}	12.09^{c}	13.53^{c}
B3	7.29^{b}	8.16^{b}	8.30^{b}	8.69^{b}
B4	10.70^{c}	12.68^{c}	12.74^{cd}	12.98^{c}
B5	11.27^{c}	13.32^{c}	13.50^{cd}	13.63^{c}

A value of 0 indicates that there was no growth, the numbers followed by the same letters in the same column indicates that there was no significant difference in the result of LSD test at 5%, the notation was obtained from the transformation result of $\sqrt{x+1}$, DAG: Days after grafting

Table 3: Number of grafting shoot leaves in different treatments

Treatments	No. of grafting shoot leaves (leaves) at age (DAG)			
	45	60	75	90
B0	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
B1	11.12^{c}	12.29^{c}	12.88^{c}	12.88^{c}
B2	13.63^{d}	13.50^{c}	13.75^{c}	13.75^{c}
B3	8.83^{b}	10.04^{b}	10.17^{b}	10.63^{b}
B4	12.54^{cd}	12.50^{c}	12.75^{c}	13.04^{c}
B5	12.04^{cd}	12.96^{c}	13.08^{c}	13.08^{c}

A value of 0 indicates that there was no growth, the numbers followed by the same letters in the same column indicates that there was no significant difference in the result of LSD test at 5%, the notation was obtained from the transformation result of $\sqrt{x+1}$, DAG: Days after grafting.

Table 4: Grafting shoot diameter in different treatments

Treatments	Grafting shoot diameter (cm) at age (DAG)			
	45	60	75	90
B0	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
B1	0.42^{c}	0.49^{c}	0.50^{c}	0.54^{c}
B2	0.41^{c}	0.43^{bc}	0.48^{bc}	0.52^{c}
B3	0.36^{b}	0.38^{b}	0.40^{b}	0.42^{b}
B4	0.45^{c}	0.48^{c}	0.50^{c}	0.53^{c}
B5	0.42^{c}	0.48^{c}	0.50^{bc}	0.51^{c}

A value of 0 indicates that there was no growth, the numbers followed by the same letters in the same column indicates that there was no significant difference in the result of LSD test at 5%, the notation was obtained from the transformation result of $\sqrt{x+1}$, DAG: Days after grafting

Grafting shoot leaf areas: According to Table 5, it can be seen that the acceleration treatments of scion growth on the

Fig. 1: Grafting scion growth in the observation on the 90th day after grafting

Table 5: Grafting shoot leaf areas of pummelo in different treatments

	Grafting shoot leaf area (cm^2) at age (DAG)			
Treatments	45	60	75	90
B0	0.00^a	0.00^a	0.00^a	0.00^a
B1	12.89^{cd}	26.58^d	31.53^d	34.12^d
B2	10.91^{bc}	23.01^c	25.77^c	29.23^c
B3	9.19^b	17.68^b	20.19^b	21.96^b
B4	9.99^b	26.20^d	29.81^d	31.95^{cd}
B5	14.69^d	25.58^{cd}	28.08^{cd}	30.16^c

A value of 0 indicates that there was no growth, the numbers followed by the same letters in the same column indicates that there was no significant difference in the result of LSD test at 5%, the notation was obtained from the transformation result of $\sqrt{x+1}$, DAG: Days after grafting

Pummelo citrus plants had significantly affected the grafting shoot leaf areas in 45-90 DAG. Treatment of B1, B2, B3, B4 and B5 resulted in greater leaf areas than treatment B0 (Fig. 1).

DISCUSSION

The untreated rootstock causes the scion to remain dormant. However, the scion is still green and continues to grow if it receives nutrients from the rootstock photosynthesis results but the growth requires a longer period of time. The removal of apical dominance in the rootstock is predicted to cause nutrient accumulation in the grafting scions that causes buds to sprout. Mason *et al.*[9] suggested that basically the apical dominance maintained in the whole plants (untreated) limits the axillary scions to absorb sugar. However, after the tip or shoot is removed, the sugar quickly accumulates in the axillary scions. Thus, because the sugar content in the axillary scions has exceeded the limit, the buds are released.

Bud sprouting occurs after the scions are linked to the rootstocks thereby resulting in the smooth distribution of nutrients from the rootstocks to the scions through tissues that have been perfectly attached either through the roots or leaves and then the nutrients are used for callus formation. The results of Mahunu *et al.*[10] showed that fast and adequate callus formation is important for scion or grafting bud survival and the formation of cambium and vascular tissues.

The formation of callus in the grafted part is influenced by the hormone auxin and cytokines which work together. It is explained that the high cytokinin content increases shoot development, if auxin is higher, it increases the root development, while if the concentration between auxin and cytokinin is balanced, it produces callus formation[11]. The presence of the cambium contact in the rootstocks and scions is the key needed for the callus formation and shoot increase[12]. The vascular cambium tissues from the rootstocks and scions must be placed in contact precisely and both tissues must stay alive until the grafting has been carried out. The faster the linking, the faster the scions receive nutrients, so that the growth of shoots will be faster, especially the appearance of the first leaf[13]. The observation results showed that the treatments of the scions affected significantly on the percentage of living seedlings. The earlier and better cambium contact between the scions and rootstocks supports the healing process, so the growth begins earlier[14].

The growth of shoots is basically very closely related to the bud sprout time. The faster the buds sprout, the faster the shoots grow along with the fulfillment of the elements needed for growing. During the bud sprouting, carbohydrates are transferred from the roots and woody organs to shoot growth, supplying new leaves for growth. Young shoots and leaves utilize elements and carbohydrates for the growth to reach one third of their ending size[15].

At the age of 75 DAG, the treatment of cutting the stems gave the longest length of plant shoots because the concentration of cytokines is higher to stimulate lateral shoot growth. According to Xing *et al.*[16], in the curved stems, higher levels of cytokines are found in the lateral shoots than in the apical shoots thereby forming more buds.

The shoot lengths of Pummelo plants at the age of 45, 60 and 90 DAG showed that the treatments of bending, cutting, layering and combination of cutting and bending the scions above grafting give significantly different results from the treatment of wounding the scions. In general, the apical dominance that is inhibited can stimulate the growth of grafting shoots. Leaf removal on the rootstocks limits the formation and accumulation of bud sprout inhibitors, due to changes in the level of endogenous hormones and growth factors that optimize the bud sprouting[17].

The appearance of more leaves and the maximum leaf size are related to faster growing shoots. The different growth speeds of grafting shoots also cause different numbers of leaves growing in the grafting shoots. According to Rodriguez *et al.*[18], the shoot length and the number of leaves are closely related because new leaves synthesize giberelin, activate the production of cytokines which are responsible for stimulating a meristematic activity and nutrient mobilization and create a good photosynthetic balance that is beneficial for the shoot growth. Chander *et al.*[19] stated that conditions supporting the grafting shoot growth can cause the growth of more leaves and a higher photosynthetic activity in the grafting shoots leading to the formation of more nutrients that facilitate the thickness of the grafting shoots.

The higher numbers of leaves and root systems increase the absorption of minerals and water from the soil to produce more carbohydrate, to assimilate vegetative growth and to increase it[20]. The increasing shoot diameter shows that the survival of the plant during this period is related to the supporting compatibility and conditions of the plants[21]. Treatment to the scions above the grafting can stimulate the activity of the meristem which is actively involved in cell division and growth by increasing the hormone content in the plant organs such as leaves which continuously increase the number of cells. According to Gonzalez *et al.*[22], increasing cell numbers are the main reason for the increasing leaf area. In normal cell division, leaf area growth also occurs to maintain cell sizes. A leaf area is related to many agronomic and physiological processes including growth, photosynthesis, transpiration and interception[23].

The final results of the study showed that the technique of accelerating the shoot growth by cutting the rootstocks above the grafting area gave a significant effect on the percentage of live seedlings. Meanwhile, the treatment of cutting the rootstocks at 4 or 20 cm above the grafting area and bending them significantly gave more results compared to the treatment of cutting the rootstocks at 4 cm above the grafting part. A higher percentage of plants ready for planting as a result of the grafting treatments is presumably because rapid healing and nutrient uptake are already greater from the beginning of growth.

CONCLUSION

The treatments of cutting and layering the scions above the grafting significantly and more effectively accelerate the bud sprouting in grafting compared to other treatments. Moreover, the treatment of cutting the scions above the grafting also produces the best percentage of living seedlings by 100%.

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