



Success of Siompu Orange Plant Shoot Cuttings With Natural Growth Regulators and Arbuscular Mycorrhizal Fungi (AMF) Application

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ABSTRACT. Siompu tangerine (*Citrus nobilis* Lour.) is an endemic plant from Siompu Island, This plant is rare and slow to bear fruit due to generally being cultivated generatively. This study aims to determine the effect of natural growth regulators combined with Arbuscular Mycorrhizal Fungi on the success of Siompu tangerine shoot cuttings. This research was conducted in the plastic house of the Indonesian Mycorrhizal Association (AMI) Southeast Sulawesi Branch Universitas Halu Oleo (UHO) and the Kendari Forestry and Environmental Sciences Laboratory Unit, which lasted for 5 months from September to February 2025. This study used a Randomized Block Design consisting of 9 treatments, namely control (a), aloe vera solution (b), shallot solution (c), AMF Inoculum of Kaimbulawa Village (d), AMF Inoculum of Lapara (e), Aloe vera solution + AMF Inoculum of Kaimbulawa Village (f), Aloe vera solution + AMF Inoculum of Lapara (g), Shallot solution + AMF Inoculum of Kaimbulawa Village (h) and Shallot solution + AMF Inoculum of Lapara (i) each treatment consisted of three replications and each replication consisted of five plants so that the total plants used were 135 plants. The results showed that the treatment of natural growth regulator combined with AMF had not provided significant results on the Percentage of living cuttings, the percentage of cuttings that sprouted, and the number of leaves. In the Kaimbulawa AMF treatment, it can increase the rate of rooted cuttings, and the 100% natural shallot PGR treatment combined with Lapara FMA increases root length and the percentage of AMF colonization.

Keywords : *Siompu Orange, Natural growth regulators, Arbuscular Mycorrhizal Fungi and Shoot Cuttings.*

INTRODUCTION

Orange tangerine Siompu (*Citrus nobilis* Lour.) is one of the mainstay horticulture types in Southeast Sulawesi and a superior national (Husna *et al*, 2023) and is a woody plant originating from the Rutaceae family (Manurung and Warsodirejo, 2019). Oranges Siompu also has potential development in system agroforestry that supports the effort of rehabilitation of land and forestry social (Mulyani *et al*, 2019). Conditions Orange Siompu moment This Already almost extinct and its productivity is low (Husna *et al*, 2022), and Orange Siompu is generally cultivated in a way generative through seed, but this condition is what causes rare seeds and slows in bearing fruit. One of how to propagate seedless oranges and speed up the age of harvest oranges is a technique of vegetative multiplication way grafting, grafting and cuttings (Zainun and Yuniati, 2021). In line with research (Batubara and Marpaung, 2022) that orange can multiplied in a way vegetatively by the method of cuttings. Shoot cuttings are a method of plant propagation using separated plant parts from its parent. Advantages of multiplication vegetative by the method of cuttings include getting new plants in large numbers in a relatively short time. In addition, it obtains the same nature from its parent (Hariani *et al*, 2018). The propagation through cuttings own problem is that rooting is difficult to grow, so that needed substance regulator growth (ZPT) is needed to stimulate root

growth. One of the substance regulator grow natural substances that can be used in nursery orange Siompu by using cuttings shoot is shallot and aloe vera, because shallot and aloe vera contain substance regulators that have *auxin*, *gibberellin*, and *cytokinin* (Afifudin *et al*, 2022).

Growth regulator of shallot and *Aloe vera* plants, just not yet enough to improve the ability to uptake of nutrients and water by roots, seed cuttings, shoot orange siompu, because the range of absorption of the root fiber is limited only to the part on the surface land, just for that. Still need support through the provision of arbuscular mycorrhizal fungi. Mycorrhizal fungi arbuscular form a symbiosis mutualism between the roots and the plant level height (Yuliyanto *et al*, 2016). However, a Study using natural Growth regulator shallot and aloe vera and application of FMA on cuttings of shoot orange Siompu Not yet Once done. Based on the description on so need to hold a study on propagation using cuttings shoots with giving substance regulators to grow naturally, and FMA applications on citrus Siompu, which was previously not yet Once done. Formulation The problem in this study is whether there is an influence of natural growth regulator

MATERIALS AND METHODS

Place and Time of Research

This research was carried out at the Home Plastic Association Indonesian Mycorrhiza Institute (AMI) Southeast Sulawesi Branch, Faculty of Forestry and Forestry Sciences Environment (FHIL) Halu Oleo University (UHO), and Forestry and Science Laboratory Unit Environment Kendari for five months, from September 2024 to February 2025.

Research Design

This research designed use Grouped Randomized Block Design based on position shoots consisting of of 9 treatments, namely:A= Control, B= Solution Aloevera 100%, C= Solution shallot 100%, D= AMF inoculum of Kaimbulawa village (*Cloroideoglomus cloroideum*, *Funneliformis dimorphicus* dan *Glomus* sp.2), E= AMF inoculum of Lapara (*Cloroideoglomus cloroideum*, *Sclerocystis sinousa* dan *Glomus* sp.2), F= *Aloe vera* solution + AMF Kaimbulawa), G= *Aloe vera* solution + AMF Lapara, H= Solution shallot + AMF Kaimbulawa and I= Solution shallot + AMF Lapara

Research Procedures

1. Making Cover

Initial steps taken before the research is by making cover with size length 300 cm, width 98 cm and height 73 cmas well as installation paranet at home plastic.

2. Preparation of Planting Media

Planting medium used is mixture soil , cocopiet , charcoal husk and sand . Next soil, *cocopiet*, charcoal husk and sand mixed (1:1:1:1) and incorporated into the portrait, then portrait entered into the hood plastic and arranged on the table nursery.

3. Selection of Cutting Materials

Cutting materials used are part shoot vertical (*orthotropic*) originating from from Nursery Association Mycorrhiza Indonesia (AMI). M has size 5 bud eyes . Cutting material cut use scissors sharp cuttings . The cutting material taken cut into $\frac{3}{4}$ pieces on try to make it even and part bottom cut at an angle (45° angle) This intended to enlarge surface water absorption and giving chance growth balanced , long roots offspring No more from 5 cm . With the criteria material cuttings used freely from pests and diseases.

4. Preparation Onion and Tongue Solution Crocodile

Substance regulator the growth used comes from from plant onion red and tongue crocodile as much as 100 grams. Red onion and shallot which is used in form pure (100%) and refined using a blender. After that , cuttings shoot orange the brain dipped into the



solution the as deep as 2 cm for 5 minutes Then planted in the existing planting medium prepared.

5. Maintenance

To maintain humidity of media and materials cuttings , then watering done 2 days once and depending on the conditions plants . Watering by using a hand sprayer. Average daily temperature in hood or shade used in tuning This between 21°C to 26°C is good for rooting cuttings . Measurement temperature done by placing thermometer in the cover . If the plant experience drought or humidity decrease can seen from water dew on the surface part in hood There is or no . And if temperature No achieved so will done treatment by means of thicken plastic or cover and moment temperature exceed provision so will do watering on the part on cover.

6. Harvesting Shoot Cuttings Seedlings Orange Siompu

Before done harvesting, seedling orange Siompu documented through taking picture based on each treatment . Furthermore , harvesting done by opening pottray and cleaning root plant use running water. Young roots Then cut use scissors sterile and inserted to in film bottles for further use in the coloring process root.

7. Observation Colonization

Observation of the colonization process root can seen through the coloring process roots (Brundrett *et al .*, 1996 in Hadianur *et al .*, 2017). First root from every plant washed with water until clean , then soaked in 10% KOH solution for 24 hours until root colored white or yellow clear . The 10% KOH solution is discarded and the roots washed with running water until clean . Then The roots were soaked in a 2% HCl solution for 30 minutes . The 2% HCl solution was discarded . Next The roots are soaked in a staining solution (glycerol , acid) lactate and distilled water in a ratio of 2: 2: 1 and added powder *trypan blue* 0.05) for 24 hours. Staining solution (*glycerol* , acid lactate and distilled water in a ratio of 2:2:1 and added powder *trypan blue* 0.05) was discarded (Setiadi *et al .*, 1992; Sangadji , 2004). Then roots soaked in solution Glycerol 50% Last root from each treatment that has been done given solution furthermore root cut into 1 cm pieces, then arranged on glass preparation 1 glass preparation for 10 pieces roots , every 5 pieces root covered with cover glass, then observed internal and external hyphae using microscope light.

Research Variables

1. Percentage cuttings life

Percentage grow counted at the end observation using formula :

$$\text{Percentage cuttings life} = \frac{\Sigma \text{Number of Live Cuttings}}{\Sigma \text{Total Number of Cuttings Planted}} \times 100\%$$

(Ekamawati) *et al .*, 2023).

2. Percentage of Cuttings That Germinate

Percentage sprout counted at the end observation using formula :

$$\text{Percentage of Cuttings That Germinate} = \frac{\Sigma \text{ number of cuttings that sprout}}{\Sigma \text{ total Number of Cuttings Planted}} \times 100\% \text{ (Nasir, 2021).}$$

3. Percentage of Rooted Cuttings

Percentage cuttings rooted counted at the end observation using formula :

$$\text{Percentage of Rooted Cuttings} = \frac{\Sigma \text{ Number of Rooted Cuttings}}{\Sigma \text{Jtotal Number of Cuttings Planted}} \times 100\%$$

(Ekamawati) *et al .*, 2023).

4. Leaf number (blade)

Amount leaf counted every ages 4, 6, 8, 10, 12, 14, 16, 18 and 20 weeks after Planting (MST).

5. Root Length (cm)

Measurement long root done one time at a time harvest . Measurement done with method with use method measurement root namely *the* Grid Intersection Method (Rowel, 1966) in (Nusantara *et al .*, 2012) has develop formula count long root as following :

$$R = \lambda \times N$$

Where;

R = length the root through which the cutting line passes (cm),

λ = constant 0.786 For grid width 1 cm (cm),

N = number of secant lines passed root

6. AMF colonization (%)

AMF colonization was calculated at the end of observation by using formula : FMA colonization = [Σ field view mycorrhizal / S total field observed view] x 100 % (Brundrett *et al .*, 1996 in Husna *et al .*, 2015) .

Data analysis

Analysis used is analysis variance analysis (F test). If treatment influential real so will continued with a difference test treatment according to the Duncan Multiple Range Test (DMRT) at the level 95% confidence . The data application used is SAS application version 9.4.

RESULTS AND DISCUSSION

Percentage of Living Cuttings , Sprouted Cuttings and Rooted Cuttings

Cuttings sprouting, and cuttings rooted in cuttings shoot orange, the brain presented in Table 1. Table 1 shows that there are no differences in the treatment of variables, cuttings live, and cuttings sprout. However, on the variable cuttings rooted show there is a difference in treatment, where AMF inoculum of Kaimbulawa treatment gives mark the highest and different real value with treatment other than in the treatment solution shallot 100% and treatment solution aloevera 100% + AMF inoculum Kaimbulawa. The visualization of plant cuttings is presented in Figure 1.

Table 1 Influence Combination of natural growth regulator and AMF on percentage cuttings live, cuttings sprouting and cuttings rooted in cuttings shoot orange the brain 5 months old.

Treatment	Live cuttings (%)	Cuttings sprouting (%)	Rooted cuttings (%)
A (Control)	33.33 ± 6.67	33, 3 3 ± 6.67	26.67 ± 6.67bc
B (Solution aloe vera 100%)	60,00 ± 11.5	53.33 ± 13.33	40.00 ± 13.33 bc
C (Solution shallot 100%)	53.33 ± 6.67	26.67 ± 6.67	46.67 ± 6.67ab
D (AMF Kaimbulawa)	66.67 ± 6.67	46.67 ± 6.67	60.00 ± 11.55a
E (AMF Lapara)	60.00 ± 11.5	26.67 ± 6.67	26.67 ± 6.67bc
F (B + D)	66.67 ± 6.67	33.33 ± 6.67	46.67 ± 6.67ab
G (B + E)	53.33 ± 6.67	26.67 ± 6.67	26.67 ± 6.67bc
H (C + D)	46.67 ± 6.67	26.67 ± 6.67	20.00 ± 6.67c
I (C + E)	40.00 ± 6.67	20.00 ± 6.67	20.00 ± 0.00c
Pr > F	0.1317	0.0535	0.0162

Description : Numbers followed by same letter No different real according to the DMRT test ($\alpha = 0.05\%$).



Figure 1. Growth cuttings shoot orange Siompu who was given Substance Regulator Grow natural combined with AMF.

Number of Leaves, Root Length and AMF Colonization

Distance test results Duncan's multiple sum leaves, long roots and AMF colonization on cuttings shoot orange the brain five months old presented in Table 2. Table 2 shows that No there is a difference in influence treatment of variables amount leaves. On the variables long root, shows there is a difference in treatment Where treatment solution shallot 100% + AMF Lapara different real with treatment other except in the treatment solution *Aloe vera* 100% + AMF kaimbulawa. On Variables AMF colonization, indicating there is a difference in treatment. Where treatment solution shallot 100% + AMF Lapara different real with treatment control, solution shallot 100% and solution *Aloe vera* 100% except for treatment other .

Table 2 Influence Combination of natural PGR and AMF on the amount leaves , long roots and AMF colonization on cuttings shoot orange the brain 5 months old .

Treatment	umber of leaves (blades)	Root Length (cm)	colonization (%)
A (Control)	5.3 ±0.33	11.5 ±0.94 b	6.7 ±0.83c
B (<i>Aloe vera</i> 100% solution)	3.1 ±0.55	9.7 ±0.31cd	23.3 ±9.61b
C (shallot 100% solution)	7.7 ±0.88	8.6 ±0.81d	26.1 ±8.89b
D (FMA Kaimbulawa)	5.0 ±0.67	9.6 ±0.59cd	37.2 ±6.34ab
E (FMA Lapara)	3.0 ±0.33	11.5 ±0.68b	35.8 ±3.63ab
F (B + D)	4.3 ±0.33	8.6 ±0.41 d	35.7 ±5.86ab
G (B + E)	3.2 ±0.44	12.0 ±0.79ab	35.0 ±7.64ab
H (C + D)	3.7 ±0.33	10.7 ±0.42bc	47.5 ±2.50a
I (C + E)	2.0 ±0.67	12.8 ±0.14a	50.0 ±8.33a
Pr > F	0.0502	0.0061	0.0015

Description : Numbers followed by same letter No different real according to the DMRT test ($\alpha = 0.05\%$).

Based on research results show that there is an influence treatment of cuttings and cuttings. This can be caused by factors environment, such as humidity, temperature, and possible conditions of the planting media more influential towards the growth process beginning cuttings compared to the treatment given. According to Gardner *et al.* (2017), the development of good plants is affected by the interaction between various internal factors and external factors. Capabilities cuttings for sprout are also influenced by the physiological condition plant parent, such as natural hormone content, reserve nutrition, and levels of stress

before cutting cuttings, Role determines the success of growth shoots (Ahmad *et al.*, 2022). Cuttings rooted show that treatment AMF Kaimbulawa, Solution shallot 100%, and *Aloe vera* 100% solution + AMF Kaimbulawa produce cuttings rooted the highest. This shows that AMF Kaimbulawa's ability to colonize has a good and fast start in forming symbiotic connections with root plants. Mycorrhiza arbuscula capable increase the availability of nutrients and water in the phase of beginning growth, as well as produce metabolites that play a role as stimulants physiological (Wu *et al.*, 2019). While that solution shall contain compound auxin, flavonoids, and phenolic compounds, which physiologically can increase the speed and quality of rooting cuttings plants (Wibowo *et al.*, 2020). In line with the research of Rahayu and Rismunandar (2020), the solution extract of shallot increases the number of roots and speeds the emergence of roots in some types of plant cuttings. The treatment combination solution aloe vera + AMF Kaimbulawa can form root, because of the existence synergistic effect between the compound bioactive in the solution aloe vera with the activity of mycorrhiza. Solution aloevera contains hormone growth, vitamins, enzymes, and antibacterial, natural, supportive formation root new roots (Afifuddin *et al.*, 2022). The combination with AMF helps form roots and enhancement the absorption of nutrients. In line with the research of Wahyuni *et al.* (2019), it was proven that a combination treatment with organic mycorrhiza speeds up root formation and enhances the quality of cuttings and seeds. Based on research results show that there is an influence treatment of the amount of leaves. This matter due to because the plant is still at the stage of beginning growth, where is reserve nutrition more Lots used more for the formation of roots compared to the production of leaves (Taiz and Zeiger, 2010). In line with research by Zhu *et al.*,(2020) that's wrong One factor reason main that is domination effect treatment to part plant others, such as the root where condition environment not optimal, then plant will more prioritize growth root or maintain network that already exists There is than to form leaf new.

On the variable long root, show treatment Solution shallot + AMF Lapara and Solution aloevera + AMF Lapara show the best results. This result shows that a combination treatment can optimize root growth, because the existence of improved production hormone growth, natural hormones that play a role in root elongation. In Suryanto *et al.* (2020) that shallots contain natural auxin, especially *Indole Acetic Acid* (IAA), which plays a role in stimulating division and cell elongation. While in the solution, aloe vera contains amino acids, vitamins, enzymes, and compounds that support antimicrobial metabolism and protect the root network from attack by pathogens. Condition This allows roots to grow faster and longer, although not as strong as the effect of Auxin in onions red (Afifuddin *et al.*, 2020). In line with the research of Wahyuni *et al.* (2019), it was proven that giving the solution of aloe vera on cuttings betel plant in a way significantly increases long the root. Compound active in solution aloe vera supports extension root through improvement activity physiological and hormonal activity in tissue plants, with the presence of FMA Lapara strengthening the effectiveness of both natural growth regulator by providing a network of hyphae external expanding Power absorb roots to water and nutrients. Muryati *et al.* (2017) explained that structure long and fine AMF hyphae allow penetration into in pores of land that is not accessible to roots, especially in poor, fertile media. Synergy between natural growth regulator and AMF Lapara. This is what supports the best results on the variable long root. And AMF Lapara's ability to colonize or produce more hormone production is tall compared to FMA Kaimbulawa.

Based on the results of colonization. The highest AMF colonization is treatment ZPT shallot 100% + FMA Lapara, with the percent colonization best with the value of 50.0%. The presence of the AMF structure in the treatment control is allegedly colonized by natural AMF through wind or irrigation water and soil media colonized by AMF. AMF through hyphae externally can increase the absorption area of nutrients, especially and protect the plant from



biotic and abiotic factors (Indriani *et al.*, 2016). The structure of fungi is in the form of hyphae inside root plants and soil, capable of increasing the area for exchange of nutrients and water between plants and hosts (Prasetyo *et al.*, 2019). The existence of this AMF colonization is allegedly capable of increasing the growth of plants (Plasta and Rini, 2017).

CONCLUSION

Based on the results of observations can concluded that AMF Kaimbulawa treatment, can increase percentage cuttings rooted, natural ZPT treatment shallot 100% and solution Aloe vera 100% combined with AMF Lapara, increases long Roots and Natural ZPT Treatment shallot combined 100% with AMF Lapara and AMF Kaimbulawa increase percentage AMF colonization.

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