e-ISSN: 3089-5081 | p-ISSN: 1979-8911
Vol. 14 No. 2 December 2025: 44-51
DOI: 10.15575/istek.v14i2.1339

THE EFFECT OF POLYVINYL PYROLYDONE (PVP), EXPLANT TYPE, AND EXPLANT PLANTING POSITION ON CALLUS GROWTH OF CIKONENG ST LARGE ORANGE (*Citrus maxima* (Burm.) Merr) AND SWEET ORANGE (*Citrus sinensis* (L.) Osbeck)

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Article Info

Article history:
Received April 28, 2025
Revised Ocotober 10, 2025
Accepted November 11, 2025

Keywords:

Abaxial Adaxial
Cikoneng ST Big Orange Sweet Orange
Callus
Tissue Culture

ABSTRACT

Cikoneng ST Orange (*Citrus maxima* (Burm.) Merr) is a germplasm of Sumedang area, which has been scarce for the past few years. The high percentage of browning in Cikoneng ST Orange explants is considered to be the cause of the low percentage of research success. Sweet Orange (Citrus sinensis (L.) Osbeck) is a type of orange that has been widely used as a potential orange explant in tissue culture. This study was conducted to determine the comparison of growth percentage by conducting cross-species research between Cikoneng ST Orange and Sweet Orange. The study was conducted at the Tissue Culture Laboratory of the Agrotechnology Department, Sunan Gunung Djati State Islamic University, Bandung from March to June 2017. The media used was a combination of MS media, 2 mg L-1 2.4 D, and 0.5% PVP. The explants used were leaves and leaf shoots of Cikoneng ST Orange and Sweet Orange. The planting positions of the explants included abaxial and adaxial planting positions. The study was analyzed using a descriptive method. The results showed that the administration of PVP in MS media and 2 mg L-1 2.4 D could induce callus of Sweet Orange at 3 DAI and Cikoneng ST Orange at 5 DAI. The use of leaf shoot explants and adaxial planting position produced the best friable callus up to 28 DAI. This study also found that Sweet Orange had the fastest callus growth time and a low browning percentage compared to Cikoneng ST Orange explants.

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1. INTRODUCTION

Cikoneng ST Orange (*Citrus maxima* (Burm.) Merr) is a germplasm of Sumedang Regency, West Java, which has experienced a shortage of superior seeds for the past few years. Propagation of Cikoneng ST Orange plants is generally carried out conventionally. According to Janick and Moore (1995), propagation of large orange plants has so far only been carried out using seeds, grafting, and budding. The decline in the population of Cikoneng ST Orange requires intensive research to improve plant quality and prevent species extinction. Conventional cultivation of Cikoneng ST Orange is no longer considered the right propagation solution. Other efforts are needed to propagate Cikoneng ST Orange plant seeds, one of which is by using tissue culture techniques.

According to Nurwahyuni (2013) tissue culture or in vitro culture techniques are a good way for plant propagation, plant quality improvement, and bioconservation. According to Gamborg and Shyluk (1981) in Suminar et al. (2007), the success of in vitro culture is influenced by plant growth regulators (PGRs), nutrients, and explant sources used and the physical environment of tissue culture. These three things will affect the regeneration process in explants. Non-conventional breeding focuses on the ability of plant cells and tissues to grow and develop into complete plants. This growth occurs through direct regeneration from explants, or indirect regeneration through callus (Kihhundu et al., 2012).

Plant growth regulators (PGRs) are organic compounds that are not nutrients that in small amounts can support (promote), inhibit, and change the physiological processes of plants (Abidin, 1995). One of the PGRs that is often used in in vitro culture is 2,4-Dichlorophenoxy Acetic Acid (2,4-D). According to Rahayu et al. (2003), the addition of 2,4-D to the culture medium can stimulate cell division and enlargement and can also trigger the formation and growth of callus. Callus is a collection of cells that develop from the growth of explants and is included in the embryogenesis phase. According to Blanc et al. (1999) the embryogenesis phase is a profitable technique for multiplying species. Callus growth begins from the initiation stage where the explant will gradually experience morphological changes into a collection of actively dividing cells.

Each explant has different flavonoid levels based on the type, age, and part of the plant used. Leaf shoots are young plant organs with meristem tissue that is still actively dividing. According to Yusnita (2003) the use of explants with meristematic properties can trigger successful culture. Young plant tissue has higher regeneration power, cells that are still actively dividing, and are relatively clean. The use of leaf shoots as explants also needs to be reviewed considering the possibility of high flavonoid levels in young tissue which can increase the percentage of browning.

Flavonoids are secondary metabolites that are characteristic of a plant. Phenol is one type of flavonoid that dominates citrus plant organs. Accumulation of phenol in a culture process will cause browning which can inhibit explant growth. According to Roostika et al. (2015), phenol accumulation can be suppressed by adding polyamide compounds in the form of Polyvinyl Pyrrolidone (PVP) to the culture medium. According to Hutami (2008), protein, amide, and polyamide compounds are able to react with phenol and restore enzyme activity that is inhibited by phenol compounds in the browning phase.

The use of leaves in in vitro culture is also related to the availability of stomata. According to Hariyanti (2010), the distribution of stomata on leaves is related to the speed and intensity of leaf transpiration. The more pores on the leaves, the more it will trigger the evaporation process. The distribution of stomata on leaves is influenced by the position of the leaves including the upper leaves (adaxial) and the lower leaves (abaxial). The use of various leaf planting positions as explants is necessary to obtain the best growth results.

Propagation of Cikoneng ST Orange seedlings using tissue culture techniques has not had a sufficient percentage of success. The main cause of this is possibly due to the lack of compatibility of the explant part, type of media, and concentration of ZPT used. One way to overcome this is by conducting comparative research on other types of oranges.

Sweet orange (Citrus sinensis (L.) Osbeck) is a type of orange that has been widely used as a potential citrus explant. This study is a cross-species study between Cikoneng ST Orange and Sweet Orange. The use of sweet orange as a comparative explant is expected to provide results to determine the effectiveness of using different explants and explant planting positions.

Research on Cikoneng ST Orange at Sunan Gunung Djati State Islamic University (UIN) Bandung has been conducted by Wartika (2015) and Maharani (2016). According to Maharani (2016), MS media combined with 2,4-D 2 mg L^{-1} is able to induce callus of Cikoneng ST Orange leaf explants from conventional propagation, but the callus induction still has constraints on browning activity and callus growth.

Based on this, further research is needed on the effect of adding PVP, types of explants with different ages, and various explant planting positions to trigger callus growth. The addition of PVP, the use of leaf tips and mature leaves, and adaxial and abaxial planting positions are expected to be able to trigger the best callus growth on Cikoneng ST Orange and Sweet Orange explant

2. METHODOLOGY

This study employed a experimental methodology. The research was conducted from March to June 2017 at the Tissue Culture Laboratory of the Agrotechnology, Faculty of Science and Technology, UIN Sunan Gunung Djati Bandung

3. RESULT AND DISCUSSION

1. Maintenance of Cikoneng Orange and Sweet Orange Parent Plants

Maintenance of parent plants in tissue culture activities is intended to prepare pest and disease- free explants. Plant maintenance includes conventional cultivation procedures carried out intensively. Maintenance carried out includes watering, weeding, application of NPK fertilizer (once every 2 weeks), and application of fungicides and bactericides (once every 2 weeks). Fertilizer application functions to supply nutrient content in the soil that can trigger plants to grow faster. Application of fungicides and bactericides as much as 2% is carried out to break the chain of pathogenic microorganisms in plants and keep plants healthy. The fungicide used has an active ingredient in the form of Propineb 70%. The bactericide used has an active ingredient in the form of Streptomycin 20%. The results of observations from the maintenance that has been carried out on the parent plant, obtained whitefly pest attacks but did not have a major negative impact on the growth of the parent plant.

2. Incubation Room Environmental Conditions

Lighting settings in the incubation room determine the high and low temperatures. Callus formation generally requires low light intensity. According to Hendaryono and Wijayani (1994), low light intensity treatment can trigger an increase in the embryogenesis and organogenesis process. Optimal callus formation is generally carried out in dark environmental conditions.

The condition of the culture room can affect the growth of explants. The average temperature range obtained during the study was 23.83oC with an average humidity of 67.11%. According to Yusnita (2003), tropical plants can grow optimally in in vitro culture media with a temperature range of 24°C - 32°C, but the optimization of the incubation room temperature also needs to be considered with the growth time of the explants. Based on research conducted by Wartika (2015), during the callus formation period, the optimal temperature is in the range of 19°C - 22°C. The callus growth phase requires a temperature that is not too high. Temperatures that are too high will inhibit metabolism so that the callus is unable to grow optimally. Based on the average temperature range of 23.83oC obtained during the study, it is known that this value has passed the optimum level for the callus growth phase, however, this value has not met the optimum level for the type of tropical plant explant culture. The average humidity range of 67.11% also shows that this value has not reached the optimum level of callus growth, but both of these things do not cause stagnation in the explants to enter the maximum callus induction stage.

3. Explant Contamination Level

The phase after initiation is the initial phase of explants and media entering the adjustment stage with the presence or absence of pathogenic microorganisms. Research shows that the level of contamination caused by fungi tends to be greater than bacterial contamination. The type of fungus that dominates contamination is Mucor with characteristics that are in accordance with the statement of Susilowati and Shanti (2001) that fungi of the genus Mucor have morphological characteristics of colonies in the form of white thread-like hyphae, with certain parts appearing as sporangia and sporangiophores in the form of black dots (Figure 1)

Figure 1 Mucor Fungal Contamination in Cikoneng ST Large Orange Leaf Explants

This type of Mucor fungus generally first appears at 5-7 DAI, but in some explants contamination was also found at 3 DAI. The different times of contamination may be caused by different physiological

characteristics of the fungus. In some explants, fungi were found to form sporangiophores more quickly within 5-7 days. The study also showed that explants originating from mature leaves were more susceptible to contamination compared to leaf shoot explants. Mature leaf explants had a higher contamination percentage of 40% compared to shoot explants (Table 1). The use of younger explants is known to be able to reduce the percentage of contamination by up to 10% compared to mature explants. The results of this study are in line

with Yusnita's statement (2003) that young tissue explants are relatively more sterile.

Table 1. Percentage of Explant Contamination up to 28 DAI
Description: E1 = Leaf Shoot Explant; E2 = Mature Leaf Explant;

EXPLANATION		AMOUNT (Bottle)	CONTAMINATION (%)	TOTA (%)
E1	Big Orange Cikoneng ST	20	20	30
	Sweet Orange	20	10	
E2	Big Orange Cikoneng ST	20	20	40
	Sweet Orange	20	20	

4. Explant Browning Level

Increased phenol levels that arise as a response to injury during initiation can result in browning which can cause stagnation and death of explants. The results showed that the Cikoneng ST Orange explants had a higher browning rate compared to the Sweet Orange explants (Table 2). According to Vandercook et al. (1996), Cikoneng ST Orange has a higher phenol content in the form of the dominance of the Naringin compound which is a characteristic. Apart from the type of plant, the part of the plant used is also a factor causing browning. The research that has been conducted shows that the percentage of browning in leaf shoot explants is higher compared to mature leaf explants (Table 2). The difference in the percentage of browning is due to the difference in the age of the explants used. The age of the explant affects the phenolic content in the plant organs used. According to Nasution (2013), phenolic reactions are generally greater in young plant tissue. Young plants have meristem tissue that is still actively dividing. As long as the plant has a good metabolism, the phenolic compounds will continue to be produced.

Table 2. Percentage of Explant Browning up to 28 DAI

EXPLANATION		AMOUNT (Bottle)	*BROWNING* (%)	TOTA (%)
E1	Big Orange Cikoneng ST	20	60	90
Sweet Orange		20	30	
E2	Big Orange Cikoneng ST	20	50	70
Sweet Orange		20	20	

Description: E1 = Leaf Shoot Explant; E2 = Mature Leaf Explant;

5. The Effect of PVP Use on Callus Induction

Callus induction is the process of callus formation that occurs as a response to injury. Pujawati (2008) stated that callus growth and development lead to the formation of organs such as shoots or roots called organogenesis or become embryos (new individual candidates) called embryogenesis. In addition to forming callus, activation of phenol compounds is also a response to explant injury. Phenol is the largest secondary metabolite produced by plants. This compound is produced in plants through the shikimate pathway and phenyl propanoid metabolism (Widyastuti, 2010). The shikimate pathway is a pathway formed from the breakdown of amino acid molecules. The shikimate pathway produces essential acid compounds that are generally characteristic of secondary metabolites in plant tissues. In citrus explants, the shikimate pathway produces phenyl propanoids which are active phenolic compounds.Large accumulations of phenolic compounds in tissue culture will cause browning which can trigger stagnation.

In this study, it was found that the addition of 0.5% PVP to MS and 2.4 D 2 mg L^{-1} media can trigger explants to produce callus faster and suppress the percentage of browning. This is in accordance with the statement of Hutami (2008) which states that the addition of 0.5% PVP is known to be able to inhibit the percentage of browning in explants. Based on research conducted by Maharani (2016), it is known that the Cikoneng ST Orange explant only formed callus at 7 HSI on MS and 2.4 D 2 mg L^{-1} media. This provides a comparison of callus growth time and proves that the administration of 0.5% PVP can trigger explants to be induced into callus faster. The results of the research that has been carried out show that the Cikoneng ST Orange explant can be induced at 5 HSI and the Sweet Orange explant can be induced at 3 DAI.

6. Effect of Plant Parts on Callus Induction and Growth

The selection of explant types based on different plant parts affects the callus induction time. Explants originating from young organs tend to have faster callus induction times compared to organs that have entered the maturity stage. Tissues in young plant organs tend to still be actively dividing and have a better survival percentage (Table 3).

Table 3. Effect of Plant Parts on Callus Induction

EXPLANATION		AMOUNT (Bottle)		CALLUS INDUCTION (%)
INDUCTION TIME (HSI)				
1	Big Orange Cikoneng ST	5	20	40
	Sweet Orange	3	20	70
2	Big Orange Cikoneng ST	6	20	50
	Sweet Orange	4	20	80

Description: E1 = Leaf Shoot Explant; E2 = Mature Leaf Explant;ormed Consent Applying

The leaf shoots used are terminal shoots that have grown for 2-3 days on the parent plant. The mature leaves selected are leaves that grow 5-10 days after passing the shoot phase. The research that has been carried out shows that leaf shoot explants have better potential to produce callus quickly at 5 DAI for Cikoneng ST Orange explants and 3 DAI for Sweet Orange explants. Leaf shoot explants have a faster callus growth rate, and leaf explants generally experience stagnation when passing the initial induction phase. According to Karjadi and Buchory (2007), terminal shoots are parts of plants that contain many meristem cells. Meristem cells are cells that are still actively dividing. The large number of meristem cells in shoot explants allows for better cell development and growth. This allows explants originating from the shoot to produce callus quickly and optimally (Figure 2).

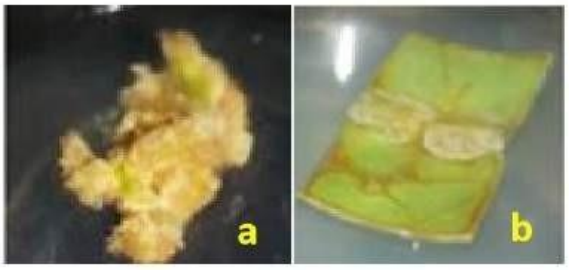

Figure 2 Comparison of Cikoneng ST Orange Explant Callus at 2 MSI

a. Leaf Shoot Explan
b. Leaf Explant

Not all induced calli can grow into calli that can be recultured. In the Cikoneng ST Orange explant, it was found that up to 14 DAI, leaf shoot explants can form friable calli with characteristics in accordance with Yelnititis's statement (2012) in the form of rapid formation of crumb cells and having a white to yellowish color (Figure 6a), while leaf explants only form calli around the wound area and have not developed into calli that can be recultured (Figure 6b). The results showed that up to 28 DAI, more uniform friable calli were obtained from shoot explant

7. The Effect of Explant Planting Position on Callus Growth

Stomata are a crucial part of the metabolic process in plants. Stomata are found on the upper (adaxial) and lower (abaxial) parts of the leaf. According to Muhuria (2007), the abaxial part of the leaf contains more stomata than the adaxial part. The study was conducted by placing explants in adaxial and abaxial positions (Figure 3).

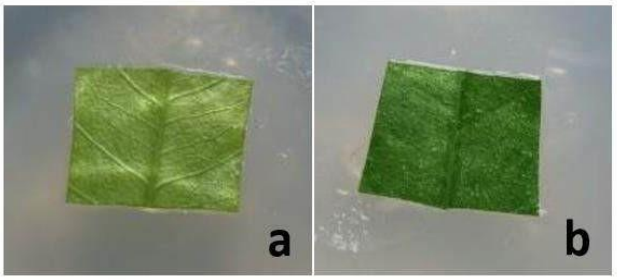

Figure 3 Placement of Explant Position

a. Adaxial
b. Abaxial

The adaxial position allows the stomata on the lower part of the leaf to come into direct contact with the air so that respiration occurs which supports the metabolism process perfectly. In the adaxial position, the lower epidermis tissue which has a thin cuticle layer is able to position the stomata to capture air optimally. The large number of stomata in the lower epidermis layer also triggers faster callus growth. The results of the study showed that adaxial treatment explants were able to form better callus compared to abaxial treatment explants. Up to 28 DAI, it was found that explants with adaxial treatment were able to form callus with a higher percentage of 50% - 80% compared to explants with abaxial treatment which were only able to form callus with a percentage of 30% - 60%. Adaxial explants originating from leaf tips are known to be able to form friable callus optimally on Cikoneng ST Orange and Sweet Orange plants at 28 DAI. This is different from abaxial explants which have not formed friable callus perfectly up to 28 DAI (Figure 4). Callus formed from adaxial explants is a type of friable callus that is ready to be cultured at the next stage of explant development. According to Yelnititis (2012), friable callus with white to yellowish color has the potential to develop into embryogenic.

Table 4. Effect of Explant Planting Position on Shoot Callus Growth up to 28 HSI

POSITION OF PLANTING EXPLAN		AMOUNT (Bottle)	CALLUS GROWTH (%)
A1	Big Orange Cikoneng ST	10	50
	Sweet Orange	10	30
A2	Big Orange Cikoneng ST	10	80
	Sweet Orange	10	60

Description: A1 = Cikoneng ST Big Orange; E2 = Sweet Orange;

8. Comparison of Citrus Plant Types on Callus Growth

Cikoneng ST Orange is one of the various types of Large Orange plants that have a difficult growth intensity, both conventionally and through tissue culture. The high content of phenol compounds in the plant made this orange almost extinct. The obstacles faced in the research process on Cikoneng ST Orange tissue culture have always focused on browning and explant growth time. The research that has been conducted focuses on comparing the callus growth of Cikoneng ST Orange and Sweet Orange. During 28 DAI, the results were obtained in the form of various orange plant calli (Figure 4). Good callus growth was obtained in Sweet Orange plants but not in Cikoneng ST Oranges. Callus development in Cikoneng ST Orange explants tends to be slow and leads to stagnation. The cause of this is possible from the morphology and different phenol compound content of the two types of oranges.

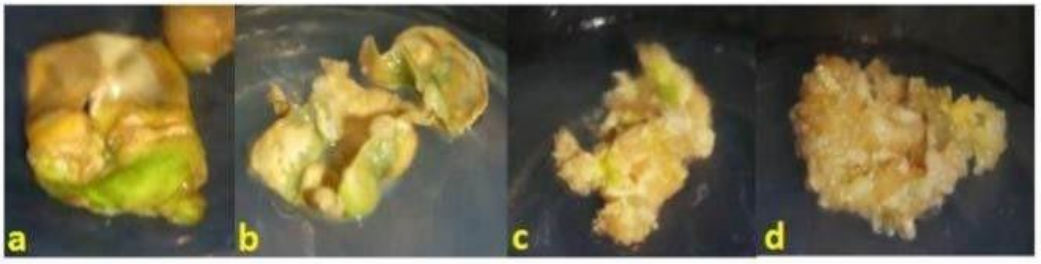

Figure 4 Comparison of Friable Callus between Citrus Species at 28 DAI

a. Abaxial Cikoneng Orange
b. Adaxial Cikoneng Orange

c. Abaxial Sweet Orange
d. Adaxial Sweet Orange

The results of this study are in line with the background of the results of the study conducted by Vandercook et al. (1998) which showed that the species of Orange contains high amounts of flavonoids. In 1 leaf of the Orange species, flavonoid compounds were found as much as 1.54 mg/g and 1.00 mg/g in the leaves of the Sweet Orange species. The group of compounds that dominate in the Orange species is the Naringin compound. Naringin is a phenolic component found in large amounts in various organs of the Orange plant. The content of this flavonoid compound is higher than the content of the Sweet Orange flavonoid compound.Naringin compound in the Orange species is a characteristic that is not possessed by all types of oranges. Naringin dominates the fruit organs of Orange but there is also a fairly large amount in the leaves. Naringin is acidic (Vandercook et al., 1998). According to Hutami (2008), acidic compounds in explants that are injured during initiation will oxidize rapidly and inhibit the work of enzymes to carry out metabolism in plants. This reaction will cause browning to stagnation in explants

4. CONCLUSION

The use of PVP and the selection of plant parts affect the callus induction time and browning percentage. Leaf shoot explants can be induced into callus at 5 DAI while mature leaf explants are only induced at 6 DAI. Leaf shoot explants also have a browning percentage of 90% compared to leaf explants which only have a browning percentage of 70%. The adaxial position is the best planting position to optimally induce friable callus up to 28 DAI.

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