

Conservation Of Juwet (*Syzygium Cumini*) Plant Using In Vitro Culture Techniques

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Abstract

Juwet (*Syzygium cumini*) is a plant that has benefits as a medicine for type II diabetes mellitus, lungs, coughing, laxative urine. However, juwet is a scarce plant, it needs cultivation as a form of conservation. One of them is by utilizing biotechnology, which is of plant tissue culture. This study aims to determine the effect of the combination of 2,4-D and BAP growth regulators on the growth of juwet embryonic callus and the effect of the combination NAA and BAP on the growth of axillary bud juwet as a conservation effort. This research is experimental. Using a Completely Randomized Design (RAL) with combination 2,4-D (0; 0.5; 1; 1.5; 2; 2.5; 5) mg/L, and BAP (0; 0.25; 0.5; 0.75; 1) mg/L and combination NAA (0; 0.25; 0.5; 0.75; 1) mg/L and BAP (0; 0.5; 1; 1.5; 2) mg/L. Analysis by Two Way ANAVA test $\alpha = 5\%$. If there is a significant difference, the Duncan Multiple Range Test (DMRT) test with a significant level of 5%. Addition of 2.5 mg/L and 3 mg/L 2,4-D without BAP can induce intermediate callus, brownish yellow and there is a large cell nucleus in each cell. While the results of axillary bud growth is treatment in BAP 1 mg/L without NAA is the most effective interaction on the emergence of buds is 26.6 days after planting with the total of buds as much as 6.66, and the highest buds 5.37 cm and the highest total of leaves, namely 8.33 strands..

1. INTRODUCTION

Juwet is a local Indonesian plant, many people know it's a mystical plant because it grows wild and lush. However, juwet has the value of a local wisdom which can be utilized

as one of them as traditional medicine, it can be seen in the Bune indigenous people (Katili, 2015).

Juwet plants can live in a high geographical range. Juwet is found in tropical and

subtropical regions (Ayyanar & Subash-Babu, 2012). Juwet fruit (buni fruit, oval), young fruit is green, after cooking it is red purplish red in color and has a strong sour taste. Juwet seeds one in one fruit, oval, hard, white. Have taproots, sympodial branches and light brown stems (Dalimarta, 2003).

According to Mudiana (2007) states that the parts of bark, seeds, and leaves of Juwet plants are efficacious to reduce blood glucose levels in patients with type II diabetes mellitus. In addition, with the taste of sweet and sour fruit, it is cool, strong astringent, aromatic smelling efficacious to lubricate the organs of the lungs, stop coughing, laxative urine. Ayyanar & Subash-Babu, (2012) juwet contains antioxidants, one of which is anthocyanin and flavanoid.

So many benefit of juwet make this plant a high interest. But now in Indonesian the availability of juwets is low. because juwet plants belong to scarce plants, because of lack of attention and cultivation of the community (Dalimarta, 2003).

Cultivation through seeds will produce plants that have low viability compared to their parents. Whereas if using a graft or connecting technique requires seeds with large amounts and up to 90 days. Juwet seedlings can be planted during the rainy season. (Mudiana, 2007) is still little information about the growth of juwet. Not many juwet production centers or juwet cultivation areas. Knowledge of the growth and development of a type of plant is very necessary to know how to handle and maintain juwet species.

In vitro culture techniques one alternative that can solve this problem. In vitro culture is a technique to develop plant parts, both in the form of cells, tissues and organs in aseptic conditions in vitro. This technique is able to produce quality, uniform seedlings, identical to the parent and capable of multiplying plants in a relatively short time (Gunawan 1992).

Factors influence in the Vitro culture are media, growth regulating substances and explants used (Lestari, 2011). The growth

regulator substances in In Vitro culture are auxin (2,4-D, NAA etc.) and cytokines (BAP, kinetin, IBA etc.).

One of the uses of In Vitro culture as plant propagation is embryogenic callus and multiplication of axillary buds (Admojo, 2014 and Gunawan, 1992). Based on the existing problems, the research is carried out, it is hoped that it will increase juwet plants to be sustainable by utilizing biotechnology.

This study aims to determine the effect of the combination of 2,4-D and BAP to grow embryogenic callus and determine the effect of the combination of NAA and BAP on the growth of axillary buds. This is a form of plant conservation juwet (*Syzygium cumini*).

2. MATERIALS AND METHODS

This study included experiments and was conducted in March-August 2018 in the tissue culture laboratory in the Department of Biology, Maliki UIN Malang. Samples from plants that have been incubated at the Green House until new leaves appear. This research was started by taking explants, sterilizing the planets, planting, incubation, morphological and histological observations, then analyzing the data.

The media used are MS, sugar, gelatin powder, 2,4-D, NAA and BAP growth regulator. growth regulator for induction of embryogenic callus is 2,4-D (0 mg/L; 0.5 mg/L; 1 mg/L; 1.5 mg/L; 2 mg/L; 2.5 mg/L; 3 mg/L) and BAP (0 mg/L; 0.25 mg/L; 0.5 mg/L; 0.75 mg/L; 1 mg/L). growth regulator for axillary bud induction is NAA (0 mg/L; 0.25 mg/L; 0.5 mg/L; 0.75 mg/L; 1 mg/L,) and BAP (0 mg/L; 0.5 mg/L; 1 mg/L; 1.5 mg/L; 2 mg/L).

Sterilization for embryogenic callus formation, young leaves are taken and then washed, 5 minutes detergent, 30 minutes fungicide and 60 minutes of water flow. sterilization in laminar air flow using chlorok 30%, 20% and 10% each for 10 minutes and rinsed sterile water 3 times each for 5 minutes. then the leaves are cut 1x1 cm and soaked betadine for 1 minute. 1 bottle containing 3

explants, each treatment had 3 replications and incubated for 45 days.

Sterilization for axillary bud growth. Materials sterilization whit detergen, bacterisidal, dungisicid, alcohol 70%, chlorox and betadine (Bazlina, 2016). Take the juwet stem that has been cut and 30 minutes of water flowing, 1 minute detergent, 60 minutes of water flow, 5 minutes bactericidal, 10 minutes of water flow, 120 minutes fungicide,

60 minutes of water flow. sterilization in laminar air flow using alcohol 70% 1 minute, chlorok 20% and 10% each for 10 minutes, rinsed.


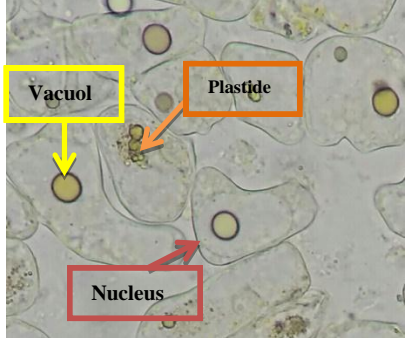

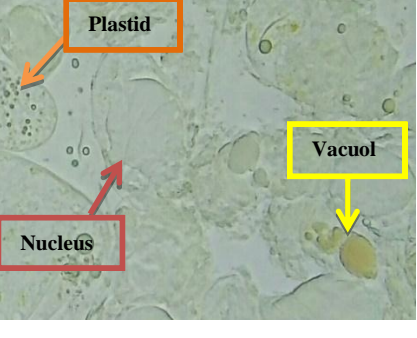
3. Results

Result from this research would can see in Table 1.

Table 1. DMRT 5% Results Effect of 2,4-D and BAP Combination on the Growth of Juwet (*Syzygium cumini*) Embryogenic Callus

Treatment (mg/L)		Callus emerge day (Day)	Treatment (mg/L)		Callus emerge day (Day)
2,4-D	BAP		2,4-D	BAP	
0	0	40.33i	2	0	33.00defg
	0,25	34.66efgh		0,25	26.00abcd
	0,50	32.66defg		0,50	27.00abcd
	0,75	28.66abcde		0,75	32.00cdefg
	1	28.66abcde		1	24.33ab
0.50	0	36.33fgh	2.5	0	36.00fgh
	0,25	25.00abc		0,25	30.33abcdef
	0,50	27.33abcde		0,50	28.33abcde
	0,75	32.66defg		0,75	27.00abcd
	1	33.00defg		1	30.00abcdef
1	0	38.66gh	3	0	33.00defg
	0,25	33.00defg		0,25	27.33abcde
	0,50	29.66abcdef		0,50	23.33a
	0,75	2.66abcd		0,75	28.00abcde
	1	23.33a		1	31.00bcdef
1.5	0	28.66abcde			
	0,25	28.00abcde			
	0,50	23.33a			
	0,75	25.66abcd			
	1	26.00abcd			

Figure 1. The results of observations Morphological and Histological of Juwet (*Syzygium cumini*)

Treatment Results 2.5 mg/l 2,4-D + 0 mg/l BAP		Treatment Results 3 mg/l 2,4-D + 0 mg/l BAP	
Morfology	Histology	Morfology	Histology
			
Intermediate texture Yellowish white color		Intermediate texture Color Brownish yellow color	

Embryogenic Callus after incubation for 45 days

Table 2. Results of 5% DMRT Effect of Combination of NAA and BAP on Induction of Juwet Axillary Buds (*Syzygium cumini*)

Treatment (mg/L)	Budding Day (day)	total of Aksilar shoots	axillary bud height (cm)	Total of Leaves (Strands)
NAA 0 + BAP 0	40.3333 h	2.3333 a	2.3333 a	0000 a
NAA 0 + BAP 0,5	34.3333 g	4.3333 cd	4.3067 d	6.6667 gh
NAA 0 + BAP 1	26.6667 abc	6.6667 g	5.3700 gh	8.3333 i
NAA 0 + BAP 1,5	29.0000 cde	5.3333 de	4.2233 d	6.3333 fgh
NAA 0 + BAP 2	31.3333 ef	4.3333 cd	3.1867 b	5.6667 defg
NAA 0,25 + BAP 0	34.6667 g	4.3333 cd	4.2133 d	2.3333 b
NAA 0,25 + BAP 0,5	28.3333 bcde	4.3333 cd	4.32.33 d	6.3333 fgh
NAA 0,25 + BAP 1	26.3333 abc	5.6667 ef	5.2167 fgh	8.3333 i
NAA 0,25 + BAP 1,5	29.3333 cde	4.6667 de	4.7133 defg	6.6667 gh
NAA 0,25 + BAP 2	31.0000 ef	3.3333 bc	4.2633 d	6.0000 efgh
NAA 0,5 + BAP 0	24.6667 a	5.3333 de	5.3433 gh	5.3333 cde
NAA 0,5 + BAP 0,5	27.3333 abcd	5.3333 de	5.6667 hi	5.6667 defg
NAA 0,5 + BAP 1	25.6667 ab	6.0000 fg	6.1667 i	6.6667 gh
NAA 0,5 + BAP 1,5	28.3333 bcde	5.3333 de	5.2733 fgh	5.3333 cde
NAA 0,5 + BAP 2	29.3333 cde	4.3333 cd	4.3933 de	5.0000 cde
NAA 0,75 + BAP 0	30.3333 de	4.3333 cd	4.3967 de	4.6667 cd
NAA 0,75 + BAP 0,5	28.3333 bcde	5.0000 de	4.4833 def	4.6667 cd
NAA 0,75 + BAP 1	25.6667 ab	6.6667 g	5.1767 efgh	6.6667 gh
NAA 0,75 + BAP 1,5	28.6667 bcde	5.6667 ef	4.3533 d	7.0000 h
NAA 0,75 + BAP 2	29.3333 cde	4.3333 cd	3.3600 bc	6.3333 fgh
NAA 1 + BAP 0	33.3333 fg	3.0000 ab	3.4300 bc	2.3333 b

NAA 1 + BAP 0,5	29.3333 cde	4.6667 de	4.0967 cd	5.3333 cde
NAA 1 + BAP 1	27.6667 abcd	4.3333 de	4.8467 defg	6.6667 gh
NAA 1 + BAP 1,5	28.6667 bcde	4.6667 de	4.2167 d	4.6667 cd
NAA 1 + BAP 2	30.0000 de	3.3333 bc	3.1633 b	4.3333 c

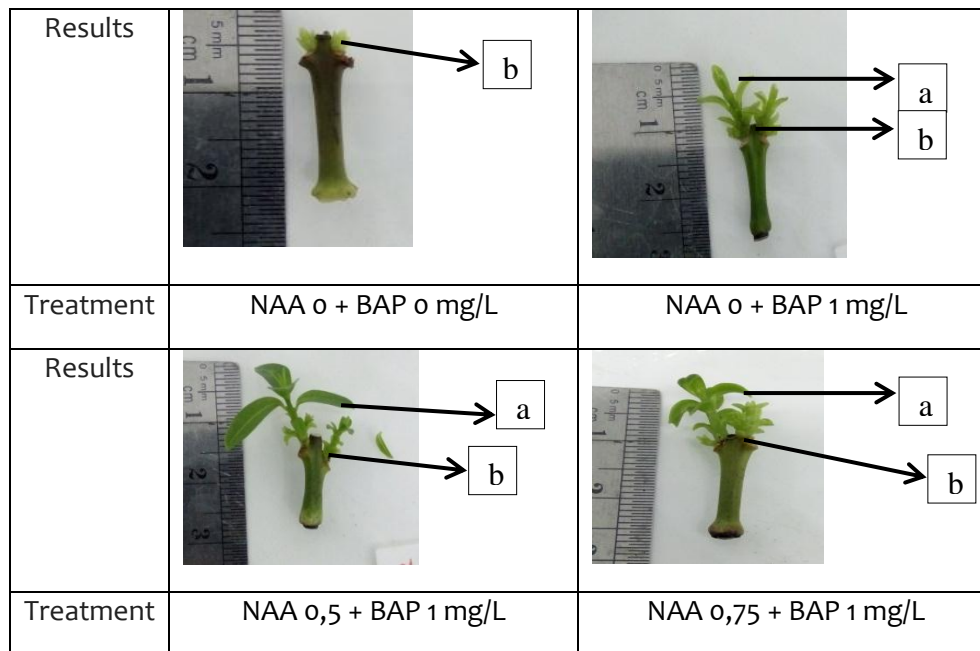


Figure 2. The Results of juwet (*Syzygium cumini*) induction Axillary Buds after incubation for 25 days. Information: a) Leaves, b) Axillary buds

4. DISCUSSION

a. Embryogenic Callus Formation

Based on the ANAVA test that has an effect on embryogenic callus formation is the Callus emerge day. The concentration of 1 mg/L 2,4-D + 1 mg/L BAP has the most significant value. In this combination the Callus emerge day on the 23 day after planting. Rosyida (2014) the right and balanced combination of auxin and cytokinin treatment will be produce optimal callus.

Quality embryogenic callus is a callus that has a crumb texture. However, in the observation results there were no crumbly callus. At concentrations of 2.5 mg/L 2,4-D + 0 mg/L BAP and 3 mg/L 2,4-D + 0 mg/L BAP have intermediate textures, while at the other concentrations they are compact. So that intermediate textured callus is considered the best texture in embryogenic callus formation and is supported by histological observations.

The best color observation results on embryogenic callus were at a concentration of 2.5 mg/L 2,4-D + 0 mg/L BAP and 3 mg/L 2,4-D + 0 mg/L BAP. At a concentration of 2.5 mg/L 2,4-D + 0 mg/L BAP has a yellowish-white color, at a concentration of 3 mg/L 2,4-D + 0 mg/L BAP has a brownish yellow color. According to Mahadi (2016) Characteristics of embryogenic callus are yellowish white. While according to Al-Gendi (2013) the characteristics of embryogenic callus are brownish yellow. The color of embryogenic callus produced by each different plant explant will have a different color. This is because the pigmentation, light intensity and plants are different.

The histology callus at a concentration of 2.5 mg/L 2,4-D + 0 mg/L BAP and 3 mg/L 2,4-D + 0 mg/L BAP showed embryogenic callus. At this concentration there is a large cell nucleus in each cell, there is a clear vacuole but its size is smaller than the cell nucleus and there is a

clear plastid. Ardiyani (2015) the characteristics of embryogenic callus in histological observations are the presence of tissue consisting of living cells, namely plasma and cell nucleus that are clearly visible. Gunawan (1987) added that there were solid cytoplasm, small vacuoles and starch grains.

b. induction Axillary Buds

Effect, so that all variables are followed by a 5% DMRT test. The concentration of 1 mg/L is the most effect concentration in all variables. On the buds emerge day with an average of 26 HST, the number of axillary buds with an average value of 6.06, the height of axillary buds with an average value of 5.35 cm and the number of leaves in axillary buds with an average value of 7,33 barley.

The concentrations of 0.5 mg/L NAA + 0 mg/L BAP were the most significantly different concentrations of buds emerging with an average value of 24.66 days after planting. At a concentration of 0 mg/L NAA + 1 mg/L BAP gave the most effective results for the number of axillary buds with an average value of 6.66. At concentrations of 0.5 mg/L NAA + 1 mg/L BAP the most effective results for axillary buds height with an average value of 6.16 cm. And in the treatment of 0 mg/L NAA + 1 mg/L BAP gave the most effective results for the number of leaves in axillary buds with an average value of 8.33 strands.

Based on the picture (Figure 2) above, it can be seen that the most results are shown in the concentration treatment of NAA 0 mg/L + BAP 1 mg/L. The results of these statements, it is suspected that the high percentage of buds formation without addition of NAA growth regulator can grow, this is possible because physiologically the content of endogenous NAA from juvenile stem explants is sufficient for buds formation. So that the NAA treatment in low concentrations of explants was able to induce buds. Zulkarnain (2009) more cytokines than auxin will form buds, whereas if auxin is more than cytokinin, roots will form.

5. CONCLUSION

The addition of 2.5 mg/L and 3 mg/L 2,4-D without BAP can induce quality embryogenic callus with yellowish white and brownish yellow, intermediate texture, and is a large nucleus cell in each cell. The addition of BAP 1 mg/L without NAA was most effective against the buds emerge day which was 26.66 days after planting, number of buds 6.66, highest buds of 5.337 and the highest total of leaves was 8.33 strands.

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