THE IN VITRO PROPAGATION OF OLIVES IN CENTRAL SULAWESI USING PLANT GROWTH REGULATORS NAA AND BAP

1st Asri Pirade Paserang
Departement of Biology, Faculty of Mathematicsand Natural Science, Tadulako University
Palu, Indonesia
Corresponding Author,email: asripaserang72@gmail.com

2nd Akwila Oktavin
Departement of Biology, Faculty of Mathematicsand Natural Science, Tadulako University
Palu, Indonesia
email: akwilaoktavin@gmail.com

3rd Moh. Iqbal
Departement of Biology, Faculty of Mathematicsand Natural Science, Tadulako University
Palu, Indonesia
email: iqbalmoh89@gmail.com

***Abstract*—** The research of in vitro propagation of olive plants (Olea europaea L.) using plant growth regulators NAA and BAP took place at the Biotechnology and Genetics Laboratory. Aimed to get the right concentration and the best explant source to induce callus. The method used was Randomized Complete Block Design (RCBD) with two factors. The first factor was the explant type, which consists of two levels: leaves and axillary shoots. The second factor was 3 level combinations of NAA and BAP (B1 = 0,5 ppm NAA and 0,5 ppm BAP; B2 = 1 ppm NAA and 1 ppm BAP; B3 = 1,5 ppm NAA and 1,5 ppm BAP). Each treatment was repeated three times, so there were 18 experimental units. The results showed that the source of leaf explants with a combination of 1 ppm NAA and 1 ppm BAP was the best treatment for callus induction, which was 13,67 DAP.

***Keywords*—** *BAP, Callus, NAA and Olea europaea L.*

I. INTRODUCTION

The olive tree (*Olea europaea* L.) is one of the most important fruit crops in the Mediterranean basin [1], 2004). The olive, together with the fig and the date, is one of the oldest fruit trees in the Mediterranean area [2]. The species, originating in the eastern part of the Mediterranean basin, belongs to the genus Olea, which is one of over 30 genera of the Oleaceae family [3].

Plant Tissue Culture is a process that uses plant material in a growing medium to grow new plantlets. The initial plant material is cultured and developed in a specific and tightly controlled environment. Otherwise known as micropropagation, the Tissue Culture Process helps you to grow multiple uniform plants in quick succession. This process is beneficial for developing countries looking to increase crop yield, a private at-home grower interested in producing consistent quality, as well as businesses looking to produce exact replicas of a species for profit.

Sulawesi Island has the potential as an olive cultivation area. The provision of olive plant seeds can be carried out through tissue culture. Tissue culture is an in vitro propagation technique that can meet the demand for plant seeds on a large scale through callus induction using leaf explants and axillary shoots in a relatively short time.

This study used a combination of growth regulators (ZPT) in the form of BAP from the cytokinin group and NAA from the auxin group. The aim of this study was to obtain the right concentration and the best explant source for the induction of olive callus (*O. europaea* L.).

II. RESEARCH METHOD

The olive plant material was obtained from the biology garden of Faculty of Mathematics and Natural Sciences,Tadulako University, Palu. The explant criteria used in the experiment were the colour of fresh green leaves, the stem is a bit whitish because it is still young, if the stem is brown then it contains a lot of tannins.

This study uses an experimental method, research using a Completely Randomized Group Design (RKLT). Each source of leaf explants (A1) and axillary shoots (A2) was given 3 treatments (B1, B2 and B3) with 3 replications so that 18 bottles of culture were observed. The composition of the treatment media as follows:

Table 1. design of treatment

Treatment	Explant	NAA (ppm)	BAP (ppm)
A1B1	Leaf	0,5	0,5
A1B2	Leaf	1,0	1,0
A1B3	Leaf	1,5	1,5
A2B1	Axillary shoots	0,5	0,5
A2B2	Axillary shoots	1,0	1,0
A2B3	Axillary shoots	1,5	1,5

Explants were taken by cutting axillary shoots and young leaves using a sterile cutter. Then the explants are ready to be sterilized. The sterilization steps include soaking 2 drops of tween for 20 minutes, 0.5 g fungicide and bactericide for 60 minutes, clorox 10% and 12% for 10 minutes and immersion in alcohol 70% for 1 minute.

Explant were grown on medium, concentration of MS [4], supplemented with 30 g/L sucrose and 8 g/L of pure agar. Each treatment bottle was added with hormones according to the list in table 1. For callus induction, explant on medium incubated in dark conditions at 24°C for 7 days and light conditions for 7 days.

III. RESULTS AND DISCUSSION

The results obtained at all concentrations (B1, B2 and B3) can induce callus by leaf explant sources (A1). At the source of axillary shoot explants (A2), only treatment with concentrations of B2 and B3 induced callus, while the

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treatment with concentration of B1 only experienced swelling of the axillary shoots (not causing callus).

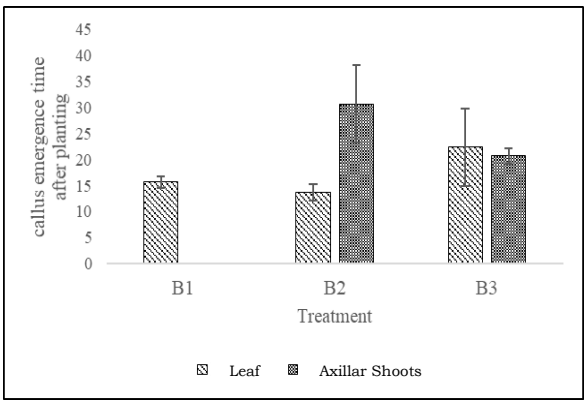

Figure 1. Effect of hormone on treatment of axillary shoots and leaf explants on callus emergence time after planting.

Callus appears initiated by changes in the surface of the explant. The explant curves and then swelling occurs, then small clumps of cells appear in the injured area. Source of leaf explants (A1) can form callus in treatments B1 (0.5 ppm), B2 (1 ppm) and B3 (1.5 ppm). Explant sources from axillary shoots (A2) succeeded in inducing callus in treatments B2 and B3. while treatment B1 formed callus after 62 days after planting.

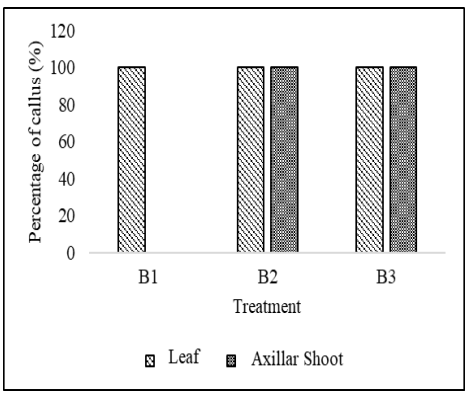

Figure 2. Percentage of callus formation except treatment A2B1

This may be due to the low concentration of the hormone in the media. [5], the addition of inappropriate hormones causes the process of plant morphogenesis to be hampered. Callus that was not successfully induced was caused by the medium given low auxin and cytokinin, so that more hormones were needed[6][7][8]. The appearance of callus is influenced by the work of endogenous and exogenous auxins and cytokinins which are correlated with each other.

The fastest callus formation was on day 13.67 at a concentration of 1 ppm. According to research by [7], reported on olive plants with anther explants it takes 14-21 days for callus formation. While the longest leaf callus induction was 1.5 ppm on day 22.33 after planting, because the concentration given was the highest concentration of the other treatments. High hormone concentrations are more inhibitory than inducing callus on leaves. Meanwhile, in the explant treatment of axillary shoots, callus induction was effective at a concentration of 1.5 ppm on day 20.67. The longest callus induction was treated with axillary shoot explants at 1 ppm on day 30.67.

The percentage of explants forming callus

The percentage of callus formation was based on the number of explants that had successfully induced callus. The percentage of callus formation is 100%. Both the axillary shoot and leaf explant sources, except for the A2B1 treatment. The induced callus was dominant in the explants that were in direct contact with the media. This is in accordance with research [9]. Succeeded in inducing tobacco callus with a percentage of 100% at various concentrations of combinations of growth regulators NAA and BAP.

Callus color

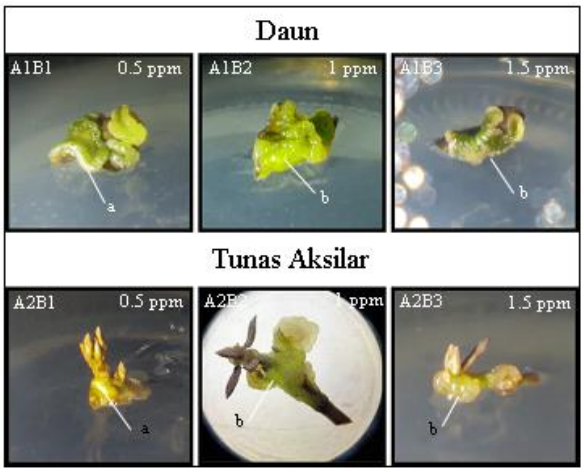

Figure 3. Callus color. a. Callus, not growing, b. Callus

The successfully induced callus had a visually observable color (Figure 3). Observation of this color using the RHS Color Chart.

The successfully induced callus had different color responses[10]. Color can be an indicator of callus representation that still has cells that are actively dividing or are heading to the stage of death. Callus color can be seen in (Table 2). Callus that is white is still actively dividing compared to callus with green color which is ready for subculture to induce shoots and roots [11].

Table 2. Callus color

Treatment	Callus color
A1B1	Greenish white
A1B2	Greenish white
A1B3	Greenish white
A2B1	Not growing
A2B2	Yellowish white
A3B3	Yellowish white

IV. CONCLUSION

The conclusions that can be obtained from this research are:

1. The best concentration of PGR combination for olive callus induction was 1 ppm NAA and 1 ppm BAP.
2. Leaves are the best source of explants for olive (*Olea europaea* L.) callus induction.

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