Shoot Regeneration of Sandalwood (*Santalum album* L.) by Different Media and *Benzile Amino Purine* (BAP) Concentrations

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ABSTRACT

Sandalwood (*Santalum album* L.) has a variety of benefits. The crops are harvested sandalwood to be used is the roots and the wood as much to contain the essential oil. Many benefits of sandalwood is not matched with the success of the cultivation of sandalwood judged is still low. The condition encouraged to make a breakthrough the provision of the seeds of sandalwood en masse throught techniques is in vitro. The composition of media and the addition of the regulator is grow affect success in the culture of tissues. The Woody Plant Medium (WPM) and Murashige and Skoog (MS) a basic media that contains the source of food contain the compound organic and inorganic to growth explant. Sterilization of sandalwood explant is done by washing with detergent, rinsed with flowing water, immersed in fungicide, bactericida, NaCl 5% and ethanol 70% solutions. Sandalwood explant then planted in the WPM and MS media with the addition of regulator is grow Benzile Amino Purine (BAP). Sandalwood explant that grow in the WPM medium with the addition of BAP 2 ppm provide optimum results in parameters day of the emergence of shoots, the long of shoots and the number of leaves. While parameters the number of shoots optimally if it is grow the MS medium with the addition of BAP 2 ppm.

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1. INTRODUCTION

Sandalwood (*Santalum album* L.) is a plant grow naturally in Indonesia, especially in region East Nusa Tenggara (NTT). Sandalwood has a variety of benefits, namely wood and the oil often used to ceremony customs and a death. Other benefits of sandalwood plant is the wood mostly used to the raw materials craft like the statue, rosary and fun (Herawan, 2012). The crops are harvested sandalwood to be used is the roots and the wood as many as to contain the esensial oil (Brand and Jones, 1999).

According to the Surata and Idris (2001) the success of the cultivation of sandalwood judged is still low less than 50%. In addition to the provision of the lamd lack of adequate there are other causes about a lack of success sandalwood plants is based on the nature of crops were easy to die. The death of the sandalwoods (Surata 2006). The condition encouraged to make a breakthrough the provision of the seed of sandalwood en masse.

To overcome these problems, then techniques propagation vegetative in culture network is expected to be able to meer the provision of the seeds and masse is in a short time. Lestari (2011) states that advantage procurement seedlings through the culture networks between else can be obtained of the plants excelled in large quantities and uniforms, in addition can be obtained sterile (mother stock) so that can be used as a material for next propagation. While according to the Welsh (1981) creation of new is efficient, cheap and free from the virus

and fungi.

The composition of the effect success media also the culture of tissues, because the media as a source of food should contained the compound organic and inorganic, like a nutriet macro and micro in the levels of certain and comparison, sugar, water, amino acid, vitamins and ZPT. Important factor that shouldn't be ignored is ion ammonium and potassium (Santoso and Nursandi, 2004).

2. RESEARCH METHOD

2.1 Plant Material

Plant used in this research is axilar shoot from sandalwood plant. Sterilization process started by washing explant with detergent then rinsed with flowing water within 1 hour. Explant then placed inside laminar air flow cabinet, soaked in NaOCl 5 % solution for 5 minutes then in ethanol 70% solution for 30 seconds. Last, explant then rinsed 3 times with sterile water.

2.2 Treatment of Growth Medium and Plant Growth Regulator

The medium used in this research is solid basic medium Woody Plant Medium (WPM) and Murashige and Skoog (MS). Prepared WPM and MS were dissolved in aquades up to 1 liter, and added with 3% sugar and 7 gr/l agar. To induce bud generation, Plant Growth Regulator *Benzyl Amino Purine* (BAP) was added as much as 0, 1,75 and 2 ppm into WPM and MS media. Acidity of media was set up to 5,8 by adding NaOH or HCl solution. Growth media then sterilized using autoclave at 121^{o}C and 1 atm pressure within 15 minutes. Sterilized media then stored in sterile room.

2.3 Observation

Observartion is done daily to discover the day of the emergence of sandalwood bud. While observation of bud quantity, bud length, and leaf quantity is done at week 6**.**

3. RESULTS AND DISCUSSION

3.1. The Day of The Emergence of Shoots

The day of emergence of a roots is one of the important factor to increase a plant of net tissue culture method. Make it faste of the emergence of a shoots so it is faster to produced material to increase the plant. The shoots what is formed is the different result to explant. The average when the sandalwood appears the plant explant shoots in various media and concentration BAP is served on the table 1.

Table 1. The influence of the type of media and concentration BAP to the day of emergence of a sandalwood shoots (*Santalum album* L.)

The Handling	The emergence of shoots
WPM 2 ppm	8,33 a
MS 2 ppm	10,33 b
WPM 1,75 ppm	10,67 b
MS 1,75 ppm	10,67 b
WPM 0 ppm	12,33 c
MS 0 ppm	15,33 d

The description: the numeral what followed the same letter indicate is not the real different is based on the DMRT test at the level 0,05%.

WPM is the fastest medium to induce sandalwood is compared with MS medium. According to the Pardal *et al*., (2004) WPM much used in a range of species of plant, because it has the content of the ion low, but the sulphate content is high. The elements of macro located in the WPM like an element of magnesium high very supportive in the growth of tissue plants.

The addition of regulator grow BAP in the media is also very influential on the speed of the emergence of a shoots. BAP with a concentration 2 ppm can accelerate day of the emergence of a shoots of sandalwood explant. According to Kurnianingsih *et al*., (2009) BAP instrumental spur the occurrence of the synthesis RNA and the protein at a variety of the network next to encourage the cell division.

Figure 1. Day of the emergence of shoots day to 8 treatment WPM 2 ppm

3.2 The Number Of Shoots

The formation of a shoots can indicated as success multiplication in a culture of tissue plant. The more shoots formed the increasingly high levels of the multiplication. An average the number of shoots sandalwood explant in a variety of media and concentration BAP served at table 2.

Table 2. The influence of the type of media and concentration BAP to the number of sandalwood shoots (*Santalum album* L.)

The Handling	The number of shoots
WPM 0 ppm	1,67 a
WPM 1,75 ppm	2,00 ab
WPM 2 ppm	2,00 ab
MS 0 ppm	2,00 ab
MS 17,5 ppm	2,33 ab
MS 2 ppm	3,33 b

The description: the numeral what followed the same letter indicate is not the real different is based on the DMRT test at the level 0,05%.

Based on the table 2, can be known that the MS medium be able to induce growth shoots more compared to the WPM medium. It is a possible because MS medium has concentration high salt minerals and compounds N in the form of NO3- and NH4+, so can induce shoots more quikly compared to the WPM medium (George and Sherrington, 1984).

The addition of regulators grow from the cytokines class proved to be able to induce more shoots. It can be seen on the table 2, where the use of BAP higher (2 ppm) can induce shoots of the most. According to George and Sherrington (1984) that the concentration cytokines higher compared with a consentration auxin will be spurred multification shoots.

A combination of the use of the MS medium with the addition of BAP 2 ppm produce the number of the highest shoots. According to the Hoesen (1998) success culture *in vitro* determined by substances regulator grow and the basic media used.

Figure 1. The number of shoots treatment MS 2 ppm

3.3 The Long of Shoots

The observation of long shoots was done at the end of the observation namely at week 6 after planting. The average of the long shoots explant sandalwood survived at tables 3.

Table 3. The influence of the type of media and concentration BAP to the long of sandalwood shoots (*Santalum album* L.)

The Handling	The long of shoots (cm)
MS 1,75 ppm	0,32 a
WPM 0 ppm	0,35 a
MS 2 ppm	0,39 a
MS 0 ppm	0,45 a
WPM 17,5 ppm	1,12 b
WPM 2 ppm	1,22 b

The description: the numeral what followed the same letter indicate is not the real different is based on the DMRT test at the level 0,05%.

The shoots of the sandalwood explant that grow is in the media WPM longer than in the MS medium. It is allegedly because the elements of Ca contained in the media WPM pretty high compared to the MS medium, where the elements of Ca instrumental is in cell growth plants. In addition to it, the WPM medium is the media that used in the culture of tissue in various types of plant. Wetherell (1982) added that in the media should be contained minerals, sugar, vitamins and hormone by comparison needed was right. Allegedly WPM medium has the content of the nutrition enough to support the formation of shoots.

3.4. The Number Of the Leaves

The leaves is an organ vegetative of plant, growth leaves influenced by the content of nitrogen located in the media growing up. In the addition to the leaves is an important organ in the growth of plants because the leaves is as a the occurrence of photosynthesis. The greater number of leaves, indicate growth explant is getting better (Acima, 2006). The results of the observation against the number of leaves served at tables 4.

Table 4. The influence of the type of media and concentration BAP to the number of the leaves sandalwood (*Santalum album* L.)

The Handling	Number of the leaves
WPM 0 ppm	1,77 a
MS 0 ppm	1,83 a
MS 17,5 ppm	2,13 a
MS 2ppm	2,47 ab
WPM 17,5 ppm	2,77 ab
WPM 2 ppm	3,53 b

The description: the numeral what followed the same letter indicate is not the real different is based on the DMRT test at the level 0,05%.

Based on the tables 4, can be know that sandalwood explant that grow in the WPM medium produce leaves with the highest. This allegedly is in the WPM medium contained elements of magnesium with the number of enough to the growth of leaves. In addition there are elements of calcium nitrates, the elements of this there is not in the MS medium. Altought the elements of this contaied in the leaves but with the addition of media then growth leaves can walk more optimally, so that allegedly elements of magnesium and calcium contained in the WPM medium can be used in the efficiency by explant to the establishment of and growth leaves. According Wijayanti (1994) elements of Mg can increase the content of fosfat in the plants. Fosfat is raw meterials for the formation of a number of protein. With the formation of protein then growth leaves also will be well.
The addition of regulator is grow BAP giving positive response towords the establishment of the leaves. The number of the leaves become in tandem with an increasing number of BAP that added in the media. According to the Yelnititis *et al.*, (1999) the addition of cytokines may drive a growing number of and the size of the leaves.

With the addition of cytokines (BAP) in the media may drive cells meristem on explant to divide and influence other cells to develop into a shoots and from of leaves.

CONCLUSION

The influence of base type media and BAP concentrations on shoots induction on sandalwood explant has give result that the explant grown on WPM medium with addition of BAP 2 ppm has give optimal result on parameters day of appearance shoots, shoots length and number of leaf. For the number of shoots more optimal than WPM, sandalwood plant growth on MS medium with the addition of BAP 2 ppm.

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