A method for aseptic culture of bud explants pogestemon cablin benth Var Tapak Tuan, Aceh, Indonesia

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Abstract. Pogostemon cablin Benth var Tapak Tuan, Aceh, Indonesia is a plant herb potentially to be developing with culture method. However, culture of this cultivar (stem and leave of bud0 explants sampled from outdoor trees is sometimes challenges due to contamination not overcome by standard surface-sterilization. An Improve method, involving a 5-minute immersion of explants in 5% (v/v) detergent, 1% fungicide and bactericide, 5% NaClO and 0.5% antibioticswhich does not interfere with normal explant and callus development, is described in detail.

1. Introduction

Patchouli is an aromatic plant that has been utilized as raw material in perfume industry primarily. It also plays a role in area of food, cosmetic and pharmacology because of patchouli oil content (up to 5%). Patchouli oil has therapeutic properties viz. anti-inflammatory, antiseptic, fungicide, insecticide, aphrodisiac, antidepressant, decongestant, astringent, carminative, diuretic, febrifuge, sedative and tonic (Swamy and Sinniah, 2015). Currently, this plant has been cultivated extensively in Indonesia, Malaysia, China and Brazil. Approximately 80 - 90% of today's global production of 1200-1300 metric tonnes per annum is realised in Indonesia (Beek and Joulain, 2017; Idris *et al*. 2014; Yahya and Yunus, 2013) with the price level is US $ 63-150 per kg for good quality oil and predicted to attain a value of $ 75 million by 2020 (Beek and Joulain, 2017).

There are three types of patchouli that are cultivated in Indonesia, namely *Pogostemon heyneanus*, *Pogostemon hortensis* Backer and *Pogostemin cablin* Benth. with the familiar name of Patchouli Aceh. *P.cablin* Benth. that classified into some sub-varieties (Tapak Tuan cultivars (Fig. 1), Lhokseumawe cultivars, Sidikalang/ Aceh Tamiang cultivars) (Harunsyah, 2012) are leading export commodity that is famous and favoured by American and European due to its quality (Schduw dkk, 2012; Beek and Joulain, 2017). *P.cablin* is believed from Philippines and grows wild in hot and humid climatic conditions with height of 1-1.2 m. The

broad leave is 0.85 inches; margin is lobed and abundant hairs on its dorsal surface. Essential oil is accumulated in the glandular trichomes and rarely flowering (Chakraphani *et al*., 2013; Swamy and Sinniah, 2014).

On the other hand, cultivation and production of patchouli oil processing in Indonesia, particularly Aceh is has been performed conventionally using simple technology. The high demand of seedlings with outstanding of short time for cultivation and harvest, free of various microorganisms, regulated scale area and standard method for extraction process is not fulfilled yet the international response; as consequent, the oil produced is difficult to accept in the world indeed lower price (Idris, *et al*,, 2014; Muharam *et al.*, 2017).

Several studies have been reported for patchouli propagation using tissue culture. However, the sterilization method for P. *cablin* Benth var Tapak tuan is not reported yet. Therefore, the objective of this study is to standardize the aseptic culture of bud of steam and leaves bud explants for propagation of P. *cablin* Benth var Tapak tuan.

Fig 1. Pogestemon cablin Benth var Tapak Tuan

2. Materials and Methods

2.1. Materials

The study was conducted at the YAHDI Tissue Culture Laboratory with plant material was derived from Aceh Selatan, Tapak Tuan. Other materials were MS medium, alcohol, sterile distilled water, detergent, clorox (NaOCl), antibiotics, fungicides, bactericides, BAP and IAA. Standard tissue culture tools were used for this research.

2.2. Methods

Sterilization of explants

Explant sterilization was carried out using explant material from outdoor, namely stem and leave buds of patchouli. The leaves of patchouli that are used as explant sources are cut and wash the explants with 5% detergents and brush with a toothbrush gently. Subsequently it was washed in running water, soaked in different sterilizing and different times (fungicide and bactericide) as tabulated in Table 1. These steps were conducted in outside of Laminar Air Flow (LAF) cabinet. The further steps were performed inside of LAF including (a) washing in distilled water 2-3 times, soaked in 10% of clorox with different time, then washed in sterile distilled water and soaked in amoxilin (Harahap *et al*., 2015)

Table 1. Sterilization procedure with sterilant name and duration of soaking

Procedures	Treatment	Sterilizing Name and Treatment	Duration (minutes)
1	Outside LAF	5% Detergent	3
	Outside LAF	1% Mancozeb as Fungicide, 1% Streptomycin sulphate as Bactericidal Sterile distilled water (explants were washed 3 times)	30
	In LAF	5% NaClO (Sodium Hypochlorite) Sterile distilled water (explants were washed 3 times)	5
	In LAF	0,5 % amoxilin antibiotics Sterile distilled water (explants were washed 3 times)	30
2	Outside LAF	5% Detergent	3
	Outside LAF	1% Mancozeb as Fungicide, 1% Streptomycin sulphate as Bactericidal Sterile distilled water (explants were washed 3 times)	30
	In LAF	10% NaClO (Sodium Hypochlorite) Sterile distilled water (explants were washed 3 times)	10
	In LAF	0,5 % amoxilin antibiotics Sterile distilled water (explants were washed 3 times)	30
3	Outside LAF	5% Detergent	5
	Outside LAF	1% Mancozeb as Fungicide, 1% Streptomycin sulphate as Bactericidal Sterile distilled water (explants were washed 3 times)	45
	In LAF	5% NaClO (Sodium Hypochlorite) Sterile distilled water (explants were washed 3 times)	5
	In LAF	0,5 % amoxilin antibiotics Sterile distilled water (explants were washed 3 times)	45

Observation

The parameters observed were morphological observations which included explant conditions that was contaminants or those that were not contaminants**.**

3. Results and Discussions

Sterilization is the most critical factor in the successful of tissue culture processes. Generally, plant material from outdoor contains a lot of impurities that allow contamination. Impurity explant would contain and grow up fungi and bacteria. As a consequent, the explant will be died at the same time. Thus, the right sterilization method without turning off the

tissue cells should be selected. There are 2 stages of sterilization, namely sterilization outside LAF and inside LAF.

In the first stage of the sterilization process using detergent which serves to remove impurities that exist on the surface of explant material. The active surfactant that is present is detergent having molecules that do not like water so that they can remove oily dirt, while other molecules that like water function to relax dirt from the substrate so that the dirt does not stick back. Then the explants will be immersed in an alcohol solution, where alcohol is an organic solvent and a strong sterilizer that functions as a germ killer. The reason for using alcohol with a concentration of 30% for 10 minutes is to prevent tissue death in the leaves. Whereas for fungicides and bactericides is a liquid solution that is quite effective in reducing microorganisms. Giving antibiotics is to treat wounds when cutting explant from its parent material. The last one is soaking Clorox which contains the active ingredient NaOCl (Sodium hypochlorite) (Figure 2). The function of NaOCl is disinfectant that cleanses contamination in explants, the reason being that this compound effectively kills bacteria and viruses (Suratman, 2013). Besides, NaOCl was able to clean microorganisms bound in plant material, remove soil particles, dust, etc. Santoso and Nursandi (2001) stated that every immersion process always ends with a washing process using sterile water until it is certain that the treatment given previously is gone.

The results of this study were exhibited in Figure 3 that the procedure (1) occurs contamination after 4 days, and the procedure (2) occurs contamination after 6 days, and the procedure (1) absent of contamination (observation was performed after 17 days). Contamination is a condition where the explants planted experience interference in the process of growth, usually overgrown with fungi or bacteria.

Fig 2. The sterilization process of *P. cablin* benth. var Tapak Tuan

Fig 3. Comparison the growth of explant after sterilization in different method sterilization after (a) 4 days, (b) 6 days, (c) not contamination after 17 days

4. Conclusion

The study describe the sterilization method for invitro culture particularly the explant that come from outdoors.

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