

10. Disinfecting technology of Camellia sinensis L inoculants through in vitro culture

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Disinfecting technology of *Camellia sinensis* L inoculants through *in vitro* culture

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Abstract. Leaves of the *Camellia sinensis* plant is a very economically valuable commodity and can be applied to various fields. Such as in the fields of agriculture, food and beverage and medicine. The problem that is found in the land area is the presence of plants that are more than tens of years old that need to be plant rejuvenated. Rejuvenation with grafting and cutting techniques is strongly influenced by the climate and extensive agricultural land. To overcome this problem, *in vitro* culture techniques are applied as an effort to conserving/producing plant seeds that are free of disease pests, controlled environments and with narrow land or laboratory scale. This research aimed to determine the inoculant of *Camellia sinensis* which gave the best results on the disinfecting through *in vitro* culture technique. The method used in the research was using two types of inoculants, they are shoot buds and young shoots in the disinfecting process, and each process was repeated fifteen times. The results obtained were that the disinfecting of *Camellia sinensis* plants with shoot bud inoculants showed the lowest level of contamination with the number of growing inoculants close to 85 percent in twelve weeks of inoculant harvesting.

1. Introduction

Camellia sinensis is a plant that has high economic value because its leaves can be used in various life activities. In agricultural activities, the leaves are used in a plant-based way as an anti-repellent against *Empoasca* sp. [1]. In health, activities can be as antibacterial, preventing cancer, as anti-obesity, and can be as an antioxidant. The food and beverage sector is useful for natural dyes related to their chlorophyll content [2-6].

Besides having a variety of benefits, there is a problem that is found in the leaves of *Camellia sinensis* that are plants that are decades old [7] to hundreds of years that need rejuvenation. Plant rejuvenation by replanting plants both generatively and vegetatively (grafting and cuttings) is strongly influenced by the environment, climate, and extensive agricultural land. The problems of old plants can be overcome by preparing seedlings through *in vitro* culture as an effort to conserve which produces seeds that are expected to be resistant or disease-free. The advantages of *in vitro* culture include being free from disease as well as being easy to control the environment or not dependent on climate, biomass can be harvested all the time [8] and can be done on limited land on a laboratory scale or can be made on an industrial scale.



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One of the factors that must be considered in the application of in vitro culture is the use of inoculants that are used related to purification activities. In general, the selection of inoculants is very important in the in vitro culture of shoot bud inoculants consisting of meristem tissue that will be more responsive in cell division. Older inoculants tend to require a long time to splitting up and will affect cell division and cell regeneration. Sterile inoculants are also important in in vitro culture technology, which can prevent or eliminate contaminants so that inoculants can be further prepared for research purposes such as crop production, plant conservation [9], storage, genetic resources, and biomass production.

The purpose of this research was to obtain *Cammelia sinensis* inoculants which provide the best results in disinfection process with *in vitro* culture techniques.

2. Methods

This research was conducted in the biotechnology laboratory of Agriculture faculty of UPN "Veteran" East Java, Indonesia using a descriptive method with a reason to describe the conditions in each research situation based on the reality that emerged at the time of the study. This research used two types of inoculants namely shoot buds and young shoots cut in a square shape before being planted on Murashige and Skoog/MS media [10], in purification activities following the method [11]. The first stage of the research was disinfecting the two types of inoculants in a 2 g L⁻¹ bactericide bath for 60 minutes [12] then rinsed off with distilled water.

The second stage of the research was inoculants soaked in a 2 g L⁻¹ fungicide solution for 90 minutes and then rinsed off with distilled water. The third research stage was soaked in 20% NaOCl for 10 minutes and in 5% NaOCl for 20 minutes [13] then rinsed off with distilled water. The fourth stage of the study was soaked in 70% alcohol for 5 minutes and then rinsed off with distilled water 3 times. After that, the inoculant soaked in a 3% vitamin C solution for 5 minutes and rinsed off with distilled water. Sterile inoculants were grown on Murashige and Skoog/MS media supplemented with 5% sucrose and BAP 3 mg L⁻¹ [14]. Each inoculant in the research activity was repeated 15 times.

2.1. Tools and materials

2.1.1. Tools. The tools that used in this research were: olympus CX 31 stereo microscopes, autoclaves, Petri cup, culture bottles, oven, storage tanks, 0.001 mg sensitivity analytical scales (Shimadzu), magnetic stirrers, beaker glass, hot plates, and chemical spoons, label paper, and aluminum foil.

2.1.2. Materials. Bud shoots and young shoots of the *Cammelia sinensis* plant taken from the greenhouse for inoculants materials. The materials in the form of macro elements that used were: NH₄NO₃, KH₂PO₄, CaCl₂, 2H₂O, KHO₃, MgSO₄.7H₂O. The materials in the form of micro elements that used were: ZnSO₄.7H₂O, H₃BO₃, KI, CuSO₄.5H₂O, CoCl₂.6H₂O, MnSO₄.4H₂O, Na₂MO₄.2H₂O, elements of Iron (FeSO₄.7H₂O, Na₂EDTA.2H₂O). The ingredients in the form of vitamins elements and water elements that used were: pyridoxine-HCl, Glycin, Thiamine-HCl, myo-inositol, sterile distilled water.

3. Results and discussion

The results of research conducted, obtained information that shoots buds inoculants contamination was 27%. This contamination is probably derived from a type of fungi, hyphae that appear as white fibrous fibers that form spore points with the growth of denser white threads. The next acknowledge of contamination is white mucous liquid that resembles milk liquid which is above the medium around the inoculant. Image of contaminants of these fungi types and bacteria in Figure 1.

In Figure 1A, it can be seen that contaminated inoculants are surrounded by a form of white cotton which has hyphae / white threads caused by fungus/fungi. This is because it can be from a culture environment such as air from a room contaminated by officers that are carried from outside the culture

room. To overcome this problem, it is necessary to use a tool or use a container that is tightly closed when sterilizing and separated from one another [15].

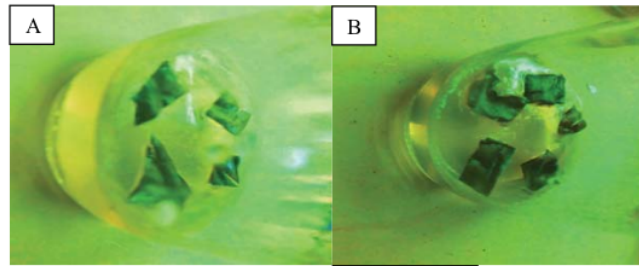


Figure 1. (A) Fungal contaminated inoculants, (B) bacterial contaminated inoculants (Scale bar=5mm).

The results of research using young shoot inoculants, contamination data obtained by 15 % which is less than using bud shoot inoculants. In addition, data on the percentage of young shoot inoculants that is growing by 85 % and data on the percentage of shoot buds inoculants that are growing by 73% are obtained Figure 2.

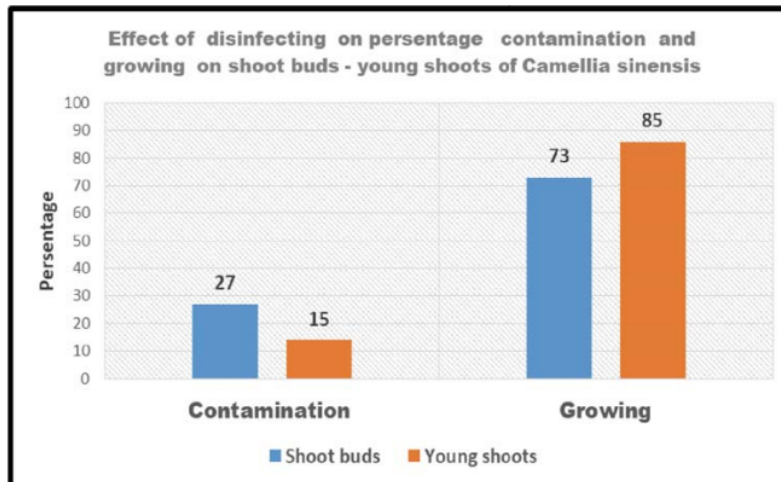


Figure 2. Effect of disinfecting on Percentage contamination and growing on shoot buds- young shoots.

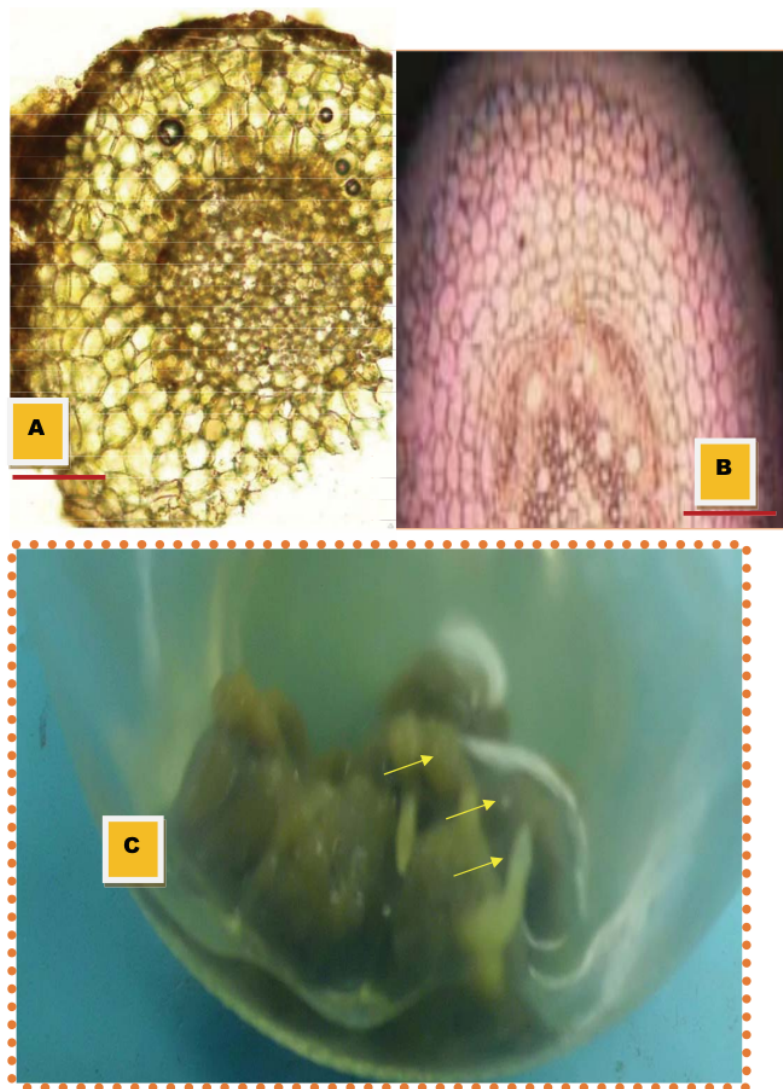


Figure 3. Inoculant root structure of *Camellia sinensis* young shoots that exists (A), plant root structure - as standard (B), and macroscopic from inoculant roots of *Camellia sinensis* young shoots that exists / yellow arrows (C), (Scale bar = 5mm).

In Figure 2 the use of shoot buds obtained contamination data of 27%, this is because, in shoot buds, its inoculant shape is in the form of leaf buds that are still curled. The curled shape and humidity

conditions cause the fungus to multiply very fertile, so it accelerates the contamination. To overcome this contamination, according to Monokesh et al. [16] it is necessary to optimize the use of various anti-contaminant agents with various concentrations in order to obtain optimum conditions to prevent contaminants. Bacterial contamination in Figure 1B includes latent contaminants which include contamination from the original plant or in the inoculant itself because it can grow on inoculants that are more than 3 weeks old [17]. Meanwhile, to overcome contamination caused by bacteria need to be done with bleaching powder combined with broad types of antibiotics such as amoxycillin, 70% alcohol and mercuric chloride [18].

The root structure of the young bud inoculants of *Camellia sinensis* living macroscopically - microscopically, was observed with an Olympus CX 31 stereo microscope, and the root structure of the plant (as standard) is shown in Figure 3. In young shoots inoculants, obtained inoculants that grow by 86% (Figure 2) and shown by the appearance of the root structure in Figures 3 A and C. The growth of inoculants in young shoots is because the cells are actively splitting and the cells respond to growth regulators which are added to inoculation media [19].

4. Conclusion

The conclusion of this research is that the technique of disinfecting with bud shoot inoculant of *Cammelia sinensis* L plants showed the lowest level of contamination with the number of growing plants that reached 85%.

Acknowledgment

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