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Use of Sterile Materials and Soaking Time For Banana (*Musa Spp*) Explant

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ABSTRACT
NaOCl is often used in various sterilization techniques with various concentrations and soaking times. In addition to NaOCL which is used for explant sterilization, 70% alcohol is also often used for the explant sterilization process. Usually mushrooms or fungi will die if exposed to a 70% alcohol solution. The aim of the study was to determine the method of sterilization with NaOCl concentration followed by immersion in alcohol on the acquisition of sterile explants of kepok banana plant. The research was conducted at the Biotechnology Laboratory, Faculty of Agriculture, UPN Veteran, East Java. The research design used a non-factorial Completely Randomized Design (CRD), the treatment consisted of 6 sterilization treatments namely (S1), (S2), (S3), (S4), (S5), (S6). The results showed that there was no effect between the concentration of NaOCl sterilization and the duration of immersion in alcohol on the percentage of live explants and the percentage of contaminated explants, and the sterilization method used can produce live and sterile explants with an average yield of below 50%, but in treatment (S6) 25% NaOCl + 70% alcohol for 30 minutes is able to produce 30% live and sterile explants. Various sterilization methods used gave good results on the percentage of stagnant explants.

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INTODUCTION

Indonesia's banana production from 2016 to 2019 was 7.007.111 tons; 7.162.678 tons; 7.264. 678 tons; and 7.280.658 tons. Based on these data, banana production continues to increase from year to year (BPS, 2019). Curently the source of banana seeds planted by farmers us usually from one mother plant, so the number of seeds produced is quite limited and it is difficult to find seedse that are not attacked by bacteria or Fusarium wilt which is commonly found in banana trees. One of the efforts to abtain seeds quickly, wihtout bacterial attack, in large and uniform quantities can be done by in vitro culture technique. One of the obstacles encountered in conducting in vitro culture is culture sterilization to obtain sterile inoculum that is free from contamination.

According to Sandra (2002), sterilization is one of the things that can determine the success rate of in vitro culture, especially in the sterilization of explants from the field.

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Choosing the right sterilization method is important for optimization. This still needs to be considered because the explants remain sterile and do not damage the explants due to the concentration of the disinfectant used (Pancaningtyas, 2011). Felek *et al.* (2015), several disinfectants commonly used in explants sterilization are benlate, alcohol, chlorox, sodium hypoclorite, $HgCl_2$, detergent and tween. Perochena *et al.* (2015), NaOCl is higly recommended because of its ability to influence microbial activity and is also relatively safe for humans and plant tissues. Although it is relatively safe fpr transplantation, caution must also be exercised whe using the NaOCl compound, acourding to Setiani *et al.,* (2018), of the sodium hypoclorite compound is used at low concentrations, it will not be affected. Very effective in controlling pollution, but if given in hight concentrations will cause problems. These compounds can inhibit tissue growth in culture. Alcohol is often used for sterilization at a concentration of 70% because at that concentration it has the effect of breaking down the protein of microorganisms (Handoyowati, 2016).

The use of NaOCl and alcohol 70% as material for sterilizing banana explants at the right concentration and soaking time is expected to produce a high level of explant sterility. The aim of this study was to determine the effect of the sterilization method using NaOCl concentration followed by alcohol 70% soaking time on the acquisition of sterile explants of kepok banana plant.

METHODS

This research was conducted at the Biotechnology Laboratory, Faculty of Agriculture, UPN Veteran East Java from October 2022 to March 2023. The tools materials used were autoclaves, LAF (Laminar Air Flow), stoves, glassware (culture bottles, glass cup, measuring cup, erlenmeyer, glass funnel, petri dish, spatula), shaker, tweezers, scissors, scalpel. Test pipette, hot plate, magnetic stirrer, bunsen, sprayer bottle, pH meter, culture rack, refrigerator, aluminum foil, plastic wrap, analytical balance, heating pan, banana weevil, alcohol 70%, NaOCl, sterile aquadest, betadine, agrept, dithane, MS media, IAA, BAP, activeted charcoal, $HgCl_2$, tween 20, paper labels, rubber bands, and tissues.

This study used a completely randomized design (CRD) with one foactor, namely the sterilization treatment of kepok banana weevil explants. The treatment consisted of 6 sterilization treatments namely (S_1), (S_2), (S_3), (S_4), (S_5), (S_6). The data obtained were analyzed using variance (ANOVA). If the result of the analysis shows significantly different trarments, then proceed with the Least Significant Difference (LSD) test at the 5% level.

Methods

The implementation of the activities in this research includes the preparation stage and the implementation stage. The preparation stage includes initiation or taking explants from the part of the banana weevil to be cultured, sterilizing the work environment, sterilizing tools and materials, and making culture media. The implementation stage is the culture of kepok banana explants, culture maintenance and observation.

Sterilization outside the LAF

The banana weevil that has been separated from its mother is cleaned of dirt by washing it with detergent and rinsing under running water until clean. The tubers that have been cleaned are peeled off the sheath down to the smallest layer or the humps are cut until

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the remaining size is ± 2 cm x 2 cm from the base. Then the cobs were soaked in 2 g/l Dithane fungicide solution for 30 minutes and then rinsed with sterile water 3 times. Then the cobs were soaked again in a 2 g/l Agrept bactericidal solution for 30 minutes using a shaker, then rinsed again with sterile water 3 times.

Sterilization in LAF

Banana weevils that have been soaked with fungicide and bactericide are then sterilized in the LAF. The stages of sterilization in LAF were banana weevils soaked in a mixed solution of tween as much as 5 drops with NaOCl according to the treatment, namely 15%, 20% and 25% for 10 minutes and rinsed with sterile distilled water 3 times. Then the explants were immersed again in a mixture of 10% NaOCl and 2 ml/100 ml of HgCl2 for 10 minutes and rinsed again with sterile distilled water 3 times. Then the explants were immersed in 70% alcohol according to the treatment, namely soaking for 20 and 30 minutes, and rinsed again with sterile distilled water 3 times. The explants were then cut into small pieces until the remaining size was ± 1.5 cm x 1.5 cm. The final stage of the explants was soaking in betadine solution (5%) before being planted in culture media.

RESULT AND DISCUSSION

The results of the analysis of variance showed that there was no effect between the sterilization treatment of NaOCl concentrations with 70% alcohol immersion time and the percentage of live explants of kepok banana explants. The results of the average percentage of the number of live explants of kepok bananas are shown in Table 1.

The results in table 1 show that the percentage of living explants was not significantly different from the sterilization treatment. The average live sterile explants produced were below 50%, this was because the total explants that were treated gave an average yield of explants that experienced stagnation. On average, the highest number of live sterile expanses occurred in the sterilization treatment (S6) 25% NaOCl + 70% alcohol for 30 minutes, which was 30%.

The percentage of live explants is the number of explants that can survive under sterile conditions with the appearance of new leaves, roots, stems, or shoots and showing no symptoms of browning or contamination. Explants that can survive under sterile conditions only have one bud and several leaves that have opened and have roots growing around them as shown in Figure 1.

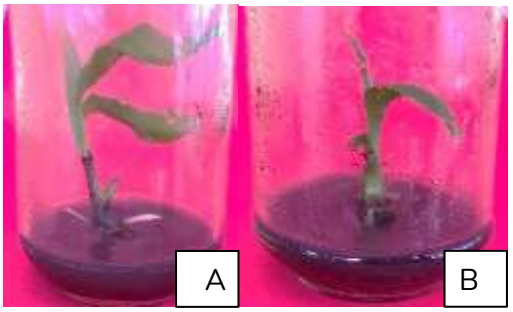

Figure 1. Appearance of live kepok banana explants under sterile conditions 56 days after planting: a) S5 treatment (25% NaOCl + 70% alcohol 20 minutes), b) S6 treatment (25% NaOCl + 70% alcohol 30 minutes).

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Browning Explanation Percentage

The results of variance showed that the sterilization treatment of NaOCl concentrates with 70% alcohol immersion time had no effect on the browning percentage of kepok banana explants. The results of the average percentage of browning explants in each treatment are presented in Table 1.

Table 1. showed that the results of the average percentage of browning explants were not significantly different from kepok banana explants. On average, banana explants experienced browning of 20% as a result of the sterilization treatment given. Browning of explants will show a change in color from green to brown to black (Figure 2). This can inhibit the growth of explants and cause the explants to die.

Figure 2. Appearance of Kepok Banana Explants with Browning

The use of NaOCl and alcohol is quite often used during the explant sterilization process, wich aims to kill microorganisms carried by the explants while they are still in the field. The treatment using sterilization with various concentrations of NaOCl followed by a long immersion in alcohol showed results that were not significantly different in almost all parameters. Some sterilants can be toxic or toxic to explants if the concentration and duration of immersion are not appropriate. In addition, the part and type of explants used can also affect the effectiveness of sterilization.

Browning of explants is thought to occur because explants that are sterilized after soaking with sterile material and rinsing with sterile distilled water are not optimal, as a result, phenolic compounds released from the explants due to injury cannot come out of the explants. Explants can also become browing due to the addition of $HgCl_2$, adding disinfectants can damage explants, according to Farooq *et al*., (2002), $HgCl_2$ is toxic if used in high concentrations and for a long time. This will cause the explants to turn brown or even die.

The lower the explant browning intensity, the greater the chance of explant regeneration. Conversely, if the intensity of explant browning is high, it can inhibit explant regeneration. According to Hutami (2008), explants that turn brown can inhibit growth and cause plant tissue death.

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Table 1. Average Percentage of Live Explants and Percentage of Browning Explants Treatment with NaOCl Concentration with 70% Alcohol Soaking Time.

Sterilization Treatment	Percentage of Living Explants (%)	Percentage of Browning Explants (%)
(S1) NaOCl 15% + 70% alcohol for 20 Minutes	17	20
(S2) NaOCl 15% + 70% alcohol for 30 Minutes	17	20
(S3) NaOCl 20% + 70% alcohol for 20 Minutes	7	20
(S4) NaOCl 20% + 70% alcohol for 30 Minutes	10	10
(S5) NaOCl 25% + 70% alcohol for 20 Minutes	20	13
(S6) NaOCl 25% + 70% alcohol for 30 Minutes	30	10

Note: NaOCl = Sodium Hypochlorite.

Percentage Of Contaminated Explants

The results of the analysis of variance showed that there was no effect of the sterilization treatment of NaOCl concentrations with 70% alcohol immersion time on the percentage of explants contaminated with both fungus and bacteria of kepok banana explants. The results of the average percentage of the number of live explants of kepok bananas are shown in Table 2.

The average percentage of explants that experienced fungal contamination in the sterilization treatment with concentrations of NaOCl with alcohol immersion time experienced the highest fungal contamination in the treatment (S2) 15% NaOCl + 70% alcohol for 30 minutes and (S5) 25% NaOCl + 70% alcohol for 20 minutes that is as much as 13%. While the average percentage of explants that experienced bacterial contamination in the sterilization treatment with NaOCl concentrations with the highest alcohol immersion time (S5) 20% NaOCl + 70% alcohol for 30 minutes was 20%.

Figure 3. Appearance of Kepok Banana Explants Contaminated by Fungi and Bacteria

Plant control carried out while the plants are still in the field in an appropriate way can reduce the initial contamination level and will reduce the concentration of the disinfectant used, thereby reducing the impact of pollution that is detrimental to the explant tissue. (Habibah et al., 2013).

The presence of contamination that occurs in these explants can cause the growth of the explants to be stunted and the explants to die. Contamination can occur due to internal and external factors. Contamination that occurred in various treatments began to appear on

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the seventh day after initiation until the end of the observation. Contamination occurs mostly by fungi that are white to black in color, and is also caused by the presence of bacteria.

The basic principle of sterilization is to remove contaminating microorganisms without killing the culture, so the use of disinfectants and soaking time must also be considered. The rinsing of the specimen after immersion in sterile material, both NaOCl and alcohol, is carried out so that the previously sterilized material is not carried away by the inoculum which is then immersed in the next sterile material. 70% alcohol can reduce the rate of fungal or bacterial infections. Alcohol can be used to fight infections caused by bacteria, viruses or fungi, but it is not effective at killing spores. Alcohol has the ability to inhibit spore formation and prevent spore germination, but this effect does not last long (Yoo, 2018).

Table 2. Average Percentage of Fungus Contaminated Explants and Percentage of Bacteria Contaminated Explants Treatment with NaOCl Concentration with 70% Alcohol Soaking Time.

Sterilization Treatment	Percentage of Fungus Contaminated Explants (%)	Percentage of Bacteria Contaminated Explants (%)
(S1) NaOCl 15% + 70% alcohol for 20 Minutes	3	0
(S2) NaOCl 15% + 70% alcohol for 30 Minutes	13	13
(S3) NaOCl 20% + 70% alcohol for 20 Minutes	0	10
(S4) NaOCl 20% + 70% alcohol for 30 Minutes	10	20
(S5) NaOCl 25% + 70% alcohol for 20 Minutes	13	17
(S6) NaOCl 25% + 70% alcohol for 30 Minutes	17	13

Note: NaOCl = Sodium Hypochlorite.

Descriptive Observations

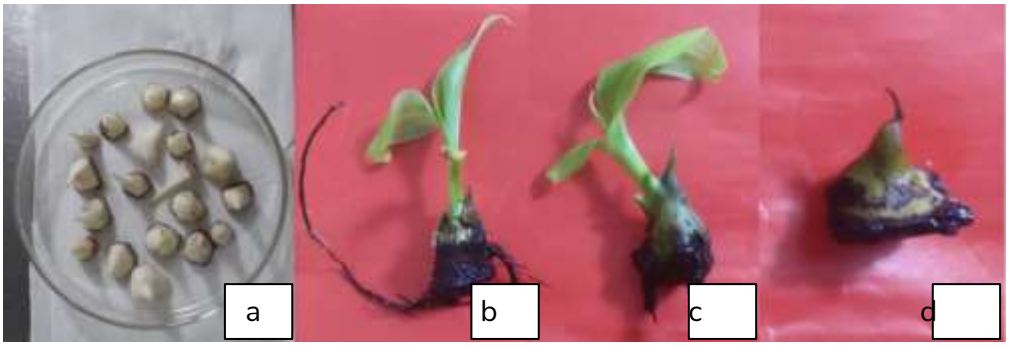

Figure 4. Appearance of Kepok Banana Explants: a) Kepok banana explants used, b) Kepok banana explants that are live and sterile and have long roots and leaves, c) Kepok banana explants that are alive and sterile and have small roots and leaves, d) Kepok banana explants that have stagnated and have no growing roots and leaves.

Figure 4 shows the initial condition of the kepok banana explants that had been cleaned from the sheath to a size of 1.5 cm x 1.5 cm which would be followed by the sterilization treatment that had been prepared. The sterilization treatment given to kepok banana explants gave different results in each treatment. It can be seen qualitatively that the

sterilization treatment was able to produce live and sterile explants of kepok bananas with the presence of roots and leaves, but the sterilization treatment could also give different results, namely the presence of stagnation experienced by the explants of kepok bananas. In addition, explants that are able to live under sterile conditions have not been able to produce new shoots.

CONCLUSION

Based on the results of the research that has been done, various sterilization methods used did not affect the results of sterile explants. The sterilization method used could produce sterile explants with an average yield of below 50%, but in treatment (S6) 25% NaOCl + 70% alcohol for 30 minutes was able to produce the most sterile and viable explants, namely 30%. Various sterilization methods used gave good results on the percentage of stagnant explants. Sterilization treatment (S4) 20% NaOCl + 70% alcohol for 30 minutes and (S6) 25% NaOCl + 70% alcohol for 30 minutes gave the lowest percentage of explants that experienced browning, namely 10%, and in treatment (S1) NaOCl 15 % + 70% alcohol for 20 minutes gave the lowest percentage of explants contaminated with bacteria, namely 0%. and sterilization treatment (S3) 20% NaOCl + 70% alcohol for 20 minutes gave the lowest percentage of fungal contamination, namely 0%.

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