Effect of liquid biological fertilizer on dwarf coconut embryo culture

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Abstract. Organic or biological fertilizer liquid is generally used to improve crop quality in plants grown in-vivo. Liquid organic and inorganic fertilizers have been used as in vitro culture media for orchids and patchouli; it is suspected that liquid biological fertilizers can also be used as in vitro growth medium. The research was conducted to determine the effect of liquid biological fertilizer (LBF) on the growth medium and development of dwarf coconut embryo culture. The study was conducted in laboratory tissue culture Palma Crops Research Institute, December 2020 - May 2021. The study used live combinations of culture mediums, and each used ten explants of dwarf coconut embryos. The results showed that on Eeuwens Y3 100% + 100% sugar medium, all experienced browning, three green shoots (live), and seven brown shoots (dead), the average shoot length was 2.9 cm. Medium LBF 100% without sugar does not undergo browning, and the embryo does not develop. LBF 50% + Eeuwens Y3 50% + 50 % sugar best medium: a minor browning, the highest number of green shoots (8 shoots), average shoot length was 4.1 cm.

Keywords: Browning, green shoot, medium combination, shoot length

1. Introduction
Coconut is a plantation commodity that is mostly owned by farmers who have high economic value because almost all parts of the plant can be used to meet the needs of human life. Until now, its production has not been optimal/still low, so tissue culture is needed; tissue culture is expected to produce quality seeds, uniform in large quantities and in a short time. In conventional agriculture, to increase crop production, it is necessary to apply standard crop cultivation such as irrigation, fertilization, pest and disease control. The types of fertilizers used to increase production are generally chemical fertilizers such as compound fertilizers (Nitrogen/N, Phosphate/P, Potassium/K), urea, Potassium Chloride/ KCl, Tripel Super Phosphate/TSP but organic fertilizers, both solid and liquid, and biological fertilizers can also be using. The use of organic fertilizers in early Dwarf coconut nurseries can accelerate leaf breakage [1]. The application of liquid organic fertilizer to mustard plants can increase the wet weight production of mustard plants [2], increasing the production of Ratun rice [3]. The application of liquid biological fertilizers in lowland rice increases the percentage of grain content [4], increasing the production of cucumber plants [5]. The application of liquid biological fertilizers increases the percentage filled grain paddy rice [4], increasing production in cucumber plants [5]. Giving the correct dose of biological fertilizer on palm seedlings showed good growth in the phase of pre-nursery [6].

Propagation of coconut generally uses conventionally grown seeds, but not all coconut seeds can be grown conventionally like kopyor coconut. Kopyor coconut seeds cannot be used as a source of seeds
planted like regular coconuts because the kopyor coconut fruit does not form endosperm as a food reserve [8]. Kopyor coconut can occur because the coconut does not have the enzyme α-D-galactosidase, which affects the formation of fruit flesh [9]. In vitro tissue culture technology offers an alternative to overcome kopyor coconut propagation that cannot be done conventionally by using embryo rescue culture techniques to produce kopyor coconut seedlings [10, 7].

In general, tissue culture media consist of macro, micro, FeEDTA, and vitamins which are chemical elements, where these chemical elements are also found in organic and liquid biological fertilizers with different concentrations. Organic fertilizers and liquid biological fertilizers are generally used to fertilize plants directly to increase the quality and quantity of crop production. Liquid organic and inorganic fertilizers have been used in tissue culture media in vitro, such as the results of [11] research showed that liquid organic fertilizers effect increase leaf length and leaf number of orchid plantlets grown in vitro. Hyponex inorganic fertilizer media increased the number of roots and root length in patchouli plants [12]. It is suspected that liquid biological fertilizers can also be used to grow plants in vitro through tissue culture techniques. The purpose of this study was to determine the effect of liquid biological fertilizers such as Bionet, Ecofarming, Bioboost, Biolang, Nasa, Humagold, Tricotech, Dinosaur on the growth medium and development of Dwarf coconut embryos.

2. Materials and methods

The research was conducted at the tissue culture laboratory, Palma Research Institute, in December 2020 – May 2021. Materials used include: Eeuwens Y3 medium, Liquid Biological Fertilizer (LBF), sugar, swallow agar, distilled water, alcohol, Clorox, embryos yellow Dwarf Bali coconut. Eeuwens Eeuwens Y3 medium contains macro nutrients: NH4Cl 535 mg/l, KCl 1492 mg/l, KNO3 2020 mg/l, NaH2PO4.2H2O 276 mg/l, CaCl2.2H2O 294 mg/l, MgSO4.7H2O 247 mg/l; micro nutrients: KI 8.30 mg/l, H3BO3 3.1 mg/l, MnSO4.7H2O 11.2 mg/l, ZnSO4.7H2O 7.2 mg/l, CuSO4.5H2O 0.25 mg/l, Na2MoO4.2H2O 0.24 mg/l, MnCl2.6H2O 0.024 mg/l; NaEDTA: NaEDTA.2H2O 37.2 mg/l, FeSO4.7H2O 13.9 mg/l; Vitamins: Thiamine HCl 0.5 mg/l, Nicotinic acid 0.05 mg/l, Pyridoxine HCl 0.05 mg/l, Biotin 0.05 mg/l, Folic acid 0.05 mg/l, Glycine 0.01 mg/l. The LBF used is Bionet which contains: Azospirillum sp., Azotobacter sp., Pseudomonas sp., Bacillus sp., P solubilizing bacteria, N-fixing bacteria, carbohydrates, fats, proteins, vitamins, and hormones (Auxin, Gibberellins, Kinetin, Zeatin).

The study used a single block method without replication, namely 5 combinations of culture medium and, each planted ten coconut embryos Yellow Dwarf Bali.

The five medium combinations are:

- M1: Eeuwens Y3 100% +100% sugar,
- M2: LBF 100% without sugar,
- M3: LBF 75% + Eeuwens Y3 25% + 25% sugar,
- M4: LBF 50% + Eeuwens Y3 50% + 50% sugar,
- M5: LBF 25% + Eeuwens Y3 75% + 75% sugar.

Explants were sterilized and implanted aseptically in laminar airflow. Explants (embryos) were sterilized by immersing them in 10% chlorox solution + 2 drops of liquid soap for 10-15 minutes while stirring. Furthermore, the explants were immersed in 75% alcohol for 1 minute, then the explants were washed with sterile distilled water 3 times. The explants were planted aseptically, then incubated in the culture room at a temperature of 25 – 27°C. Observations were carried out once a month for live months. The data were analyzed by description, and the observed variables were: contamination of the medium/embryo, embryos that sprouted, the condition of the medium did browning or not, shoot color, rooted plants.

3. Result and discussion

The application of liquid fertilizer to plants affects plant growth and development, such as using liquid organic fertilizer for in vitro growth medium on the Vanda limbata Blume x Vanda tricolor Lindl...
orchid plantlet, which affects the increase in leaf length and the number of leaves [11]. The use of liquid biological fertilizers (LBF) for culture medium, based on Table 1, shows that on M1, M4, and M5 medium, embryos have sprouted, while on M2 and M3 medium, none have sprouted. This is because, since in the growing medium (M1, M4, and M5) where the embryos germinate is due to the sufficient availability of nutrients (Eeuwens Y3 medium), and the medium that grows shoots is a mixed medium between Eeuwens Y3 + LBF with a minimum Eeuwens Y3 content of 50%. All media at the age of 1 month after planting has not experienced browning. In M1 media, there was 1 shoot that was brown/dead, thought to be caused by an immature embryo, the influence of physiological factors, so that the shoot did not develop and eventually died. The results of research [13] on the germination of Bido coconut seeds showed that about 15% of the seeds did not germinate; when germinated can not develop fully and eventually die.

Table 1. Number of explants, medium contamination, number of embryos that germinated, condition of the medium, the number of green or brown shoots at the age of 1 month.

| Medium | Number of explants | Medium contamination | Number of embryos that germinated | Condition of the medium | Number of shoots | Brown/dead |
|--------|--------------------|----------------------|----------------------------------|-------------------------|-----------------|------------|
| M1     | 10                 | 0                    | 6                                | 0                       | 10              | 5          |
| M2     | 10                 | 0                    | 6                                | 0                       | 20              | 0          |
| M3     | 10                 | 0                    | 2                                | 0                       | 10              | 2          |
| M4     | 10                 | 1                    | 4                                | 0                       | 10              | 1          |
| M5     | 10                 | 1                    | 2                                | 0                       | 10              | 0          |

Note: M1: Eeuwens Y3 +100% sugar, M2: LBF without sugar, M3: LBF 75% + EeuwensY3 25% + 25% Sugar, M4: LBF 50% + Eeuwens Y3 50% + 50% sugar, M5: LBF 25% + EeuwensY3 75% + 75% sugar.

Based on Table 2., all embryos had germinated in embryos grown on M1, M4, and M5 medium, except for contaminated embryos. On M3 medium: only three embryos sprouted from 6 mediums that were not contaminated. Embryos planted on M2 media have not yet germinated, possibly due to a lack of nutrients. M1, M4, and M5 growth medium have browned, especially for M1 medium have 100% experienced browning, while M2 and M3 growth medium have not or have not experienced browning. In the medium M2, and M3 have not experienced browning suspected of microorganisms contained in the medium, in which the microorganisms can decompose compounds issued explants cause color changes in the medium. In the M4 and M5 media, browning occurred. It was suspected that the microorganism was not sufficient to decompose all the compounds released by the explants. In tissue culture, to reduce browning in the culture medium, activated charcoal was added [14]. Activated charcoal can remove browning by absorbing and oxidizing phenolic compounds and inactivating peroxidase [15]. Browning that occurs in tissue culture medium and explants, is familiar in woody plant species. Browning generally occurs due to phenolic compounds released by explants due to injury, inhibiting tissue growth and death [16, 15].

Table 2. Number of explants, medium contamination, number of embryos that germinated, condition of the medium, the number of green or brown shoots at the age of 2 months.

| Medium | Number of explants | Medium contamination | Number of embryos that germinated | Condition of the medium | Number of shoots | Brown/dead |
|--------|--------------------|----------------------|----------------------------------|-------------------------|-----------------|------------|
| M1     | 10                 | 0                    | 10                               | 10                      | 0               | 9          |
| M2     | 10                 | 0                    | 0                                | 0                       | 10              | 1          |
| M3     | 10                 | 4                    | 3                                | 0                       | 6               | 3          |
| M4     | 10                 | 1                    | 9                                | 1                       | 8               | 9          |
| M5     | 10                 | 2                    | 8                                | 3                       | 5               | 7          |
Note: M1: Eeuwens Y3 +100% sugar, M2: LBF without sugar, M3: LBF 75% + EeuwensY3 25% + 25% Sugar, M4: LBF 50% + Eeuwens Y3 50% + 50% sugar, M5: LBF 25% + EeuwensY3 75% + 75% sugar.

Based on Table 3., three growth mediums, namely M1, M4, and M5, all embryos had germinated (excluding embryos in contaminated medium), and browning occurred in varying amounts of the medium in contrast, all embryos on M2 medium did not germinate, and no browning occurs in the growth medium. This is probably due to the absence of Eeuwens Y3 media elements in M2 media which contain nutrients. Shoots growing on M1 medium began to change the color of the shoots from green to brown as much as one shoot, and on M5 medium, there were one shoots of green turned brown shoot. In M3 medium, no browning occurred, and three green shoots sprouted embryos at the age of 3 months after planting from 6 embryos that were not contaminated. This condition is due to a lack of nutrients so that the fertility rate is low. Soil fertility affects the speed of broken leaves, where the fertile soil causes faster palm leaf broke out in the nursery phase [17].

Table 3. Number of explants, medium contamination, number of embryos that germinated, condition of the medium, the number of green or brown shoots at the age of 3 months.

| Medium | Number of explants | Medium contamination | Number of embryos that germinated | Condition of the medium | Number of shoots |
|--------|--------------------|----------------------|----------------------------------|-------------------------|-----------------|
|        |                    |                      |                                  | Browning | No | Green | Brown/dead |
| M1     | 10                 | 0                    | 10                               | 10        | 0  | 8     | 2          |
| M2     | 10                 | 0                    | 0                                | 0         | 10 | 0     | 0          |
| M3     | 10                 | 4                    | 6                                | 1         | 5  | 6     | 0          |
| M4     | 10                 | 1                    | 9                                | 2         | 7  | 9     | 0          |
| M5     | 10                 | 2                    | 8                                | 5         | 3  | 7     | 1          |

Note: M1: Eeuwens Y3 +100% sugar, M2: LBF without sugar, M3: LBF 75% + EeuwensY3 25% + 25% Sugar, M4: LBF 50% + Eeuwens Y3 50% + 50% sugar, M5: LBF 25% + EeuwensY3 75% + 75% sugar.

The growth of plants in the growing medium at the age of 4 months after planting (Table 4) showed an increase in browning on M5 medium as much as one medium from 5 medium to 6 medium. On M4 medium, where originally two shoots were brown at three months of age observation.

Table 4. Number of explants, medium contamination, number of embryos that germinated, condition of the medium, the number of green or brown shoots at the age of 4 months.

| Medium | Number of explants | Medium contamination | Number of embryos that germinated | Condition of the medium | Number of shoots |
|--------|--------------------|----------------------|----------------------------------|-------------------------|-----------------|
|        |                    |                      |                                  | Browning | No | Green | Brown/dead |
| M1     | 10                 | 0                    | 10                               | 10        | 0  | 6     | 4          |
| M2     | 10                 | 0                    | 0                                | 0         | 10 | 0     | 0          |
| M3     | 10                 | 4                    | 6                                | 1         | 5  | 6     | 0          |
| M4     | 10                 | 1                    | 9                                | 2         | 7  | 8     | 1          |
| M5     | 10                 | 2                    | 8                                | 6         | 2  | 7     | 1          |

Note: M1: Eeuwens Y3 +100% sugar, M2: LBF without sugar, M3: LBF 75% + EeuwensY3 25% + 25% Sugar, M4: LBF 50% + Eeuwens Y3 50% + 50% sugar, M5: LBF 25% + EeuwensY3 75% + 75% sugar.

The condition of the plant at the age of 5 after planted as shown in Table 5. shows that the green shoots on M1 medium are decreasing and turning brown, where at the age of 1 month after planted there are nine green shoots, and at the age of 5 months there are only three green shoots, while seven shoots are brown or dead. Shoots die due to the presence of phenolic compounds released by explants (embryos), browning causes stunted shoot growth and eventually dead tissue [15]. Green shoots that turn brown/die not at the same time; it is suspected that each shoot has a different level of resistance to phenolic compounds.
Table 5. Number of explants, medium contamination, number of embryos that germinated, condition of the medium, number of green or brown shoots, shoot length, and number of shoots rooted at the age of 5 months.

| Medium | Number of explants | Medium contamination | Number of embryos that germinated | Condition of the medium | Number of shoots | Shoot length (cm) | number of shoots rooted |
|--------|-------------------|----------------------|-----------------------------------|-------------------------|-----------------|------------------|-----------------------|
| M1     | 10                | 0                    | 10                                | Browning                | 0               | 3                | 2.69                  | 0                     |
| M2     | 10                | 0                    | 0                                 | 0                       | 10              | 0                | 0                     | 0                     |
| M3     | 10                | 4                    | 6                                 | 1                       | 5               | 4                | 2.28                  | 0                     |
| M4     | 10                | 1                    | 9                                 | 3                       | 6               | 8                | 4.10                  | 0                     |
| M5     | 10                | 2                    | 8                                 | 6                       | 2               | 7                | 4.81                  | 1                     |

Note: M1: Eeuwens Y3 +100% sugar, M2: LBF without sugar, M3: LBF 75% + EeuwensY3 25% + 25% Sugar, M4: LBF 50% + Eeuwens Y3 50% + 50% sugar, M5: LBF 25% + EeuwensY3 75% + 75% sugar.

In M3 and M5 medium, there were rooted shoots, one shoots each with a length of 2.28 cm and 4.81 cm. On M1 medium, the average shoot length was 2.69 cm. M4 medium had the greenest shoots (eight shoots) with an average length of 4.10 cm and had six mediums that did not brown. Based on the five tables above, explant subcultures can be done every month using the M1 medium because browning has not occurred in the medium. Explant subcultures can be doing every two or 3 months using the M4 medium even though there are three medium experiencing browning but there are eight shoots that are still green. It is suspected that liquid biological fertilizer can be used as a substitute for activated charcoal in tissue culture medium because it can overcome browning.

Figure 1. Conditions of 5 combinations of growing medium and growth of explants at the age of 5 months.

4. Conclusion
M4 medium (LBF 50% + Y3 50% + sugar 50%) was the best medium and it is suitable for culture of Dwarf coconut embryos and, subcultures can be done every 2 months.

Suggestion
This research needs to be continued to obtain a combination of growth mediums suitable for tissue culture and can prevent browning.

Acknowledgments
The authors thank the staff at the Tissue Culture Laboratory, Indonesian Palm Crops Research Institute, for supporting this research.
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