In vitro induction of oil palm (Elaeis guineensis Jacq.) shoot roots and their acclimatization in mycorrhiza-enriched media

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Abstract. Oil palm is a vegetable oil-producing plant (CPO) which provides the largest foreign exchange contribution compared to other crops and is widely used in food, medicine, cosmetic, and energy industries. Tissue culture technology is currently used to produce quality oil palm seeds. Oil palm shoots tend to grow and develop in clumps (groups) in vitro. Bipolar nature does not appear in all the shoots produced, so to produce plantlets it is necessary to do induction. This research aimed to obtain the right root induction media. A completely randomized design (CRD) with two factors was used with the first factor being the type of auxin (IAA, IBA, and NAA), and the second was the auxin concentration (0, 0.25, 0.5, and 0.75 ppm). In the eighth week after planting, the variables of root length, number of leaves, and shoot height were not significantly different except for the root number. The best root induction media for plantlet formation was the MS base medium with the addition of NAA type auxin at a concentration of 0.75 ppm. The plantlets formed a symbiosis with mycorrhiza which was applied at a dose of 4 g per polybag in the fourth month after planting.

1. Introduction
Palm oil is one of Indonesia's leading commodities and has contributed a lot to increase the country's foreign exchange. Palm oil is widely used in the food, cosmetics, pharmaceutical, and bioenergy industries. The current area of oil palm plantations based on the Decree of the Minister of Agriculture numbered 833/KPTS/SR.020/M/12/2019 is 16.381 million hectares [1]. Oil palm plantations in Indonesia are the largest in the world. Many oil palm plantations in Indonesia are currently entering the replanting period because the plants are more than 25 years old. To support and increase in palm oil production without expanding the oil palm area, a quality seed source is needed.

Tissue culture technology produces the clonal quality seeds of oil palm. The advantages of seed propagation using tissue culture technology include: the seeds can be produced in large quantities, the seed size is more uniform, the seeds can be produced anytime and the seeds have the same superior properties as the parent. The important thing in tissue culture is the correct selection of broodstock. Quality broodstock can produce quality seeds and is correlated with productivity [2].
The technology application applied in oil palm propagation is the somatic embryogenesis method. This method uses the initial material of young oil palm leaves through indirect embryogenesis stages. Oil palm somatic embryogenesis goes through several stages. At each stage, an appropriate medium and culture environment are required. The composition of culture media generally consists of macronutrients, micronutrients, vitamins, amino acids, and sucrose [20]. Besides, other additives are very important in culture media, namely growth regulators. These growth regulator compounds are needed as components in the media to support growth and differentiation [15].

The stages of oil palm tissue culture include callus induction, embryo induction, shoot regeneration, shoot maturation, and root induction [6]. Shoot maturation is done to prepare the shoots to enter the root induction stage. Oil palm shoots grow clam, where each shoot has a bond with other shoots to support its growth. This causes not all palm shoots to be bipolar, so to produce plantlets it is necessary to do root induction [6]. Root induction is a step to grow new roots with the help of auxin group growth regulators.

Auxins are an essential group of growth regulators and have an important effect in the process of cell division and root formation [7]. The auxin group consists of 2,4 D (2.4 Dichlorophenoxy Acetic Acid), Picloram (4-Amino-3.5.6-Trichloro Picolinic), IAA (Indole Acetic Acid), NAA (Naphtalene Acetic Acid), IBA (Indole Butyric Acid), 2.4.5.T (2.4.5 Trichloro Acetic Acid), NOA (Naphtoxy Acetic Acid), 4-CPA (4-Chlorophenoxy Acetic Acid), Dicamba (3.6 Dichloro Anisic Acid) [20].

Plantlets will be produced with good morphology if supported by the presence of strong roots as organs to help absorb nutrients and minerals in the culture media. The types of auxins that are commonly used in root induction are IAA, IBA, and NAA types. Each plant has a different response to the three types of auxins. So, to produce oil palm plantlets, a root induction medium with the right type and concentration of auxin is needed.

Mycorrhiza is a type of biological fertilizer developed to support environmentally friendly organic agriculture and sustainable agricultural management [13]. The addition of mycorrhizae to plant cultivation provides benefits to increase crop production and helps improve critical land [19]. The existence of mycorrhizae in the soil provides a symbiotic effect of mutualism, mutually beneficial between mycorrhizae and plant.

Mycorrhizae will form external hyphae and improve the root system of oil palm plants, thereby increasing the range of plants to absorb nutrients, especially phosphorus (P). The role of mycorrhizae in acid soils is very important because the availability of P is one of the limitations for plant growth in acid soils. In return, plants provide a place to live and the residual primary metabolites in the form of sugar residues as a source of nutrition for mycorrhizae [13].

This study aims to obtain the appropriate type and concentration of auxin to produce mycorrhizal oil palm seeds in vitro. Another aim was to know the right mycorrhizal dose given to the seeds at the time of acclimatization. It is hoped that the production of high-quality oil palm seeds will support the seed availability nation-wide.

2. Material and Methods
2.1. Research location
The research was conducted at the Plant Micropropagation Laboratory, Centre for Biotechnology-BPPT, Building 630 Puspiptek Area, South Tangerang, Banten Province.

2.2. Source materials
The explants used were oil palm shoots produced by propagation through somatic embryogenesis of tenera varieties. Shoot explants used in the treatment had the following specifications: vigorous shoots having at least three leaves, about 8-10 cm height, and normal morphology.

2.3. Treatment media
The culture medium used was the modified MS (Murashige and Skoog) basic medium [5] with a composition of macronutrients, micronutrients, vitamins, and sucrose. The composition of the
treatment medium was the basic MS medium added with auxin growth regulators (IAA, IBA, and NAA) with concentrations ranging from 0 (control), 0.25 ppm, 0.5 ppm, and 0.75 ppm. The media solution was adjusted to have pH 5.8, added with gelrite at about 2.2 g L\(^{-1}\), and then sterilized at 121 \(^{\circ}\)C for 15 minutes.

2.4. Planting explant
The selected shoots were prepared to be planted in the treatment medium. Shoots were cleaned and allowed to have 3 leaves only. Only shoots of about 8-10 cm in size were selected. A little scratch was made on the stump and planted in the prepared media. Incubation was carried out in the culture room at 27 \(^{\circ}\)C, with 1500 lux intensity for 16 hours per day.

2.5. Research design
This in vitro study used a completely randomized design (CRD) with two factors. The first factor was the type of auxin: IAA, IBA, and NAA. The second factor was the concentration of growth regulators: 0 (control), 0.25 ppm, 0.5 ppm, 0.75 ppm. The variables were observed every week until the eighth week after planting. The variables observed were the number of roots and root length. The data from the observations were analysed and further tested statistically using the SPSS program.

2.6. Mycorrhizal fertilizer application
The plantlets were adapted before acclimatization. Acclimatization materials such as 12 × 15 cm polybags, soil, and manure were prepared. Cover facilities were prepared as an adaptation space to strengthen the plantlets. The application of mycorrhizal fertilizers in this study used several doses: 0.2, 3, and 4 g per polybag. Observations were made every month until the fourth month after planting. The roots were isolated and observed under a microscope. Data were collected on the percentage of root parts that had been infected and the time it started to become infected. The percentage of roots infected with mycorrhizae was measured to determine the efficiency of using mycorrhizal fertilizers on oil palm plantlets.

3. Results and Discussion
Oil palm shoots produced by the somatic embryogenesis method have the characteristics of growing in groups (clump). Not all of the resulting shoots were bipolar, so they did not have direct roots. Shoots that were removed from the clump had been selected with a uniform height of about 8-10 cm. Generally, shoots that were forcibly removed from their clump would have stunted growth. Oil palm culture requires warm temperatures, as this speeds up the regeneration time.

3.1. Number of roots
Roots are plant organs and play an important role in the absorption of nutrients, which are then channelled for plant growth and development. Morphologically, there are primary and secondary roots. New roots appearance on palm shoots were observed every week. New roots began to appear in the third week after planting. The roots appeared on each shoot at different time, this was presumably because the difference of the physiology of each shoot. Physiologically mature shoots would easily induce new roots, in contrast to young shoots. The results of the data analysis on the number of roots growth showed that auxin treatment had a significant effect in the fifth week after planting, whereas the concentration treatment was not significant based on the weekly observation. The interaction between auxin type and dose treatments was significant at six weeks after planting (Table 1).
Table 1. ANOVA results of the observation data on the number of roots in the third to eighth week

| Treatment               | Week 3  | Week 4  | Week 5  | Week 6 | Week 7  | Week 8  |
|-------------------------|---------|---------|---------|--------|---------|---------|
| Auxin Type              | 0.6276  | 0.0563  | 0.0004* | <.0001*| <.0001* | <.0001* |
| Concentration           | 0.1986  | 0.7892  | 0.7685  | 0.1254 | 0.4924  | 0.4924  |
| Auxin Type * Concentration | 0.7564 | 0.7529  | 0.1073  | 0.0020*| 0.0315* | 0.0315* |

The application of auxin types in root induction media aims to ascertain which type of auxin is suitable for oil palm shoots. Several studies indicated that root induction in each plant had a tendency towards certain types of auxins. For example, in pepper culture, the highest number of roots appeared in the medium with the addition of 0.1 ppm IAA [16], whereas the root induction of Ingu culture (Ruta graveolens L) was optimal at the addition of 0.001 ppm NAA [17]. In other studies, optimum root induction of sago culture occurred on media added with 35 ppm IBA [18], while that of satoimo culture roots 3 µM IAA [24].

Observing the number of roots from the third to the eighth week showed that the use of auxin types IBA and IAA resulted in the number of roots that were not significantly different from the control (Figures 1 and 2). In contrast to the NAA, it was seen that in the fourth week the number of roots increased. The number of roots in the NAA treatment continued to increase until the seventh week with an average number of about 3.26. The number of oil palm shoots began to stabilize in the seventh and eighth weeks (Figure 1). These results indicated that the NAA responded to the maximum number of roots in the seventh week after planting. The use of NAA in oil palm clonal shoot induction was more effective. In contrast to the shoots originating from oil palm embryos, it was found that the addition of 15 µM IBA produced a root percentage of around 87% with the highest number of roots at around 1.33 [12].

![Figure 1](image_url)
Figure 2. The number of roots in the treatment media added with: a. nothing (control), b. IAA, c. NAA and d. IBA.

The addition of auxin in all treatments gave a response of an increase in the number of roots except for control (Figure 3). Young roots began to appear in the third week after planting on the media with the addition of 0.5 and 0.75 ppm auxin. The number of roots in the media supplemented with 0.5 and 0.75 ppm auxins continued to increase from the fourth week to the sixth week (Figure 4). The addition of auxins around 0.25 ppm caused the increase in the number of roots from the fourth to the seventh week. These results indicated that an increase in the concentration of auxin triggered the emergence of new roots. The number of roots induced in vitro per week was not the same. Several things that influence root emergence other than root induction media are shoot age and shoot size. In general, young shoots with a diameter of less than 0.4 cm are slower to produce roots.

Figure 3. Graph of the increase of the number of oil palm shoot roots at several auxin concentrations in the third to eighth week after planting.
Figure 4. The number of roots in the treatment media with the addition of NAA at the concentration of: a. 0 ppm (control), b. 0.25 ppm, c.0.5 ppm, and d.0.75 ppm.

The interaction of auxin types and concentrations was significantly different from the sixth to eighth week after planting. The addition of NAA at a concentration of 0.25 ppm, 0.5 ppm resulted in the number of roots that were not significantly different, but significantly different from the addition of 0.75 ppm NAA (Table 2). All treatments with the addition of NAA at all concentrations were significantly different from those with the addition of IAA. While the addition of IAA treatment was not significantly different from IBA treatment at all concentrations (Table 2). These results indicated that the addition of NAA was more effective in inducing the number of oil palm shoot roots. The most optimal treatment in inducing the highest number of roots was media added with 0.75 ppm NAA (Figure 5). The treatment that did not produce roots was the media added with 0.75 ppm IBA. These results showed that the increase in IBA concentration inhibited the formation of oil palm shoot roots (Figure 6).

Table 2. The number of roots effected by the type and concentration of auxin in the third to eighth week after planting.

| Treatment (ppm) | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 |
|-----------------|--------|--------|--------|--------|--------|--------|
| P1 (kontrol)    | 0 ± 0a | 0 ± 0a | 0 ± 0b | 0 ± 0c | 0 ± 0d | 0 ± 0d |
| P2 (NAA 0.25)   | 0.4 ± 0.54a | 1 ± 1.22a | 1.2 ± 1.30b | 1.4 ± 1.51bc | 2.4 ± 1.94bc | 2.4 ± 1.94bc |
| P3 (NAA 0.5)    | 0.2 ± 0.44a | 1 ± 1.22a | 1.4 ± 1.51b | 2.6 ± 2.07b | 2.6 ± 2.07b | 2.6 ± 2.07b |
| P4 (NAA 0.75)   | 0 ± 0a | 1.2 ± 1.30a | 2.8 ± 1.78a | 4.8 ± 1.30a | 4.8 ± 1.30a | 4.8 ± 1.30a |
| P5 (IAA 0.25)   | 0.2 ± 0.44a | 0.2 ± 0.44a | 0.2 ± 0.44a | 0.2 ± 0.44a | 0.6 ± 0.89d | 0.6 ± 0.89d |
| P6 (IAA 0.5)    | 0.4 ± 0.89a | 0.4 ± 0.89a | 0.4 ± 0.89b | 0.4 ± 0.89c | 0.4 ± 0.89d | 0.4 ± 0.89d |
| P7 (IAA 0.75)   | 0.2 ± 0.44a | 0.2 ± 0.44a | 0.2 ± 0.44a | 0.2 ± 0.44a | 0.4 ± 0.54d | 0.4 ± 0.54d |
| P8 (IBA 0.25)   | 0.6 ± 0.89a | 0.8 ± 1.30a | 0.8 ± 1.30b | 0.8 ± 1.30c | 0.8 ± 1.31cd | 0.8 ± 1.31cd |
| P9 (IBA 0.5)    | 0.6 ± 0.89a | 0.6 ± 0.89a | 0.6 ± 0.89b | 0.8 ± 1.30c | 0.8 ± 1.31cd | 0.8 ± 1.31cd |
| P10 (IBA 0.75)  | 0 ± 0a | 0 ± 0a | 0 ± 0b | 0 ± 0c | 0 ± 0d | 0 ± 0d |

Note: The number followed by the same letter shows no significant difference based on the Duncan test α = 0.05.
Figure 5. Oil palm seeds treated with 0.75 ppm NAA.

Figure 6. Oil palm seeds treated with 0.75 ppm IBA.

NAA treatment in other plants also showed an optimal root induction response, such as the Fuchsia hybrid plant which produced the highest percentage of root emergence value of 83.9% at an NAA
concentration of 0.75 ppm [22]. Many types of NAA auxins can have a significant effect on the root system, and produce straighter and thicker roots compared to the use of other types of auxin hormones. The addition of NAA plays a role in inducing the growth of the root system, delaying the aging process, and triggering an increase in nutrients through the assimilation process. According to [23], NAA affects the shape of the roots that are formed, the higher the concentration added, the thicker and bigger the roots form. Auxin is widely used in vegetative propagation with various concentrations.

The sixth, seventh and eighth weeks after planting did not show any increase in the number of roots. It was suggested that the nutrient content in the media had been depleted, so it was unable to support an increase in the number of roots. Concerning the comparison of the effectiveness of IAA and NAA in vitro culture of date palm, it was found that the use of NAA was more effective than IAA (date palm shoots on media added with 4.0 ppm NAA produced an average percentage of rooted shoots of about 11%, while the addition of IAA 0.4 ppm only 9%). Using MS medium plus auxin NAA on root induction of Ruta angustifolia gave a better response than the addition of auxin IBA [17]. It is known that the ability of plant tissues to absorb NAA is better than IBA and IAA.

Different types of plants have different responses to the types and concentrations of growth regulators. The interaction and balance between the growth regulators applied to the media with endogenous hormones in the tissue will determine the direction of culture development. Among the three types of auxins tested, it was found that NAA and IBA were more effective in root induction than IAA. This is because NAA and IBA are more stable than IAA and their mobility in plants is low [21]. NAA and IBA are two types of growth regulators that are often used in plant propagation using tissue culture techniques [4]. In its application, the use of NAA has been proven to be able to induce oil palm roots [11], where the influence of NAA is stronger than IBA [14], and the use of single NAA is considered more efficient than the combination of NAA and IBA in inducing the shoot roots of oil palm palm [25].

3.2. Root length

Another parameter observed besides the number of roots was the root length. The emergence of the root with certain number occurred as the root elongated. Root length increased with the growing time (Table 3). Roots began to appear in the third week after planting. Root length at week one to six was not significantly different in all treatments. But, the seventh and eighth-week showed that various increase in root lengths was seen in each treatment. Media with the addition of IBA produced a longer root response than those added with the other two types of auxins. The treatment of auxin IBA at the concentrations of 0.5 and 0.75 ppm produced the longest roots compared to the treatment of auxin NAA and IAA. This is because oil palm is a slow-growing plant.

Based on the analysis results, the addition of 0.5 ppm NAA and 0.5 ppm IBA showed the longest average root length compared to the other treatments (Table 2). This result was similar to the previous result [9] in which the addition of IBA gave the longest average root length in Talinum paniculatum in the fourth week after planting. The media with the addition of IBA had a longer average root length compared to those of NAA and IAA. These results were consistent with the study on Ruta graveolens L. [17] in which the use of the IBA hormone showed the longest average root length compared to NAA. The addition of a higher IBA concentration of about 1.5 ppm turned out to give a response of about 50% to the increase in the number of roots and root length in oil palm plantlets. The response of adding IBA at a concentration of 0.75 ppm or less had no significant effect on increasing the number of roots [8]. The use of auxins at low concentrations can stimulate plant root extension, whereas at high concentrations it would be phytotoxic since it could inhibit rooting [4].
Table 3. The length increase of the root effected by the type and the concentration of auxin in the third to eighth week after planting.

| Treatment (ppm) | Root Length (cm) |  |  |  |  |  |
|-----------------|------------------|---|---|---|---|---|
|                 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 |
| P1 (control)    | 0      | 0      | 0      | 0      | 0      | 0      |
| P2 (NAA 0.25)   | 0.38   | 0.54   | 0.84   | 0.88   | 1.2    | 1.44   |
| P3 (NAA 0.5)    | 0.14   | 0.38   | 0.98   | 1.86   | 2.28   | 2.82   |
| P4 (NAA 0.75)   | 0      | 0.4    | 0.92   | 1.54   | 1.94   | 2.14   |
| P5 (IAA 0.25)   | 0.38   | 0.5    | 0.86   | 1.24   | 1.6    | 1.8    |
| P6 (IAA 0.5)    | 0.1    | 0.46   | 0.7    | 0.78   | 0.78   | 0.78   |
| P7 (IAA 0.75)   | 0.04   | 0.06   | 0.12   | 0.16   | 0.24   | 0.28   |
| P8 (IBA 0.25)   | 0.38   | 1.04   | 1.62   | 2.16   | 2.36   | 2.56   |
| P9 (IBA 0.5)    | 0.14   | 0.54   | 1.36   | 2.12   | 2.52   | 2.64   |
| P10 (IBA 0.75)  | 0      | 0      | 0      | 0      | 0      | 0      |

Note: The number followed by the same letter shows no significant difference based on the Duncan test results \( \alpha = 0.05 \)

3.3. Mycorrhizal applications

The palm plantlets produced using the root induction media were subsequently acclimatized. The acclimatization stage is a process that must be passed to get oil palm seeds ready for planting in the field. Acclimatization is an adaptation process from homogeneous plantlet conditions to more heterogeneous conditions. Apart from producing genetically high-quality oil palm seeds, it is also necessary to produce seeds that have good growth in the field. Seeds that can withstand tense environmental conditions will be needed under current agricultural conditions and weather conditions. The observations found that the mycorrhizal spores that were attached and the hyphae formed symbiosis with new roots in the fourth month after planting (Table 4). Observations were made every month by isolating the young roots of the oil palm to be observed under a 10× magnifying microscope (Figure 7).

Table 4. Data from time and percentage (%) of mycorrhizal infections in young roots of oil palm seeds

| Treatment | Root Infection (%) |
|-----------|--------------------|
|           | Month 1 | Month 2 | Month 3 | Month 4 |
| Control   | X       | X       | X       | X       |
| 2 g       | X       | X       | X       | V       |
| 3 g       | X       | X       | X       | V       |
| 4 g       | X       | X       | X       | V       |

Note: based on the X sign shows there is no response and the V indicates there is already a response (hypha has been attached and multiplied by the root)

Under the microscope, the mycorrhizal hyphae attached to the roots of the oil palm and grew alongside the roots (Figure 8). The mycorrhizal hyphae colonized the roots and was an indication of the mutualistic symbiosis between the two fungi and the oil palm. Observation under a microscope was done by dividing the observation area into the left, middle, and right zones. The observation resulted in the percentage of hyphal infection to the roots in the fourth month after planting. The best
dose, indicated by the highest percentage of hyphal infection to the roots, was 4 g per polybag. The left zone produced an average of 9.7%, the middle area 20.1%, and the right side 15.8%. Doses below 1, 2, and 3 g led to the percentage of hyphal infection being lower than the treatment of 4 g (Table 5).

Table 5. Data on the percentage of hyphal infecting the roots of palm plantlets

| Treatment | Percentage Infection (%) |
|-----------|--------------------------|
|           | left | Middle | right |
| control   | 0.3  | 1.3    | 0.7   |
| 2 g       | 5.7  | 6.5    | 4.3   |
| 3 g       | 5    | 10.2   | 9.7   |
| 4 g       | 9.7  | 20.1   | 15.8  |

Table 5 shows the percentage of mycorrhizal infections in the roots of oil palm plantlets after acclimatization. The percentage of root infection determines the optimum dosage for mycorrhizal applications on oil palm seeds. Several cases reported the use of mycorrhizal fertilizers without the right technique. The incorrect mycorrhizal application can cause no mycorrhizal infection in plants, and affect growth in the field.

Oil palm is a type of plant that has a long growing period. The application of mycorrhizae to young plants showed significant morphology. Following acclimatization, the mycorrhizae-treated seeds showed very different growth compared to those without mycorrhizal treatment. The use of mycorrhizae in oil palm plantations can reduce the use of inorganic fertilizers, hence expectedly more cost effective. The results of this study (Table 5) indicated that the percentage of mycorrhizal infections in the roots increased with the increasing dose of mycorrhizae. The lowest percentage of mycorrhizal infections in the roots of control plants was due to the unsterilized acclimatization media, hence the presence of indigenous mycorrhizae. Mycorrhizae are a type of fungi that lives freely in nature and can associate themselves with plant roots.

Figure 4 shows that acclimatization caused the mycorrhizal infection to occur. The symbiosis of mutualism between mycorrhizae and plant roots causes plants to obtain many benefits such as making
it easier for plants to obtain nutrients in the soil. The presence of mycorrhizal infection was indicated by the presence of hyphae on the roots of the plant as observed under a microscope. The long, blue shape was a type of endomycorrhizal mycorrhizae that penetrated the root cortical tissue. Hyphae outside the root network were called external hyphae. External hyphae were observable under a stereomicroscope at 6× magnification. External hyphae expand the absorption area of the host plants’ roots, hence increasing uptake of essential elements like nitrogen (N) and potassium (K) [10]. External hyphae also support reproductive functions and transport carbon and other nutrients into the spores.

4. Conclusion
The auxin type NAA more effective in inducing oil palm shoot roots in vitro. The NAA concentration of 0.75 ppm resulted in a response to a greater number of roots and was ready for acclimatization. The application of 4 g of mycorrhizae in the acclimatization stage of oil palm plantlets produced more hyphae in the 4th month after planting.

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