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EX VITRO ROOTING OF OIL PALM (*Elaeis guineensis* Jacq.) PLANTLETS DERIVED FROM TISSUE CULTURE

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ABSTRACT

Plantlets of oil palm (*Elaeis guineensis* Jacq.) derived from somatic embryos sometimes do not form well developed-roots. Root formation of unrooted-plantlets can be induced with auxin during *ex vitro* acclimatization period to simplify the procedure and to reduce seedling production cost. Experiments were conducted using a completely randomized design to determine the effect of different types of auxin, i.e. indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthalene-acetic acid (NAA) at different concentrations, i.e. 0, 2, 4, 8, and 16 mM on root development of oil palm plantlets. The plantlets used were derived from somatic embryos of MK 649 oil palm clone. The basal end of the shoots was dipped in auxin solution for 10 minutes before the shoot was cultured in a small plastic pot containing a mixed growing medium. The cultures were then placed inside a closed transparent plastic tunnel (240 cm x 100 cm x 95 cm) for 12 weeks. The results showed that without auxin treatment only 15% of the shoots formed roots. Dipping in auxin solution increased significantly root frequency to more than 50%. The best root formation was found on the shoots treated with 2 mM NAA by which rooting frequency was 80%. Auxin treatments also increased root quality as indicated by more number of primary and secondary roots. IAA, IBA, and NAA treatments at all concentrations tested increased significantly shoot height on average by 42% and shoot diameter by 30% compared to control treatment, but did not influence root length. The best treatment for inducing roots of oil palm plantlets *ex vitro* was by dipping the basal end of the plantlets in 2 mM NAA solution. The result showed that rooting of oil palm plantlets could be successfully conducted *ex vitro* that would eliminate sterile rooting stage thus simplify the protocol and reduce seedling production time and cost.

[***Keywords***: *Elaeis guineensis*, oil palm, tissue culture, *ex vitro* rooting, auxin, acclimatization]

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is one of the most important estate crops in Indonesia. In the last two decades, the total area of oil palm in Indonesia has increased sharply from less than 1 million ha in 1989 to more than 7 million ha in 2008 (Directorate General of Estate Crops 2008).

Oil palm is mostly propagated by hybrid seeds (Dura x Pisifera) that are highly heterozygous. Therefore, variations with respect to yield, oil quality, and disease resistance are to be expected. Vegetative propagation of individuals with high yield, disease resistance, or other superior characters can only be done by tissue culture. Cloning of the available elite individuals was found to increase the yield by 23-39% (Subronto *et al*. 1995).

The most common technique used at present for clonal propagation of oil palm is through somatic embryogenesis on a solid medium. Calli initiated from unfolded leaves are induced to form somatic embryos. Maturation and germination of somatic embryos are then conducted to produce plantlets. Some of the plantlets are not able to form roots although they have future radicles. If the plantlets have already good aerial parts (shoots), root induction can be done *in vitro* in the laboratory with rooting frequency of 66-92% (Rival *et al*. 1997; Konan *et al*. 2007; Ibrahim *et al*. 2009; Nizam and Te-chato 2009; Riyadi and Sumaryono 2010) or *ex vitro* during acclimatization period in the greenhouse or nursery.

Ex vitro rooting of plantlets has advantages over *in vitro* rooting. *Ex vitro* rooting is conducted to simplify tissue culture protocols and to reduce the production cost (Meier-Dinkel *et al.* 1993; Borkowska 2001; Hazarika 2003; Martin 2003; Shekafandeh 2007). Furthermore, *ex vitro* rooting and acclimatization phases could be done at the same time, hence it is more efficient. Roots of plantlets produced *in vitro* are usually very weak and without root hairs (Hazarika 2006). Therefore, during early acclimatization period, the roots do not function normally to support the plants as anchors or physiological role to uptake water and nutrients. *Ex vitro* rooting of micropropagated shoots of *Rhododendron ponticum* resulted in higher survival rate of the plantlets during acclimatization period than that of *in vitro* rooting (Almeida *et al*. 2005). In addition, plantlets without roots are

much easier and faster to be planted on a soil mixture medium than those with roots at the acclimatization phase.

One of the major problems during *ex vitro* transfer is the high rate of water loss from shoots of plantlets taken out from the cultivation vessels (Pospisilova *et al*. 2007). Acclimatization of plantlets without roots would decrease the ability of the plantlets to uptake water. This disadvantage of *ex vitro* rooting can be reduced by placing acclimatized-plantlets in a closed confinement where the relative humidity is almost 100% to reduce water loss through transpiration.

Ex vitro rooting has been applied in micropropagation of various woody perennial plants (de Klerk 2002), but there is no report yet on e*x vitro* rooting of oil palm plantlets. Most reports of *ex vitro* rooting of woody species have involved treatment with exogenous auxin such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthalene-acetic acid (NAA) (Meier-Dinkel *et al*. 1993; Rival *et al*. 1997; Kim *et al*. 1998; Martin 2003; Shekafandeh 2007). Auxin is usually applied as a solution by dipping the basal end of the shoots or by drenching on the soil surface, and as powder or paste applied on the surface of plantlet base. Auxin is applied singly or in a combination at different concentrations to improve rooting frequency of plantlets in the acclimatization period.

The objective of this study was to determine the effect of different types and concentrations of auxin on the development of roots and shoots of oil palm plantlets during acclimatization period.

MATERIALS AND METHODS

Plant Materials

Study was conducted at the Plant Cell Culture and Micropropagation Laboratory of the Indonesian Biotechnology Research Institute for Estate Crops, Bogor. Plant materials used were unrooted-plantlets (shoots) of oil palm derived from somatic embryos of oil palm genotype MK 649 of the Indonesian Oil Palm Research Institute. The somatic embryos were induced from calli initiated from young unfolded leaves of mature oil palm tree (Sumaryono *et al*. 2008). The shoots were grown on a de Fossard (DF) solid medium containing 2.22 µM BAP and 1.44 µM GA_3 for 3-5 months. Average height of plantlets used as plant materials was approximately 9.5 cm with 2-3 leaves. Homogeneous plantlets in term of size and vigor were selected and used for the study.

Ex Vitro Root Induction and Culture Conditions

Selected plantlets were taken carefully from the culture tubes and the remaining agar was washed thoroughly using a small brush under running tap water. After air-dried for several minutes, the base of the plantlets was dipped in auxin solution for 10 minutes. The treatments tested were types of auxin (IAA, IBA, and NAA) at different concentrations (0, 2, 4, 8, and 16 mM). Thirty uniform plantlets divided over three replicates of 10 plantlets each were used for each experimental combination tested.

The plantlets were planted in a non-sterilized mixture of soil and organic matter (1:3 v/v) in small transparent plastic pots (10 cm height and 6.5 cm diameter). Fungicide solution was applied on the soil surface to prevent from fungal attack. The pots were then placed inside a closed plastic tunnel under 50% shading net for 12 weeks. The tunnel was made of transparent plastic in a dome shape with the size of 240 cm length, 100 cm wide, and 95 cm height. During hot afternoons, spray irrigation using a water sprinkler above the tunnel was applied to reduce air temperature and to maintain air humidity. Average temperature inside the tunnel was 26°C in the morning and reached the highest at 31°C at 1 PM. Relative humidity was 100% in the morning, decreased slowly during the day down to 73% at 1 PM, and then increased again. Light intensity inside the plastic tunnel during the day was 20-40 µmol m^{-2} $second^{-1}$.

After 12 weeks, the plantlets were harvested by removing the plantlets from the soil. Observations were conducted on plantlet height, leaf number, shoot diameter, rooting frequency, root length, and root class. Plantlet height was determined from the base of the shoot to the tip of the longest leaf. Shoot diameter was observed by a caliper at the base of the shoot. Root length was examined from the base of the root to the tip of the longest root. Root performance of oil palm plantlets was classified into five groups according to Riyadi and Sumaryono (2010) as follows:

Class 1 = plantlets without roots
Class 2 = plantlets with one primary root and no secondary roots
Class 3 = plantlets with one primary root and many secondary roots
Class 4 = plantlets with two or more primary roots and no secondary roots
Class 5 = plantlets with two or more primary roots and many secondary roots.

Statistical Analysis

The experiment was arranged using a completely randomized design. Data were subjected to analysis of variance (F test) using SPSS program. The differences among treatment means were determined by Duncan's multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

Rooting Frequency

Root formation of plantlets of micropropagated crops can be conducted during *in vitro* culture (*in vitro* rooting) or during acclimatization period (*ex vitro* rooting). Results of this experiment showed that the induction of roots of oil palm unrooted plantlets could be conducted *ex vitro* as long as the plantlets had been treated with auxin. In control without auxin treatment, only 15% of the plantlets formed roots, where as in auxin treatments regardless of its concentration, the plantlets would form roots in more than 50% of the plantlets tested (Fig. 1). The highest root frequencies at 70-80% were found in NAA at 2, 4, and 8 mM, although these were not statistically different from other auxin treatments. NAA was also used for *in vitro* root induction of oil palm plantlets (Rival *et al*. 1997; Konan *et al*. 2007; Nizam and Te-chato 2009). The NAA concentration used by Rival *et al*. (1997) and Konan *et al*. (2007) was 1 mg l^{-1} (equivalent to 5.37 µM), whereas Nizam and Te-chato (2009) used a higher concentration of NAA at 6 mg l^{-1} (equivalent to 32.2 µM). All concentrations of NAA used for *in vitro* rooting were much lower than the best concentrations for rooting found in this experiment (2-8 mM) because in *in vitro* rooting NAA was mixed into the medium for the whole culture period, while in *ex vitro* rooting the plantlets were only dipped in NAA solution for 10 minutes.

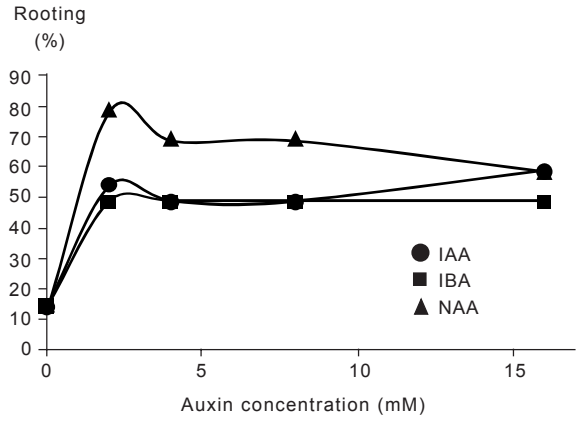

Fig. 1. Effect of IAA, IBA, and NAA at 0, 2, 4, 8 and 16 mM on root frequency of oil palm plantlets during 12 weeks of acclimatization at a closed plastic tunnel; average rooting frequency of the control treatment was 15%.

NAA was also found to be the best for *ex vitro* rooting in other woody species such as *Rotula aquatic* at 2.69 µM producing on average 5.6 roots per shoot (Martin 2003) and *R. ponticum* shoots dipped in 1 g l^{-1} NAA for 2 minutes resulted 96% rooting frequency and 6.2 roots per plantlet (Almeida *et al*. 2005). Dipping in a solution of 1 g l^{-1} IBA increased *ex vitro* rooting percentage of *R. ponticum* to 94% (Almeida *et al*. 2005) and combination of IBA and IAA increased *ex vitro* rooting percentage to 91.7% in myrtle (Shekafandeh 2007). Other rooting substance such as humic acid was found to decrease the number of plant death and to promote rooting rate and vigor in plantlets of blueberry (Zhao *et al*. 2008).

Root frequency highly correlated with root class ($r = 0.86$), shoot height ($r = 0.75$), and shoot diameter ($r = 0.74$), but did correlate with other parameters. This indicates that higher root frequency would have higher root class or quality and also better growth of the aerial part (shoot) of the plantlets such as shoot height and shoot diameter (Table 1). Therefore, the use of NAA at 2-8 mM would increase root induction and root quality in oil palm plantlets, consequently increase the growth of shoots. Konan *et al*. (2007) also stated that the development of roots facilitated the growth of the aerial part of oil palm plantlets.

Table 1. Effect of IAA, IBA and NAA at 0, 2, 4, 8 and 16 mM on shoot height, leaf number and shoot diameter of oil palm plantlets after 12 weeks of acclimatization.

Auxin	Concentration (mM)	Shoot height (cm)	Leaf number	Shoot diameter (mm)
-	0	11.1b	3.8a	2.34c
IAA	2	15.7a	4.2a	3.00ab
	4	16.4a	4.5a	3.09ab
	8	15.6a	4.8a	3.14ab
	16	16.5a	4.5a	3.16ab
IBA	2	15.9a	4.3a	3.12ab
	4	15.7a	4.1a	2.84b
	8	15.2a	4.8a	3.01ab
	16	16.2a	4.2a	3.02ab
NAA	2	16.3a	4.8a	3.35a
	4	16.8a	4.5a	2.97ab
	8	15.4a	4.7a	3.05ab
	16	14.5a	3.8a	2.72bc

Means in the same column followed by the same letters are not significantly different based on Duncan's multiple range test at $P < 0.05$.

Root Growth

Formation of root system of oil palm plantlets after 12 weeks of acclimatization was observed based on root class and root length. Root class is defined according to the number of main roots and the formation of secondary roots, thus it indicates root quality. Statistical analysis revealed that auxins used affected significantly root class of oil palm plantlets at 12 weeks of acclimatization. All types and concentrations of auxin increased root class (Fig. 2). The best root quality of oil palm plantlets was achieved on treatment of NAA at 2, 4, and 8 mM where root class on average was 3. It means that most of the roots had one or more primary roots with many secondary roots. Root class of oil palm plantlets with NAA treatments was significantly higher than those of other auxin treatments. In the control, on the other hand, root class average was only 1.4. This indicates that some plantlets were without roots (root class 1) and other plantlets were with one primary root without secondary roots (root class 2).

Root length shows how deep the roots penetrate into the soil. In this acclimatization experiment, the plantlets were planted in small plastic pots (10 cm height and 6.5 cm diameter), therefore at 12 weeks of acclimatization there were many roots already more than 10 cm in length and penetrated out from the pots through the holes at the bottom of the pots or grew in circle on the bottom of the pots. Statistical analysis revealed that there was no significant effect of all auxin treatments on root length of oil palm plantlets. Average root length of the untreated plantlets was 9 cm and of NAA at 8 and 16 mM and IBA at 16 mM was shorter than that of untreated plantlets (Fig. 3). Root length is considered not a good criterion for high quality of oil palm seedlings. A more important criterion is the formation of primary and secondary roots as indicated by root class. In addition, root length did not correlated with aerial growth such as shoot height and shoot diameter. Therefore, root quality as indicated by root class is more important criterion for growth and quality of oil palm seedlings at a juvenile phase.

Roots were formed after 3-4 weeks of acclimatization. At 6 weeks, several roots had been observed close to the transparent plastic pots; they were young primary roots with white and smooth in appearance (Fig. 4a). One to three primary roots emerged from the base of plantlets were downwardly oriented and ended in a white apex. Many secondary roots grew laterally from a primary roots especially close to

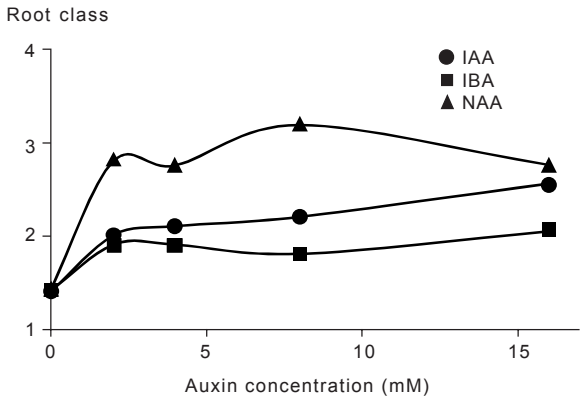

Fig. 2. Effect of IAA, IBA, and NAA at 0, 2, 4, 8 and 16 mM on root class of oil palm plantlets during 12 weeks of acclimatization at a closed plastic tunnel; root class 1 = without roots; class 2 = with one primary root and no secondary roots; class 3 = with one primary root and many secondary roots; class 4 = with two or more primary roots and no secondary roots; class 5 = with two or more primary roots and many secondary roots; average root class of the control treatment was 1.4.

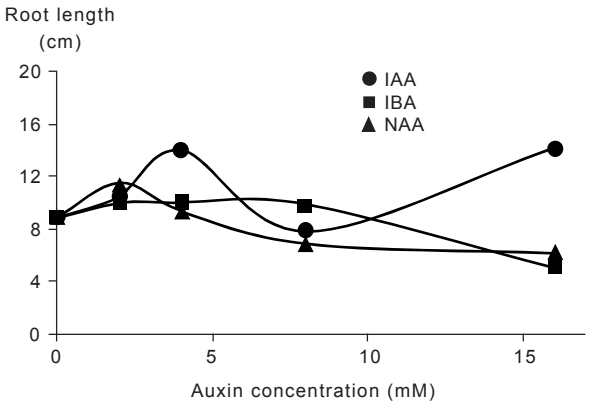

Fig. 3. Effect of IAA, IBA, and NAA at 0, 2, 4, 8 and 16 mM on root length of oil palm plantlets during 12 weeks of acclimatization at a closed plastic tunnel. The average root length shown by the control treatment was 9.0 cm.

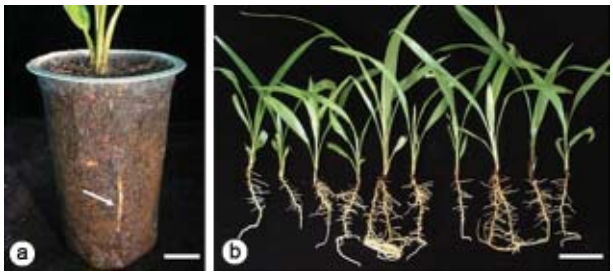

Fig. 4. The development of roots of oil palm plantlets at 6 weeks (a, arrow) and 12 weeks (b) after acclimatization. Bar = 1 cm (a) and 5 cm (b).

the base (Fig. 4b). The root system of the plantlet is similar to that of the seedling of oil palm during juvenile phase (Jourdan and Rey 1997). Good root system is considered important to support good growth of the plantlets subsequently in the nursery and field.

Shoot Growth

Compared to control, all types and concentrations of auxin tested enhanced significantly shoot height of oil palm plantlets, 12 weeks after acclimatization of initially un-rooted-plantlets. The average initial shoot height was 9.5 cm. Without auxin treatment, plantlet average height was only 11.1 cm, whereas with auxin the average heights of the plantlets were 14.5-16.8 cm (Table 1), increased by 30-47% compared to the untreated control. However, there were no shoot height differences among the IAA, NAA, and IBA at 2, 4, 8, and 16 mM treatments.

Statistical analysis revealed that there was no significant difference among all treatments on plantlet leaf number. The number of leaves was 4-5 sheets (Table 1), increased from 2.7 sheets on average at the start of the experiment. This means that there was a formation of 1-2 new leaves in 12 weeks of acclimatization. The addition of IAA, NAA, and IBA at 2, 4, 8, and 16 mM did not influence the number of leaves of oil palm plantlets during acclimatization for 12 weeks.

Diameter at the base of the shoot of oil palm plantlet increased significantly by all auxin treatments. However, there was no difference among types and concentrations of auxin used. Dipping un-rooted plantlets of oil palm in IAA, NAA or IBA at 2 mM for 10 minutes prior to planting in mixed growing media enhanced significantly shoot diameter after 12 weeks of acclimatization, on average by 30% compared to control. The highest shoot diameter was found on the treatment of 2 mM NAA. Increasing auxin concentration up to 16 mM did not further increase the shoot diameter (Table 1). This indicates that auxin treatment at 2 mM was already sufficient for shoot growth of oil palm plantlets during acclimatization. As other plant growth regulators, auxin enhances growth at lower concentrations and inhibits growth at higher concentrations (Gaspar *et al*. 2003). In this experiment, auxin at a range of 2-16 mM promoted shoot growth of oil palm plantlets and did not reach a concentration that inhibits the growth. Auxin concentrations of lower than 2 mM should be further tested to determine whether it would give better results on root and shoot growth of oil palm plantlets during acclimatization period.

Dipping the base of un-rooted plantlets of oil palm in auxin solution for 10 minutes was able to induce root formation as well as increase shoot growth. There was a high correlation between root growth (root frequency and root class) and shoot growth (shoot length and shoot diameter). It suggests that good root growth of the plantlets would support shoot growth during acclimatization period. Similar results were reported by Borkowska (2001) in micropropagated strawberry rooted *ex vitro*. Roots have a physical role as an anchor to support plantlets to stand upright and a physiological role to uptake water and nutrients for plant growth and development.

The results of this experiment revealed that root formation of oil palm plantlets could be induced *ex vitro* during acclimatization phase. The rooting frequency of *ex vitro* rooting of oil palm plantlets at 80% in 2 mM NAA treatment was comparable to *in vitro* rooting of oil palm reported by Rival *et al*. (1997) at 80-92% rooting frequency on a solid medium, by Konan *et al*. (2007) at 66% with three plantlets per tube on a solid medium, by Nizam and Te-chato (2009) at 88% on a solid medium, and by Riyadi and Sumaryono (2010) at 73.3% in a liquid medium. *Ex vitro* rooting technique would eliminate sterile rooting phase thus simplify the protocol and reduce seedling production time and cost (Meier-Dinkel *et al*. 1993; Borkowska 2001; Hazarika 2003; Martin 2003; Shekafandeh 2007). The study may have a great benefit in clonal mass propagation of oil palm.

CONCLUSION

Rooting of tissue culture-derived rootless oil palm plantlets can be induced during *ex vitro* acclimatization by dipping in the auxin solution for 10 minutes. The best treatment was in 2 mM NAA which induced rooting frequency up to 80%. The treated plantlets produced higher shoot height and diameter, and more primary and secondary roots which are suitable for oil palm clonal mass propagation.

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