DOUBLE-LAYER TECHNIQUE IN ROOTING OF OIL PALM *in vitro* PLANTETS

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The *in vitro* propagation technique of most crops is labour intensive. It relies heavily on the skill and experience of the operation personnel. This is especially prominent in the shoot elongation and rooting stages where shoots are handled individually. The process of *in vitro* rooting in the micropropagation of horticultural crops has been estimated to account for 35% to 75% of the total cost of the process (Debergh and Maene, 1981). In 1985, Maene and Debergh introduced a method that avoided or reduced the handling of individual shoots during the plantlet elongation and rooting stage. In this method, a liquid medium is added to established cultures instead of transplanting/transferring the cultures to the medium. A layer of the liquid nutrient forms above the solid medium, thereby forming two layers of nutrient media; hence the name of the technique. This labour saving approach has been widely applied in banana micropropagation in Taiwan (Lee and Hwang, 1995).

in vitro PROPAGATION IN OIL PALM

The oil palm tissue culture process involves ortet selection, sampling of explants, callus initiation and multiplication, embryogenesis, shoot regeneration, rooting and hardening of ramets and field evaluation of clones. Shoot regeneration and embryoid multiplication usually occur concurrently in the same polyembryoid (PE) cluster. Separation of these shoots is needed to allow the PE to continue to proliferate in the next subculture. These shoots are individually selected and detached from each other before transfer into fresh medium for the shoot development (SD) stage. A few months later, these shoots are again individually manipulated and transferred into another medium for the root initiation (RI) stage. Both stages require handling of individual shoots that need a substantial amount of labour. By improving the technique in both or at least in one of these stages, it is expected that production efficiency will increase.

DOUBLE-LAYER TECHNIQUE FOR ROOTING IN OIL PALM

Shoots (about 2 cm and above) from PE cultures are separated, selected and a V-shape cut is made at the base of each shoot. These shoots are evenly transferred into solid SD medium with 15 shoots per 250 ml conical flask. No fused shoots are allowed as these can later create multiple shoots with a single root system. After about two months in SD medium, these shoots grow to a height of about 6 cm and are ready to undergo the rooting stage via the double-layer technique. At this time, about 50 ml liquid rooting media are aseptically poured into the flask (*Figure 1*). This amount is sufficient to initiate rooting of 15 shoots for a period of three months. Depending on the clone, about 75%-90% shoots develop roots, and the shoots can attain more than 12 cm in height at the 2-3 leaf stage (*Figure 2*). Non-rooted shoots are picked out from the culture and subsequently transferred singly into test tubes containing fresh liquid RI medium for a second cycle of rooting. Rooted shoots are now ready for the *in vitro* hardening stage, which is done by moving the rooted cultures to the nursery environment without opening the vessel closure and placing under a half-shaded area for

Figure 1. Adding of liquid rooting medium to shoot cultures.

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Figure 2. Root development stage.

five to seven days. Hardened ramets from the flasks are pulled out under water so as to expedite the separation of shoots while minimizing root injury and attached agar removal (Lee and Hwang, 1995). After treatment with fungicide, they are planted directly in polythene bags under 75% shade.

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