6. CONCLUSION

From the results of this research work of "Isolation and culture of oil palm (*Elaeis guineensis* Jacq.) protoplasts" the following conclusions can be drawn:

Flow cytometric analysis of oil palm by using FACScalibur cytometer revealed that DNA content of *Elaeis* cv. *Tenera* is 4.18 pg when *Glycine max* cv. Polanka was used as an internal reference plant, whereas that was estimated to be 3.97 and 4.34 pg using *Lycopersicon esculentum* cv. Stupicke and *Zea may* line CE-777 as internal references, respectively. The distribution of the nuclei extracted from embryos imbibed for 48 h displayed a prominent peak at 4C (4.53-6.8%) indicating that they consisted of actively-dividing cells. This profile is in contrast to those observed for non-imbibed embryos and embryos imbibed for 24 and 72 h, calli, shoots and roots. It should be noted that the potential for cell division and regeneration was as high as the proportion of cells in phases G2/M. Since one-year-old calli and shoot meristem of seedling at the third subculture were significantly different in their DNA contents, these calli should not be used as a protoplast source.

Oil palm embryos imbibed for 48 h are the most suitable source for protoplast isolation. Protoplasts isolated from these embryos revealed a high physical and physiological stability and showed uniform appearance. Moreover, these embryos also yielded a higher number of protoplasts in isolation than other sources. Protoplasts isolated from embryos exhibited the highest mean of cell division though the difference was not statistically significant. These results suggest that embryos

imbibed for 48 hours consisted of the cells with high dividing ability, which might favored for yielding the protoplasts suitable for culture.

Incubation in enzyme solution mixture of 3% Cellulase + 1.5% Driselase + 1% Macerozyme for 3 h gave the highest number of protoplasts from basal leaf and shoot protoplasts whereas 1% Cellulase + 0.5% Driselase + 1% Macerozyme gave the highest number of protoplasts from callus when incubated on a rotary shaker for 24 h. For imbibed embryos, enzyme solution mixture of 1% Cellulase + 0.25% Driselase + 0.5% Macerozyme was chosen because it gave the highest yield of oil palm protoplasts under stationary condition during the incubation for 48 h in darkness. Protoplasts isolated by this condition still retained more than 50% viability. Agarose bead technique is the chosen technique for oil palm protoplast culture since it gave a high percentage of cell division. Although the percentage of cell division was lower than in liquid thin layer technique, continued cell divisions to microcolonies were only seen in protoplast cultures derived from bead technique. When imbibed embryo-derived protoplasts were cultured by bead technique containing different gelling agents, the technique with agarose used as gelling agent gave the highest percentage of cell division. The protoplasts embedded in agarose bead were easy to observe and withstood the gradual reduction of osmotic pressure surrounding protoplasts.

MS medium containing 8.3 μM picloram, 4.5 μM 2,4-D, 9.3 μM kinetin and 4.4 μM BA is chosen for protoplast culture since it gave the highest percentage of cell division. The concentration of auxin in this medium resembled that in callus induction medium. 2,4-D was indispensable for initial cell division whereas picloram was found to be not essential. The other auxin, dicamba, did not promote initial cell division. MS medium containing 4.5 μM dicamba and 2,4-D, 9.3 μM kinetin, 4.4 μM BA and 3.8

μM ABA is the suitable medium for cell colony induction and gave the highest rate of plating efficiency. Picloram was less effective in promoting cell colony formation. Although attempts were made to maintain the cell colonies in cell colony induction medium using different types and concentrations of growth regulator, these cell colonies did not grow beyond the 4^{th} week of culture.

The findings of this research are as follows:

1. Oil palm embryos imbibed for 48 h are the choice for obtaining seedling and callus induction efficiency. This condition is a critical step in the establishment of an *in vitro* culture system of oil palm which leads to the increased cell division of oil palm protoplasts.

2. An incubation time for 48 hours without agitation in the dark is the critical condition for protoplast isolation from oil palm embryos which leads to the increased yield of protoplasts (4.77×10^{6} protoplast. $gram^{-1}$ FW).

3. 2,4-D is the important plant growth regulator for inducing cell division of oil palm calli and protoplast which leads to the increased cell colony formation of oil palm (0.03%).

The conclusion of this research is "embryos imbibed for 48 hours are the most effective protoplast source for producing viable protoplasts and protoplast culture of oil palm".