UNIVERSITI TEKNOLOGI MARA

MICROPROPAGATION OF SUGAR PALM (*Arenga pinnata* Wurmb Merr.)

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Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Agriculture Biotechnology)

Faculty of Plantation and Agrotechnology

January 2018

ABSTRACT

A micropropagation protocol of sugar palm (*Arenga pinnata* Wurmb Merr.) through seed culture, embryo culture, organ culture (direct organogenesis), callus culture and somatic embryogenesis using different type of explants (i.e. immature embryos, tender leaf of palm heart and different parts of aseptic seedlings such as leaf, upper stem, basal stem and roots) has been developed. Cultures responded differently to the different type and concentrations of plant growth regulators (PGRs) used in the artificial nutrient medium (MS). Seed culture and embryo culture through *in vitro* germination method were carried out to facilitate rapid and uniform growth of aseptic seedlings; which were later used as source of explants. Optimal establishment of aseptic seedlings (100%) was obtained through embryo culture on MS basal medium (MS0) within 7-8 weeks following inoculation. Dormancy of mature seeds used could not be broken under *in vitro* condition. The direct organogenesis culture in sugar palm on different type of explants revealed the optimum organogenic capability of basal stem explant treated with MS + 2.0 mg/L BAP + 1.0 mg/L GA_3 to regenerate into complete plantlet. Multiplication of bud-like shoots (0.04%) was determined later from the immature embryo explant treated on MS + 2.0 mg/L BAP + 1.0 mg/L GA_3 + 1.0 mg/L $AgNO_3$. Maximized rooting of plantlets and shoot regenerants was obtained upon transfer to MS + 3.0 mg/L IBA. Through callus culture, the significant roles of auxins particularly 2,4-D and cytokinins (BAP and Kinetin) to proliferate callus on different type of explants was proven. Immature embryo explant inoculated on MS + 0.4 mg/L 2,4-D + 0.5 mg/L BAP promoted 100% frequency of fragile, beige callus within 8 weeks of culture. Increased sucrose concentration (6.0%) and the addition of 3.0 g/L casein hydrolysate (CH) to MS + 0.4 mg/L 2,4-D + 0.5 mg/L BAP maximized the embryogenic potency of callus. Somatic embryogenesis pathway began with the development of globular and heart-shaped somatic embryos on the very same medium, which later matured into torpedo and cotyledonary developmental stages on MS + 0.4 mg/L 2,4-D + 0.5 mg/L BAP + 1.0 mg/L $AgNO_3$. Improved germination of cotyledonary embryos into primordial shoots and roots was achieved on MS + 1.0 mg/L BAP + 1.0 mg/l NAA. Separate regeneration of shoots and roots of normal morphology was established on ½ MS medium + 0.1 mg/L activated charcoal. Well-developed synthetic seeds production in sugar palm was obtained by the encapsulation of somatic embryos in 3.0% Na- alginate and 100 mM $CaCl_2$. Maximum rate (50%) of synthetic seeds germination was observed on MS + 1.0 mg/L BAP + 1.0 mg/l NAA within 8 weeks of culture. Temperature at 25^{o}C was relevant for synthetic seed storage. Well-rooted *in vitro* plantlets survived the acclimatization phase at 80% following transfer to soil: peat moss: perlite (2:2:1) mixture. Exposure of *in vitro* plantlets for acclimatization under 50-100 µmol $m^{-2}s^{-1}$ light intensity resulted in 60% survival rate after 12 weeks. Established plantlets were successfully maintained under greenhouse condition with 40% rate of survival after a year.

ACKNOWLEDGEMENT

Foremost, Alhamdulillah, thanks to Allah the Almighty Who give me blessings and guidance which given me strength and inspiration to embark on my PhD study and instilling in all of my strength to complete this thesis. I submit my heartiest gratitude to my respected advisor, Assoc. Prof. Dr. Asmah bt Awal and co-advisor, Dr. Mohd Yusoff bin Abdullah for their sincere guidance and help for completing this project. I have been amazingly fortunate to have advisors who gave me freedom to explore on my own and at the same time the guidance to recover when my steps faltered.

I am deeply indebted to the dedicated staff of postgraduate laboratory, Faculty of Plantation and Technology, UiTM Puncak Alam, - En Shahfie bin Md Latep, for providing equipment and maintaining the good condition of laboratory for smooth research implementation. I am also greatly indebted to the dedicated staffs of Faculty of Health Science, UiTM Puncak Alam for allowing me to use the facilities available at their histology and cytology laboratory as well as providing information and helping hands.

Most importantly, I revere the patronage and moral support extended with love by my dearest husband, precious son, and my most beloved parents and siblings. Their love and support helped me overcome setbacks and stay focused on my study. Many friends have helped me stay sane through these difficult years. I greatly value their friendship as their support and encouragement were a great help throughout the course of the research work. I humbly extend my thanks to all concerned persons who co-operated with me in this regard.

Finally, I acknowledge the university for the financial support by 600-RMI/DANA5/3/REI (1/2013), Universiti Teknologi MARA (UiTM) Shah Alam, Selangor and Mybrain15 scholarship (MyPhD), Ministry of Higher Education, Malaysia whom funded the research discussed in this thesis.

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