

Kanchanaree Pongchawee 2006: Irradiation and Protoplast Fusion Approaches for Varietal Improvement of *Anubias spp.* Doctor of Philosophy (Aquaculture), Major Field: Aquaculture, Department of Aquaculture. Thesis Advisor: Professor Uthairat Na-Nakorn, Ph.D. 200 pages.
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Aquatic plant *Anubias nana* Engler was cultured on semi-solid MS medium supplemented with 8 μ M BA. The maximum shoot proliferation (6.80 ± 0.79 shoots/pieces) was obtained after 6 weeks of culture. The plantlets were transplanted to a greenhouse and cultured in hydroponic system. High quality plants were produced within 90 days with average weight of 7.34 ± 1.29 g, height of 7.91 ± 0.79 cm and 86 % survival rate.

The suitable gamma ray dose (GR_{30}) for induced mutation of *A. nana* was 34.56 grays and for *Anubias congensis* N.E. Brawn was 28.30 grays. The gamma ray significantly decreased height and number of shoots and roots ($p < 0.05$) of plants. Abnormal appearances were observed, i.e. dwarf plants, change of leaf shape and color. After transplantation to the greenhouse, there were 5 mutated plants exhibiting good growth. Amplified fragment length polymorphism (AFLP) markers could identify mutated plants from normal plants.

For the protoplast fusion experiments, protoplasts were successfully isolated from the *in vitro* plantlet leaves of *A. nana* and *Cryptocoryne wendtii* De Wit. Purified protoplasts of *A. nana* were cultured on KM8P medium while those of *C. wendtii* were cultured on MS medium by agarose bead with thin layer liquid culture. Micro-colonies were formed within 30 days. Protoplasts of *A. nana* \times *C. wendtii* were electrofused at a density of 5×10^5 protoplasts/ml using an alternating current (AC field) of 90 V/cm for 30 s followed by 2 pulses of 1100 V/cm direct current (DC) field and 40 μ s duration. The intergeneric hybridization produced 5.20 ± 0.97 % heterokaryons with 79.40 ± 1.92 % viability. The heterokaryons were cultured on MS medium supplemented with 0.2 mg/l (2,4 D), 1 mg/l NAA and 0.5 mg/l zeatin by agarose bead with thin layer liquid culture method. The plating efficiency of 6.89 ± 2.05 % and viability of 19.75 ± 2.87 % were observed after 10 days' culture.

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Student's signature

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