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Research Article

Effects of NAA and BAP, Double-Layered Media, and Light Distance on *In Vitro* Regeneration of *Nelumbo nucifera* Gaertn. (Lotus), an Aquatic Edible Plant

Noraini Mahmad,[1] Rosna Mat Taha,[1] Rashidi Othman,[2] Azani Saleh,[1,3] Nor Azlina Hasbullah,[4] and Hashimah Elias[1]

[1] *Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[2] *International Institute for Halal Research and Training (INHART), Herbarium Unit, Department of Landscape Architecture (KAED), International Islamic University Malaysia, 53100 Kuala Lumpur, Malaysia*
[3] *Faculty of Applied Science, MARA University of Technology, 40450 Shah Alam, Selangor, Malaysia*
[4] *Department of Agriculture Sciences, Faculty of Technical and Vocational Education, Sultan Idris Education University, 35900 Tanjung Malim, Perak, Malaysia*

Correspondence should be addressed to Noraini Mahmad; fara_aid@siswa.um.edu.my

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In vitro direct regeneration of *Nelumbo nucifera* Gaertn. was successfully achieved from immature explants (yellow plumule) cultured on a solid MS media supplemented with combinations of 0.5 mg/L BAP and 1.5 mg/L NAA which resulted in 16.00 ± 0.30 number of shoots per explant and exhibited a new characteristic of layered multiple shoots, while normal roots formed on the solid MS basal media. The double-layered media gave the highest number of shoots per explant with a ratio of 2 : 1 (liquid to solid) with a mean number of 16.67 ± 0.23 shoots per explant with the formation of primary and secondary roots from immature explants. In the study involving light distance, the tallest shoot (16.67 ± 0.23 mm) obtained from the immature explants was at a light distance of 200 mm from the source of inflorescent light (1000 lux). The plantlets were successfully acclimatized in clay loam soil after 8 months being maintained under *in vitro* conditions.

1. Introduction

Lotus is in the genus of *Nelumbo* and belongs to the family of Nelumbonaceae. The Nelumbonaceae family consists of a perennial aquatic and emergent angiosperm plant which consists of two species: *Nelumbo nucifera* Gaertn. (the Asian or sacred lotus) and *Nelumbo lutea* (Willd.) Pers. (the American lotus or water chinquapin). The former is distributed in Asia and North Australia and the latter is found in North and South America [1, 2]. Lotus is an important economic aquatic plant, not only as a dainty and ornamental flower but also as a source of herbal medicine, with strong bioactive ingredients, including alkaloids and flavonoids, and antioxidant, antisteroidal, antipyretic, anticancerous, antiviral, and antiobesity properties [3–6]. Lotus is usually propagated vegetatively through rhizome division or tuber production, but the normal propagation rate is very low [7]. It can also be multiplied through seeds but, for quick and more efficient germination, the seeds need to be scarified by rubbing the outer hard seed coat gently on sandpaper at both ends and finally immersing them in water to initiate germination. Scarified seeds were germinated after 3-4 days while normal seeds took 10–15 days to germinate. If the hard coating remains intact, the seeds will remain viable for centuries and it may take a few years for the seed to sprout if placed in water [8].

The present research is aimed at studying *in vitro* regeneration of immature (yellow plumule) explants on solid Murashige and Skoog (MS) media supplemented with different combinations and concentrations of α-napthaleneacetic