Development of a Suitable *Invitro* Protocol for *Nymphaea* Leaf Culture

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Abstract

Nymphaea is commercially important aquatic plants propagated through cross pollinated seeds cause trait variations in the offsprings, the morphological features of flowers are different from one plant to another and cause major problem by commercial producers and vendors. Therefore, multiplication through micropropagation is a viable option. Hence, this research focused on establishing a robust *in vitro* protocol for *Nymphaea* leaf culture. The young leaves of Nymphea were excised from a single mother plant and cut into (5mm x5mm) pieces were used as explants. Then five sterilization methods (Factor 1) *viz:* 0.2% of $HgCl_2$ (T1), 0.1% of $HgCl_2$ (T2), 5% NaOCl (T3), 10% NaOCl with Tween20 (T4), 10% NaOCl (T5) were employed. Then two types of media (Factor 2), namely 1/2MS (M1), comprising 50 ml of A stock, 2.5 ml of B stock, 5 ml of C stock, along with glycine, pyridoxine, nicotinic acid, thiamine, BAP, 2.4-D, Myo-inositol, and sugar, consistent and favorable results were observed across various sterilization techniques. Conversely, media 2 (M2), with a similar composition but supplemented with BA, NAA, and kinetin were employed. The data collected over the course of 12 weeks, the results indicate that T4 consistently exhibits the lowest contamination percentage of leaf across both media formulations, while T3 consistently demonstrates higher contamination rates. Furthermore, media 1 consistently yields superior results in terms of tissue culture initiation compared to media 2. By identifying optimal sterilization techniques and media formulations, this study provides valuable insights for enhancing mass propagation.

Keywords: 2.4-D, Glycine, Invitro protocol, Myo inositol, Nicotinic acid, Pyridoxine, Surface sterilization,