Optimization of Seed Sterilization Procedure for *Nymphaeanouchali* Brum.f.,

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Abstract

*Nymphaeanouchali*Brum. f., 'Nil Manel' belongs to one of the oldest Angiosperm families, Nymphaeaceae. It has become a flagship species in 1986 with the official announcement of Sri Lankan government as the national flower of Democratic Socialist Republic of Sri Lanka. However, *N. nouchali*has been utilized by the local communities for over thousand years. Pale blue petals, unique fragrance and cultural value of the flower made it a highly demanded commodity in both cut flower and ornamental aquatic plant industry in Sri Lanka. Moreover, rhizome of the *N. nouchali*has been consumed and cultivated as a food item and dried and fresh plant parts have been used as folk and aurvedic medicine to treat various illnesses.

*Nymphaeanouchali*is a native, day blooming plant grown in shallow water bodies. Naturally it is vegetativelypropagated, by erect, unbranched rhizomes or by seeds. These unbranched rhizomes normally act as time travelling organs to withstand harsh conditions such as droughts. In dry season, shallow water bodies such as fallow paddy fields, marshland or abandon tanks which habituate*N. nouchali* dry up. As a result, entire population of *N. nouchali*arewiped out without leaving any traces. Consequently, it could result a habitat destruction Furthermore, some of the introduced water lily species such as violet flowered waterlily naturally hybridize with the native species producing s more aggressive hybrids. Native *N. nouchali*is gradually suppressed from the water bodies by violet flowered waterlily and its hybrids. *N. nouchali*has been listed in the IUCN red list of threatened species. Conservation of native biological diversity is one of the major challenges and immediate actions are required to conserve this valuable plant species for future generations. Mass propagation of *Nymphaeanouchali* through *invitro* multiplication will be a reliable solution. Inthe present study, attempts were made to test the feasibility of invitro seed culture to germinate seeds obtained from isolated plant populations which are expected to be pollinated from the pollen of same species.

Sterilization was done using five different combinations of Clorox™ concentrations (10, 30, 50, 70 and 100%), each for two different time durations (10 or20 min.).Each treatment comprised of five replicate petri dishes (60× 15 mm)each containing 25 seeds in 12 mL of half strengthbasal Murashige and Skoog medium supplemented with 2% sucrose, 100 mg/L MyoInositol and 0.6% plant tissue culture agar. The cultures were observed at one week intervals and germination rate of the seeds and the contamination rate, if any, were recorded.

Considering overall results, Only Clorox™ concentrations showed significant ($P<0.001$) effect on sterilization of seeds. 100% Clorox™ for 20 min. sterilization showed lowest contamination rate (39.2±2.49). However, none of the sterilization protocols showed complete control of contamination in the cultures and 50% and 70% concentrations did not showed significant difference with 100%. Highest germination percentage (39.2±2.38) was recorded with 30% Clorox™ for 10 min. sterilization, however, almost all the germinated seeds (37.6±3.2) were contaminated with bacteria. Highest rate of non-contaminated (4.8±1.14) seedlings was observed with 50% Clorox™ for 10 min. sterilization. Considering overall results, none of the tested sterilization protocols gave an adequate disinfection rate in the cultured seeds. However, an important observation made in this experiment was contamination showed a significant ($P<0.05$) effect on germination. This could be attributed with the dormancy created by a thick coat of *N.nouchali* seeds. Therefore, high germination rate of the contaminated seeds could be due to the bacterial degradation of seed coat.

Keywords: Contaminations; Germination; *Nymphaeanouchali* Brum. f.; Sterilization protocol