

Optimization of an efficient semi-solid culture protocol for sterilization and plant regeneration of *Centella asiatica* (L.) as a medicinal herb.

ABSTRACT

The present study investigates the effects of different concentrations, as well as type of plant growth regulators (PGRs) and medium (MS, Duchefa) on the growth and development of *Centella asiatica* in semi-solid culture. In addition, a protocol for successful sterilization of *C. asiatica* explants prepared from field-grown plants highly exposed to fungal and bacterial contamination was determined. Results for sterilization treatments revealed that applying HgCl₂ and Plant Preservative Mixture (PPM) with cetrimide, bavistin and trimethoprim which were included after washing with tap water, followed by the addition of PPM in the medium, produced a very satisfactory result (clean culture $90 \pm 1.33\%$) and TS5 (decon + cetrimide 1% + bavistin 150 mg/L + trimethoprim 50 mg/L + HgCl₂ 0.1% + PPM 2% soak and 2 mL/L in medium) was hence chosen as the best method of sterilization for *C. asiatica*. The synergistic combination of 6 benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) in concentrations of 2 mg/L and 0.1 mg/L, respectively, in Duchefa medium compared with MS induced the most optimal percentage of sprouted shoots (93 ± 0.667), number of shoots (5.2 ± 0.079) and nodes (4 ± 0.067) per explant, leaf per explant (14 ± 0.107) and shoot length (4.1 ± 0.67 cm). Furthermore, optimum rooting frequency ($95.2 \pm 0.81\%$), the number of roots/shoot (7.5 ± 0.107) and the mean root length (4.5 ± 0.133 cm) occurred for shoots that were cultured on full-strength MS medium containing 0.5 mg/L indole-3-butyric acid (IBA). In this study, the acclimatized plantlets were successfully established with almost 85% survival. The findings of this study have proven an efficient medium and PGR concentration for the mass propagation of *C. asiatica*. These findings would be useful in micropropagation and ex situ conservation of this plant.

Keyword: *C. asiatica*; Semi-solid culture; Sterilization; PPM.