DEVELOPMENT OF A SOMATIC EMBRYOGENESIS PROTOCOL FOR TEA

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Micropropagation of tea (*Camellia sinensis* (L.) O Kuntze) has little progressed due to high rate of contaminations, poor rooting, low multiplication rate and its recalcitrant nature. Since somatic embryogenesis emerged as an alternative to resolve above limitations, present study intended to develop a viable somatic embryogenesis protocol for tea. Four factor factorial experiment was designed with two growth stages of three explants namely; *ex-vitro* leaves (2nd and 3rd leaf), cotyledons (immature and mature) and leaf calli (2nd and 3rd sub cultures) of TRI 2024 and 2043 cultivars with MS medium with two growth regulator combinations for each explants ((I) 2 mg L^{-1} BAP + 3.5 mg L^{-1} NAA, 2 mg L^{-1} BAP + 0.1 mg L^{-1} NAA and (II) 2 mg L^{-1} BAP + 3 mg L^{-1} NAA, 3 mg L^{-1} BAP + 0.1 mg L^{-1} NAA for leaf calli, (I) 3 mg L^{-1} BAP + 0.1 mg L^{-1} NAA, 3 mg L^{-1} BAP + 0.1 mg L^{-1} NAA and (II) 2 mg L^{-1} BAP + 3 mg L^{-1} NAA, 3 mg L^{-1} BAP + 0.1 mg L^{-1} NAA for *ex-vitro* leaf and (I) 3 mg L^{-1} BAP + 0.1 mg L^{-1} NAA, 3 mg L^{-1} BAP + 0.1 mg L^{-1} NAA and (II) 2 mg L^{-1} BAP + 0.2 mg L^{-1} NAA, 3 mg L^{-1} BAP + 0.5 mg L^{-1} NAA for cotyledons). Somatic embryos were developed via direct pathway from cotyledons and indirect pathway from *ex-vitro* leaves and leaf calli. Somatic embryos were observed only from cotyledons, while *ex-virto* leaves and leaf calli have developed only up to callus stage during the study. Significantly higher callus formation was observed in *ex-vitro* leaves in the medium II, when 3rd leaf of TRI 2024 cultivar was used as explant. Higher callus proliferation was seen in 2nd sub culture of TRI 2043 cultivar in medium II. Hence, MS medium with 2 mg L^{-1} BAP and 3 mg L^{-1} NAA is suitable for callus induction. The highest percentage of somatic embryogenesis (40%) was observed in mature cotyledon of TRI 2043 in medium I. In conclusion, somatic embryogenesis varies among different cultivars, explants and media. MS medium with 3 mg L^{-1} BAP and 0.1 mg L^{-1} NAA is suitable for embryo induction of cotyledons.

Keywords: Callus, Cotyledon, *Ex-vitro* leaves, Growth regulators, Somatic embryogenesis