In vitro studies on plant development of *Camellia sinensis* (L.) O. Kuntze (tea) with emphasis on somatic embryogenesis

Thayamini Harold Seran

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Department of Plant Sciences

Faculty of Science

University of Colombo

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Abstract

Histological and morphological studies on somatic embryogenesis were carried out for efficient *in vitro* plant development of *Camellia sinensis* (tea). To determine the suitable stage of cotyledons for direct somatic embryogenesis, the sterilized cotyledons (6 -12 mm diameter) at various developmental stages were inoculated on MS medium containing 2.0 mg L^{-1} BAP and 0.2 mg L^{-1} NAA. Results indicated that immature cotyledon (10 mm diameter, stage 3) was the most responsive stage for the production of typical (normal) somatic embryos. Cytological examinations revealed that typical somatic embryos at different stages exhibit gradual tissue differentiations. Typical mature somatic and zygotic embryos morphologically and histologically have similar bipolar structures.

Immature cotyledon segments (stage 3) were placed on different media containing NAA (0.0 – 3.0 mg L^{-1}) in combination with BAP (2.0 mg L^{-1}) to select suitable medium for efficient production of typical somatic embryos. The optimum concentrations of NAA were in the range of 0.2 – 1.0 mg L^{-1} for direct somatic embryogenesis (14.6% - 16.7%). Cotyledon-type and seed-like somatic embryos were developed in 0.2 and 1.0 mg L^{-1} NAA respectively. Further, the explants of high yielding cultivars DT1 (Estate selection in Sri Lanka) and TRI 2025 (Assam type) performed well and exhibited embryogenic potential (28.1% and 21.9% respectively).

Several types of explants obtained from *in vitro* germinated zygotic embryonic axes were cultured to select the suitable explants for the production of typical somatic embryos. Results indicated that both hypocotyl and small succulent leaf explants were

the best for the production of firm, typical somatic embryos. Histological studies showed that most somatic embryos were directly originated from the cortex tissue of the hypocotyl and upper epidermal layer of the succulent leaf explants.

Further, attempts were made to establish *in vitro* callus culture from field grown leaves. Results indicated that high frequencies (50% - 58 %) of aseptic cultures were achieved in 60% and 75% solutions of Clorox^{TM} for 30 min soaking period among varying concentrations of disinfectant (15% - 75%) and various exposure times (15 - 60 min). The rates of initiation (72.2%) and formation (56.9%) of calli from *in vitro* aseptic explants were relatively high in MS medium containing BAP (2.0 mg L^{-1}) and NAA (3.0 mg L^{-1}) among tested combinations of BAP and NAA concentrations.

Morphological and histological studies were carried out on the development of embryogenic callus from leaves of *in vitro* shoots. It was observed that collenchyma cells were developed from vegetative cells at the cut end and packed within the swelling tissues. After bursting, parenchyma cells were gradually formed as a result of cell divisions. These parenchyma cells differentiated as embryogenic cells. The average number of single embryogenic cells was significantly higher in the liquid than in solid medium. Further investigations were done to produce somatic embryos from primary embryogenic calli. Results showed that formation of somatic embryos (11.7%) was high in MS medium containing BAP (1.0 mg L^{-1}) and NAA (0.1 mg L^{-1}) in combination with GA_3 (0.1 mg L^{-1}).

Zygotic embryonic axes were coated with 3% and 4% sodium alginate matrices. The results revealed that 3% sodium alginate with 100 mM calcium chloride dihydrate provided the most suitable matrix concentrations for encapsulation. Further, naked and coated axes cultured on MS medium without (MS1) or with (MS2) 3.0 mg L^{-1} BAP and 0.5 mg L^{-1} IBA gave highest rates of survival (100%) and germination (93-100%). But efficient plant recovery from non-encapsulated (73.3%) and encapsulated (42.2%) axes was achieved on MS2 medium. A high rate (48%) of *in vitro* plantlets was survived at 8^{th} week of transplanting in soil than in a mixture of soil: sand (1:1 v/v).

Feasibility of cold storage of encapsulated embryonic axes was studied to obtain efficient *in vitro* conversion of plants and their survival under *ex vitro* conditions. Synthetic seeds and natural seeds were stored at 4 °C for 0, 4, 8, 16, and 20 weeks. Efficient *in vitro* germination and plant recovery were obtained from synthetic seeds stored for four weeks than control. Vigorous plantlets were collected from *in vitro* cultures and successfully established in soil: coconut coir dust (1:1 v/v).

To perform synchronous germination of plant material into vigorous plants under *ex vitro* conditions, sterilized zygotic embryos and zygotic embryonic axes were cultured on germination medium whereas seeds were sown in sand bed as a control. Consistent germination was observed on cultured embryonic axes *in vitro* for uniform growth where rate of germination was significantly highest (99.0%) at 4^{th} week. Further, healthy plantlets developed from embryonic axes *in vitro* had erect shoots with short internodes as well as taproots with abundance adventitious roots under nursery conditions for better adaptation in field as compared with seedling raised by conventional sexual propagation.