Establishment of *in vitro* culture to produce friable callus from leaf of *Camellia sinensis* (L.)

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Abstract: Attempts were made to produce in vitro *callus from tea* (Camellia sinensis L.) *leaves. Unfolded leaves were collected and surface sterilized in various concentrations of Clorox™ (15% -75%) in combination with various exposure times (15-60 min) to obtain optimal concentration and exposure time for sterilization of field grown leaves. Results indicated that 50% and 58% aseptic cultures were achieved in 60% and 75% solutions of Clorox™ in a soaking period of 30 min respectively. Futher, sterilized mature zygotic embryos were cultured on MS media containing 1 to 10 mg/l BAP in combination with 0.1 mg/l NAA to obtain the suitable concentration of BAP for the establishment of* in vitro *micro shoots. The result showed that 5 mg/l concentration of BAP would be suitable for the initiation of* in vitro *micro shoot cultures. At 12th week, plantlets regenerated in BAP at 5 mg/l were subcultured in the presence of 3 mg/l BAP and 0.1 mg/l NAA. Multiplication rate of first two subcultures was 3.6 ±0.2. Further leaf segments at 2nd, 3rd and 4th subculture periods were cultured on callus medium to determine the competence of friable callus initiation on leaves of newly establishing* in vitro *micro shoots. Results revealed that initiation of friable callus was fairly better on leaves obtained at 4th subculture among the tested treatments. Moreover,* in vitro *and field grown leaves were compared on the efficiency of callus initiation. A significant high frequency of callus induction (79.2%) was achieved from* in vitro *leaf explants, which were collected at 5th subculture.*

Key words: Friable callus, leaves, *in vitro* aseptic culture, micro shoots.

INTRODUCTION

In Sri Lanka, *Camellia sinensis* L. (tea) plants are grown under various types of soil and climatic conditions and also are faced with pest and disease problems. Therefore, it is necessary to provide improved planting material to growers in order to ensure highest possible yield of tea with high quality. Thus, newly elite cultivars within the existing genotypes have been mainly developed by the Tea Research Institute (TRI) for commercial planting of tea in Sri Lanka. During the past 2-3 decades, conventional breeding methods that combine high yield and good quality, pest and disease resistance and also other improved attributes have been employed. However, low seed set ability of high yielding clones like TRI 2024, TRI 2025 and DT1 (Anandappa *et al.*, 1988), quick loss of seed viability and long duration for the production of new tea cultivars by conventional methods (Anandappa, 1986) have been considered as obstacles in tea breeding programmes. Further, this traditional method has limited the potential in further tea improvement due to the