STUDIES ON ANTHER CULTURE OF TEA
(*Camellia sinensis* (L.) O. Kuntze)

by

Thayamini Kathiresampillai

Thesis submitted in parial fulfillment
of the requirement for the degree of
MASTER OF SCIENCE

Faculty of Science
University of Colombo
Sri Lanka

1998

ABSTRACT

An attempt was done to regenerate haploids from cultured anthers of tea.

The exact stage of microspores was determined by a cytological examination. But for large scale programmes, bud length and colour of anthers were used as a indicator to find out the stage of microspore. It was noticed that anthers isolated from less than 7 mm bud length has microspores at uninucleate stage.

Selection of a suitable stage of microspores to produce the callus was studied. It was observed that microspores at mid uninucleate stage was found to be the most responsive for callus induction from anthers excised from 5 mm bud length. Root initiation was noted in peripheral region of callus at 5^{th} month.

Further study was done to determine the suitable culture media and condition to produce good calli from anthers containing microspores at mid uninucleate. It was found that a high rate of callus induction was obtained in half MS medium with 2,4 D(2 mg/L) and kinetin (1 mg/L) incubated in dark (60%) and 2,4 D (2 mg/L) and BAP (1 mg/L) grown in light (53%). The mean weight of callus varied significantly ($P<0.001$) within 7 media. The callus growth was generally more in half MS media. Medium with 2,4 D (2 mg/L) and either kinetin (1 mg/L) OR kinetin (1 mg/L) and IAA (1 mg/L) cultured in dark were found to be more suitable for callus growth whereas in light, medium with 2,4 D (2 mg/L) and BAP (1 mg/L) was effective.

After transfer the calli to differentiation media, callusing was noted in all media tested but their growth was not found continuously. Embryoid and meristemoid like structures were observed on the calli formed in dark and light respectively. However, plant regeneration did not occur during the 5 months period on the shoot growth medium.

Histological and morphological studies on the anther callus development revealed that nuclei of numerous microspores began to divide unequally, forming multicellular structures during the 1st week of culture and anther lobes were swollen gradually until bursting. 89% of the anthers cultured remained alive and 6% of anthers was blacken at 1st month. The rate of callus induction was rapid during the 6-10 weeks and the callus growth was significantly increased during the 5 months. Calli became more heterogeneous with time in culture.

The determination of ploidy levels in anther callus showed that two levels of ploidy were presented in calls. When considering peak index and position at channel, peak 1 and 2 would be haploid and diploid respectively. In the callus the percentage of haploid cells was more (68%) than that of diploid (6%).

The study on comparison of callus growth in anthers of different clones indicated that the survival % of anthers of three clones such as TRI 2043, TRI 2023 and TRI 2025 was high (highest was 98%, the lowest 78%) and calli were produced in anthers of all clones used in this trial. TRI 2043 exhibited a relatively more callus formation (76.2 mg) from anther cultured in medium with 2,4 D and BAP grown in light, followed by TRI 2023, TRI 2024, TRI 2025 and TRI 777 whereas in dark, calli were significantly ($P<0.01$)

formed in anthers of four clones (TRI 2025, TRI 2024, TRI 2043 and TRI 777) than that of TRI 2023. Calli that formed in light turned to dark green, meristemoid like structures after transfer to the same medium without 2,4 D.