ESTABLISHMENT OF CELL CULTURES OF TEA (*Camellia sinensis* L.) TO STUDY PROGRAMMED CELL DEATH (PCD) IN PLANTS.

C. L. THILAKARATHNE, A.H.L.A.N. GUNAWARDENA AND M.T.K. GUNASEKARE*

Department of Agricultural Biology, University of Peradeniya
**Tea Research Institute of Sri Lanka, Talawakelle*

Tea (*Camellia sinensis* L.) is highly heterozygous and predominantly cross-pollinated woody perennial crop. Therefore, the development of improved plant materials through conventional breeding is a laborious and time-consuming task. Alternatively, cell cultures hold great promise in the production of improved cultivars of tea. As there are no studies done in the past to perfect the conditions necessary for establishing cell cultures isolated from plant tissues of tea, the present study aimed at perfecting such conditions. There is clear evidence that Programmed Cell Death (PCD) occurs during plant development and environment response. However, the signals that trigger PCD in plants are unknown. In plants, selective cell death is necessary for growth and survival. Therefore, cell cultures are used to study PCD *in vitro*.

A system was established for isolation of single cells from surface sterilized leaf tissues by digesting them in an enzyme mixture containing 1% (w/v) Macerozyme in cell and protoplast washing medium and mannitol (CPW 1.6 M) for 3 hours. Cells were purified by centrifugation followed by washing in CPW 1.6 M solution. Culture density ($5x10^4$) was adjusted by counting purified cell suspension on a haemocytometer. Purified isolated cells were then, cultured in Murashige and Skoog basal medium containing 3 % (w/v) sucrose and 1.6 % (w/v) mannitol devoid of growth regulators. Cell viability was determined by staining cells with 0.25 % (w/v) Evan's blue. Characteristics related to PCD were observed using a light microscope. For optimizing yield and viability of isolated cells, factors such as concentrations of enzymes and mannitol, stage or the age of the leaf, type of explant and digestion time were examined.

Of the factors tested, optimum yield and viability of cells were obtained when second leaf from the shoot apex was digested with 1% (w/v) Macerozyme in CPW 1.6 M for 3 hours. Under these conditions, it was possible to achieve 1.6×10^6 - 2.0×10^6 cells per gram fresh weight of leaf tissue with viability ranging from 75 - 78 %. Under similar conditions however, *in vitro* leaf tissues resulted inferior quality cells as well as more protoplasts than cells. Cell division started at 24 hours after culture. Percentage of dividing cells reached its peak 144 hours (6 days) after culture and declined afterwards. One of the characteristics of PCD, condensation of cytoplasm was observed in freshly isolated cells and dividing cells. Dead cells were stained in blue color whereas viable cells were remained unstained. For further studies of PCD characteristics, transmission electron microscopic observations are needed.