STANDARDIZING A STERILIZA TION PROT OCOL FOR STEM NODAL CULTURE AND EST ABLISHMENT OF *IN VITRO* MUL TIPLICATION RATE S OF NEW TEA CUL TIVARS (*Camellia sinensis (L.)* O Kuntze)

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Success of tea micropropagation is hindered by severe microbial contamination of explants and low multiplication rates of tea cultivars. The study aims at developing an effective sterilization protocol by combining pre harvesting sterilization measures with five different surface sterilization protocols using nodal explants from s ix new tea cultivars. Attempts were also made to identify most abundant contaminants in tea and to incorporate fungicide to the culture medium in order to control them. Multiplication rates of new cultivars were evaluated under different combinations of auxins and cytokinins. Impact on pre harvesting sterilization measures (maintaining mother bushes inside a propagator with fungicide spraying) to reduce contaminations of nodal explants was inconsistent among accessions. Among the five surface sterilization techniques tested, treatment with 0.1% $HgCl_2$ for 10 minutes yielded the best results as it improved the number of clean and alive nodal explants (45.25%). Treatments with acidified bleach and bleach, ethanol and tween 20 mixture were ineffective in reduction of contaminations (>75%). Treatment with acidified bleach and benlate mixture where cuttings were kept overnight in a refrigerator resulted severe browning of explants (44.08%). Two fungi Pestalotiopsis and Aspergillus were the most prominent contaminants those resulted 65-70% and 25% contaminations of explants respectively. When fungicides (Hexaconazole and Chlorothalonil) were added to the MS medium, high survival percentage of explants (>60%) were observed and that could be a viable alternative for hazardous surface sterilants like $HgCl_2$. Preliminary results showed significant difference in multiplication rates among new tea cultivars and accession 101 recorded the highest multiplication rate of 2.56 fold.

Highest multiplication rate which is 3.12 fold was achieved when 2.5 mgl^{-1} of BAP (6-Benzyl-aminopurine) and 0. 1mgl^{-1} of IBA (Indole-3-butric acid) were used in culture medium.

Key words: *Camellia sinensis,* Fungicide added media, Multiplication rates, Nodal explants, Pre sterilization measures