233/B

Control of shoot necrosis and plant death during anther culture of coconut (*Cocos nucifera* L.)

H. D. D. Bandupriya[1,2]* and W. W. M. A. Iroshini[1]

[1]*Departmentof Plant Sciences, Faculty of Science, University of Colombo, Colombo 03.*
[2]*Tissue Culture Division, Coconut Research Institute, Lunuwila.*

Coconut breeding is normally done either by mass selection or crossing between varieties that have high variation within a population and the resultant progenies are not true hybrids. The production of double haploids (DH) is the fastest route to produce homozygous pure lines in coconut. Successful development of an anther culture protocol for coconut is hampered by the severe shoot necrosis and dieback. In the present study, attempts were made to eliminate shoot necrosis at the shoot multiplication stage. In the first experiment transfer of the subculture of shoots to a fresh regeneration medium at an interval of three weeks was attempted, instead of sub culturing shoots at six weeks intervals. In the second experiment, to measure the effect of Calcium Chloride ($CaCl_2$) on shoot necrosis, different levels of $CaCl_2$ (2, 3 and 4 mM) were incorporated into regeneration medium and the same levels maintained until plantlets were transferred to soil. Experiments were repeated three times and the number of plants showing necrosis symptoms in each treatment was recorded. A binary logistic model was used to compare the probability of shoot necrosis as it is a binary response.The transfer of shoots frequently in to a fresh medium was not beneficial for the suppression of the necrosis problem (Chi.sq=0, p=1.00). Nevertheless, the increase of calcium chloride concentration significantly affected the alleviation of the problem of necrosis. Necrosis occurrence was significantly reduced (p=0.001) from 83.89 % to 26.78% respectively, when $CaCl_2$ concentration in the regeneration medium was increased from 2.0 mM to 4.0 mM. Transfer of shoots at very early stages and continuous sub culture of shoots in the high calcium chloride containing (4.0 mM) regeneration medium effectively reduced the occurrence of necrosis and dieback. The rooted shoots produced through the optimized protocol were acclimatized successfully. The prevention of shoot loss due to shoot necrosis will be beneficial for the further refinement of the coconut anther culture protocol.

Keywords: Androgenesis, calcium chloride, haploid, sub culture

E-mail: dbandupriya@pts.cmb.ac.lk