Studies on *in vitro* Micropropagation of *Vitis vinifera* (Grape vine) and a comparison with *Musa, Papaya* and *Citrus*

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ABSTRACT

Main emphasis of this research project was on the development of a complete micropropagation protocol for locally grown cultivars of *Vitis vinifera* (Grape), the analysis of ploidy levels of micropropagated plants and the comparison of micropropagation between *Vitis*, *Musa*, *Citrus* and *Papaya*, which are economically important fruit crops in Sri Lanka.

The first part of the present study involves establishing a micropropagation protocol for two locally available cultivars of grapevine, namely Cardinal and Israel Blue. The shoot tips of healthy plants of known origin were used as the explant to initiate proliferating bud cultures. The location of the explant in relation to the apical bud showed dramatic influence on shoot proliferation. In addition, culture media, culture conditions, regeneration procedures, acclimatisation techniques and survival of plants under field conditions were studied.

Axillary buds excised from 2^{nd} - 4^{th} nodes from the apex produced the highest percentage of establishment after an effective surface sterilization procedure. Apical buds did not produce multiple shoots. Those collected from mature stem portions (beyond the 4^{th} node) failed to survive under *in vitro* conditions. Optimum shoot initiation (8 shoots per explant after 7 days in culture) was achieved on Murashige and Skoog medium supplemented with 1.0 mg/L BAP and 0.1 mg/L IAA.

Subculturing was carried out at 2-week intervals, due to the rapid proliferation of shoots. Senescence and abscission of leaves was observed after the 3rd week in culture. The average proliferation calculated at each level of subculture indicated that it increased with each subculture. The average proliferation became significant at the fourth level of subculture in both varieties. An average of 49 shoot clusters per explant were obtained at the end of the seventh level of subculture.

To induce root initiation, shoot clusters were inoculated in liquid MS medium with 1.0 mg/L IBA. After four weeks, rooted shoots were transferred to semi-solid basal MS medium for root elongation. Rooted plantlets with an average height of 6.0 cm, were acclimatized for two weeks on sterilised stone chips, provided with a nutrient medium containing MS salts. High humidity and 50% light was provided during acclimatisation. They were then transferred to a potting mixture consisting of soil and sand in the ratio of 1:1 and were kept under glasshouse conditions for two weeks, after which they were transferred to the field.

Plant growth was observed to be normal while pruning was done according to the instructions by the Department of Agriculture. The *in vitro* grown plants took eight months to fruit, which is seven months shorter than that in conventional plants.

In the second part of the study, the ploidy level of the mother plants and micropropagated plants were compared using flow cytometry. The graphs obtained on the ploidy analyser showed a prominent peak in the same quantity class for each sample tested. It indicated that there was no difference in ploidy levels of the

specimens tested; therefore, no variations had been caused during micropropagation as far as the ploidy level was concerned.

In the next part of the study, the proliferation rate of *Vitis* was compared with those of *Citrus*, *Papaya* and *Musa* at each level of subculture. It was calculated that *Musa* showed the highest rate under present experimental conditions, while *Papaya* showed the lowest. *Vitis* and *Citrus* showed intermediate rates. Five levels of subculture were considered in this exercise. Average proliferation was seen to increase upto the tested level of subculture in all four crops, but the rate of proliferation decreased after the fourth level in *Musa*, *Papaya* and *Citrus*. It also indicated that the locally available cultivars of *Vitis* could be micropropagated, a technique that has been proved successful for the other fruit crops addressed in this study.