B-35: Some studies on development of a rapid *in vitro* propagation system for *Malus domestica* (apples)

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Apples (*Malus domestica*) an important fruit crop, is grown mainly in temperate zones. In Sri Lanka, they are grown in Nuwara Eliya and Welimada districts. Disease-free planting material of apples is scarce in Sri Lanka owing to several fungal diseases which are also responsible for the poor fruit yields at present. This study investigates the development of *in vitro* methods for rapid multiplication of local apple varieties to obtain healthy planting material while retaining the true-to-type nature of the progeny.

Although rapid *in vitro* multiplication has been successful in other countries, the response of local varieties is unknown. *In vitro* requirements can differ according to the genotype. Hence it is important to establish rapid *in vitro* methods for local varieties.

The research objectives are to find suitable media and explants for *in vitro* propagation of local apple cultivars and preparation of axenic cultures.

In the first experiment, actively growing shoot buds from 3 year old apple plants of varieties Ragala and Rahangala were used. They were sterilized in a 9% w/v solution of commercial bleach (Calcium hypochlorite) for 15 min followed by Ethyl alcohol 70% v/v for 30 seconds, and in a 0.1% w/v benlate solution for 15 min. Shoot tips were isolated and cultured in the initiation media in light intensity of about 3000 lux, at a temperature of $\pm$ 25°C and a photoperiod of 16 h light and 8 h darkness. Initiation media used, were MS (Murashige and Skoog, 1962), ½ Strength MS and Lepoivre (Lepoivre, 1978) with 0.09 mg/l BAP and 0.01 mg/l IBA as growth regulators.

In all media 0.5 g/l autoclaved benlate and 0.1 g/l filter sterilized streptomycin were used to control fungi and bacteria appearing in cultures respectively. Also (1 g/l) polyvinyl pyrrolidone (PVP_{10}) was incorporated to the medium to control the browning of media due to exudates from the explants. There were 30 replicates per treatment for each variety and the experiment was repeated twice more thus obtaining 3 sets of results.

Assessments were made of the numbers of cultures contaminated with fungi and bacteria, explants that necrosed and the numbers where shoot tips remained green and growth occurred. Where successful initiation occurred, 2 weeks and one month after culturing, measurements were taken of the height of the main stem and the length of longest leaf.

In a second experiment, shoot tips used as explants were taken from young tender buds and those from matured parts. The medium used was that of Lepoivre with the same amount of growth regulators as before and there were 40 replicates per treatment for each variety.

Thirdly, 30 dormant buds were tested with each of the 2 varieties in a medium of MS with 0.09 mg/l of BAP, 0.01 mg/l of IBA and 0.1 mg/l of GA_3

Lastly, to prevent vitrification and induce shoot multiplication, further culturing of initiated shoot tips were done into benlate free (a) Lepoivre media of same composition, (b) Lepoivre media of same composition but without streptomycin and (c) Lepoivre media of same composition with 162 mg/l phloroglucinol under same culture condition as before.

In all experiments sub culturing was done initially to media free of benlate in one week, followed by regular sub culturing to same media at monthly intervals.

Chi squared test was used to determine whether the factors tested. e.g. media effect and the state of maturity of explants had any effect on the initiation process. For growth measurements analysis of variance was computed.

The initiation of growth from shoot tips of apples was successful in both Rahangala and Ragala local varieties. Although growth could be induced in several media namely Murashige and Skoog (MS), half strength MS and Lepoivre the latter medium was the best for initiation and shoot growth of cultures.

The following were the rates of successful initiations in experiment 1, using young tender tissues disregarding the contaminated cultures:

	% Success.	
	Rahangala	**Ragala**
MS	37.7	33.9
½MS	34.3	27.1
Lepoivre	78.7	68.6

The height of the main stem and the length of the longest leaf were significantly better in Lepoivre medium than in MS or ½ strength MS, differences being more prominent after one month in culture than after 2 weeks. The growth regulator combination tested BAP (0.09 mg/l) and IBA (0.01 mg/1) was sufficient for the growth of apple shoot tips.

Fungal infection in cultures were more (14-20%) in different media compared to the bacterial infectious (4-7%).

The important result from experiment 2 was that with both varieties of apples Rahangala and Ragala, successful *in vitro* initiations occurred only with shoot tips of young tender tissue and not with those of mature parts. Success rate was 71% with Rahangala and 65% with Ragala with young tender tissues, while no success at all with older tissues from both varieties using the lepoivre medium. This indicates the importance of the physiological condition of the donor explants for culture initiation.

Shoots were also produced *in vitro* from dormant axillary buds in MS medium with growth regulators BAP, IBA and GA_3. However, further progress of the growth of these shoots were poor when subcultured to media of the same composition.

It was necessary to remove the antibiotic streptomycin in successive culturing stages of apple shoot tips, to prevent vitrification of the shoots. Phloroglucinol used in culture media to prevent such vitrification was without success.

In vitro shoot proliferation was achieved in Lepoivre medium with 0.09 mg/1 BAP and 0.01 mg/1 IBA after 3 months, with monthly sub culture intervals, giving a 3 fold multiplication rate of shoots. Initiation of shoot tips of Ragala and Rahangala varieties could be achieved. The growth and success rates of initiation were better in Lepoivre than in the other 2 media. Young tender shoot buds of apples responded well in tissue culture and not from those of mature parts, indicating the importance of the physiological condition of the donor explant for culture initiation. The dormant buds of apples could not serve as a better source of explants than young tender shoots under the conditions tried out.The vitrification of apple shoots in culture could only be overcome by removal of streptomycin from the medium and not by adding phloroglucinol.

In vitro shoot multiplication of apples could be induced in Lepoivre medium with 0.09 mg/1 BAP and 0.01 mg/1 IBA giving a three fold multiplication of shoots per month.

It was possible to prepare axenic cultures of local apple varieties despite the difficulties of contamination and browning of media.