B-34: Mass production of *Solanum virginianum L.* (katuwelbatu) through tissue culture

A M P D Attayanake, K K S Fernando
(*Plant Genetic Resources Centre, Gannoruwa, Peradeniya*)

Solanum virginianum is a small prickly shrub native to India, Burma and Sri Lanka, estimated in ayurvedic medicine as a cure for fever, coughs, asthma, colic and several other ailments. Large quantities are imported and the demand for this plant is such that it has become very rare in nature. Since it will be necessary at some point to cultivate this plant, it is useful to have ready a means of mass propagation.

Shoot tips, axillary buds and leaves of katuwelbatu from a field grown plant were used to obtain explants. Plant material was washed several times in running tap water for 10-15 min and immersed in 70% v/v ethanol for 20-30 sec. It was then transferred to 20% "clorox" (5.2% $NaOCl_2$ containing 1-2 drops of teepol/100 ml solution for 7 min. Then under aseptic conditions the plant material was washed 3 times with sterilized distilled water. Terminal and axillary buds were excised and transferred to MS Murashige and Skoog 1962) medium containing different combinations of growth regulators. In the case of leaves, approximately 0.5 cm^2 pieces were cut and inoculated on to the medium. All the cultures were incubated at 26 ± 2^0C under fluorescent light (3000 lux) for 16 h per day.

The basal medium contained MS macro and micro salts, vitamins, growth regulators, sucrose (3% w/v) and agar (0.8% w/v). Different concentrations of cytokinin (BAP 0.5 -2 mg/l), auxin NAA or IAA (0.5 - 2 mg/l) and coconut water (10% v/v) were used as growth regulators. The pH was adjusted to 5.7 with 1N NaOH and 1N HC1 before adding agar. Sterilization was done by autoclaving the media at 120^0C, 1050 N/cm^{-2} for 15 min.

Segments of the plant containing axillary buds, terminal buds and leaves were successfully sterilized (90% survival) using 20% "clorox", pretreated with 70% ethanol. Almost all the terminal buds grew into single shoots after 4-7 days of culturing *in vitro*. Axillary buds also grew into single shoots after one week of inoculation, but the rate of growth was less than in the terminal

buds. The single shoots when transferred to a medium supplemented with 1 mg/1 BAP 15-20 shoots within a week) At higher concentrations of 1.5 and 2 mg/1 BAP, the multiple shoots were short and crowded. Callus formation was minimal in media containing BAP alone, whereas media with either IAA or NAA along with BAP gave a mass of calius around the explants. Subculturing this callus onto BAP (1-2 mg/l) medium resulted in adventitious shoot formation. Medium containing 10% v/v coconut water gave enhanced callus formation. Leaf pieces cultured on BAP and IAA gave callus, adventitious shoots and roots. The shoots obtained from leaf pieces and terminal axillary buds were successfully rooted in MS medium half strength macro and full strength micro with vitamin and IAA 1 mg/1 as the growth regulator. These plants, acclimatized under greenhouse conditions. The survival rate was around 60%.

During the multiplication phase 15-20 shoots were obtained within a week when terminal buds were used as explants whereas only 10-15 shoots were obtained when axillary buds were used. When leaf pieces were used as explants callus formation was more prominent and the number of shoots per culture was less than 10. These results showed that the terminal buds were the best in establishing the explants *in vitro*.