D-01: Induction of callus from leaves of *Ophiorrhiza mungos* L.

S S Ranaweera, P M D W Sudharshani
(Dept of Botany, University of Sri Jayewardenepura, Nugegoda)

Ophiorrhiza mungos L. (Rubiaceae) contain a potent antitumor compound, camptothecin. High frequency callus induction is a prerequisite for initiation of cell suspension cultures of *O. mungos* to produce camptothecin. This paper reports the results on the induction of callus from leaf explants of *O. mungos*.

Young and mature leaves from field-grown plants and sterile leaves from plants raised *in vitro* were used as explants to induce callus. Explants were surface sterilized in 70% ethanol for 10sec. and in 1%v/v "Chlorox" for 10-15 min. Sterile explants obtained from field were inoculated on MS medium supplemented with 2,4-D (1.25: 2.5: 5.0; 10.0μM) each in combination with kinetin (0.5; 1.0μM). Leaf explants obtained from plants raised *in vitro* were inoculated on medium supplemented with different levels of 2,4-D, IBA and NAA (0.1, 0.5, 1.0, 1.5mg/1), each in combination with 3 levels of kinetin (0.1, 0.3, 0.5mg/1). All cultures were incubated at 26 ± 2°C in the dark. Each treatment was replicated 8 times (10 explants/replicate) and the experiments were repeated twice.

Increase in 2,4-D concentrations from 1.25 to 10.0μM (in combination with 0.5 μM kinetin) reduced the time taken for callus initiation from 35 to 20 days and from 40 to 27 days in young and mature leaf explants respectively. The increase of 2,4-D concentration from 1.25 to 10.0μM (in combination with 1.25 μM kinetin) increased the callusing percentage from 38% to 64% in the young explant. The highest frequency of callus induction (77.7%) was obtained from young explant with 2.5 μM 2,4-D and 0.5 μM kinetin. The callus obtained was greenish, soft and friable.

In the experiments with leaf explants obtained from *in vitro* raised plants, calli were initiated in a shorter time (10 days). High frequency formation (100%) was observed in all auxin treatments, except for the treatment with 2,4-D (0.1mg/1) and kinetin (0.1mg/1) which produced 40%. Greenish yellow, soft friable calli were induced by 2,4-D and IBA and pale yellow harder calli by NAA.

Soft friable calli induced by IBA and 2,4-D could be used to initiate cell suspension cultures of *Ophiorrhiza mungos*.