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Short Communication

Nodal bud culture in *Schleichera oleosa*: Aspectic culture establishment, explant survival and influence of plant growth regulators

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Schleichera oleosa (Lour.) Oken, known as Kusum is a multipurpose tree belonging to the family Sapindaceae. Its kernels yield a good quality of oil, young fruits are pickled and its bark, stems and seeds can also be used in curing cancer, tuberculosis etc. In India, the tree has long been prized for its best quality of lac yielding capacity than any other host. Vegetative propagation of *S. oleosa* remains problematic. Not much success has been obtained in air layering and cutting. Preparation of planting stock in large number is very expensive. Survival rate is also very low. Thus, micropropagation may be of a great help in this situation. No report is available so far on *in vitro* propagation of this species.

Single nodal segments from newly sprouted shoots of mature selected superior tree phenotypes of *S. oleosa* were used as explants and collected in summer (April to May). Explants were washed thoroughly with running tap water for 1 h followed by agitation in 0.1% Bavistin for 15 min and rinsed with distilled water. After washing explants were dipped in 70% (v/v) ethanol for 30 s, sterilized by a dip in a $HgCl_2$ solution (0.1%, w/v) for 3, 5, 7 and 10 min (sterilization termed S_1, S_2, S_3 and S_4) or in a solution of NaOCl (3%, v/v) for 5, 10 and 15 min (sterilization termed S_5, S_6 and S_7) followed by rinsing five times with sterile double distilled water. Different methods were used to control the browning in the present study are as follows:

BC_0: Control; BC_1: Culture in darkness for 24 h after inoculation then under a 16/8 h (light/ dark) photoperiod and sub-culturing in alternate days; BC_2: Use of 0.3% (w/v) activated charcoal (AC) in medium; BC_3: Culture in liquid medium; BC_4: Chilled treatment of explants with 0.2% (w/v) ascorbic acid (AA) for 1 h prior to sterilization with $HgCl_2$; and BC_5: Pretreatment of explants as stated in BC_4 and inclusion of AA (10 mg l^{-1}), citric acid (CA, 10 mg l^{-1}) and polyvinyl pyrrolidone (PVP, 50 mg l^{-1}) in medium.

The MS media [1] in different strengths a) full strength b) half-strength and c) half strength of only nitrate salts, were used. The medium was supplemented with different concentrations of plant growth regulators, *viz*., cytokinins: 6-benzyl-aminopurine (BAP) and kinetin (KN) alone or in combination with auxin naphthalene acetic acid (NAA, 0.1 mg l^{-1}). The medium was also supplemented with antioxidants (as stated in BC_5 method) to check browning. Adenine sulphate and activated charcoal were also used in some experiments for achieving elongation of shoots. The pH of medium was adjusted to 5.7 $\pm$ 0.1 prior to autoclaving. The medium was gelled with 0.8% (w/v) agar. The cultures were kept at 25 $\pm$ 2°C with a 16 h photoperiod. At least 10 cultures were raised in each treatment and all experiments were repeated thrice.

One of the major obstacles for establishment of culture specifically when explants are taken from mature trees is high contamination rates [2-4]. In our experiment, a significant high rate (about 90% after 2 weeks in culture) of visual contamination was scored in sterilization S_1, S_2, S_5, S_6 and S_7 treatments. When the explants were sterilized by 0.1% $HgCl_2$ for 10 min, about

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90% explants were not contaminated but had died may be due to excessive scorching by the chemical. Only the sterilization S_3 i.e., application of 0.1% $HgCl_2$ for 7 min produced non-contaminated alive explants (about 80%). Therefore, this sterilization method (S_3) was consistently used later on. $HgCl_2$ proved to be the best sterilant in several mature forestry tree species also *viz*., chir pine (0.075% for 8-10 min) [5], eucalyptus (0.1% for 20 min) [6], semul (0.1% for 10 min) [7] and sishum (0.1% for 15-20 min) [8]. Another major obstacle for establishment of *in vitro* cultures of several woody plants is the lethal browning of the explant and culture medium. In the present studies, all explants died in culture within a week in control (no browning control measure followed). Five different approaches (BC_1 to BC_5) were tried to overcome the problem and the results are summarized in Figure 1. Browning is generally considered to result from the oxidation of phenolic compounds released from the cut ends of the explants. In contrast to earlier successful reports on control of browning by culturing in darkness, quick subculturing [9] or culturing in liquid medium, these methods failed to control browning in our experiment. Even, when the explants were inoculated in the medium containing AC (method BC_2) significantly higher (more than 80%) explants turned brown. Of the five methods tested, pretreatment of explant with antioxidant as well as inclusion of antioxidants in medium (method BC_5) proved the best method and 77.25% of explants produced non-brown culture. This method effectively kept the cultures alive even after one month. Method BC_4 where only pretreatment of explant was done about 50% of cultures received non-brown as observed after one week of inoculation. However, most of these cultures slowly turned brown within one month. Therefore, for controlling browning the method BC_5 was consistently used later on.

Initiation of shoots was observed in all the concentrations of BAP tried alone or in combination with KN and NAA (Table 1). MS medium supplemented with BAP (5 mg l^{-1}) + KN (5 mg l^{-1}) showed maximum percentage (50.7) of shoot initiation. In most of the cases, shoot initiation completed within two weeks of inoculation. Micro shoots were subcultured on the same medium to obtain proper elongation. The elongated shoots (1.0-1.5 cm) were subsequently separated from the explant and transferred to elongation medium for further growth. The maximum shoot elongation (3.5 cm) was recorded in MS medium supplemented with BAP (5 mg l^{-1}) + KN (5 mg l^{-1}) + $AdSO_4$ (100 mg l^{-1}) + AC (3 g l^{-1}) after one month of subculturing to this medium. Addition of $AdSO_4$ promoting higher shoot length has also been observed in *Bombax ceiba* [7] and *Oroxylum indicum* [10]. Most of the clones of *Salix caprea* responded with rapid shoot development in medium containing 0.1% activated charcoal [4]. In the present study it was observed that the MS medium containing half strength of nitrate salt was favourable for shoot initiation whereas better elongation of shoot was on full strength of MS medium (data not presented). So far we did not succeed to convert these shoots to complete plantlets of *S. oleosa* by inducing roots.

Table 1. Percentage of explants showing initiation of shoots from nodal bud of *Schleichera oleosa* using different culture media after 2 weeks of inoculation

MS medium + plant growth regulator (mg l^{-1})			Shooting (%)
BAP	KN	NAA	
1	-	-	19.5 b*
1	1	-	15.6 ab
1	1	0.1	10.0 a
3	-	-	29.6 c
3	3	-	20.0 b
3	3	0.1	20.5 b
5	-	-	36.4 c
5	5	-	50.7 d
5	5	0.1	12.5 a

*Means followed by different letters are highly significant at $p < 0.05$.

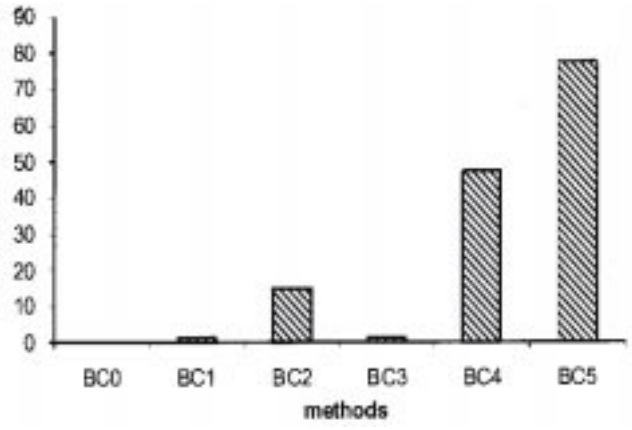

Fig. 1. Percentage of nodal explants of *Schleichera oleosa* producing non-brown culture after one week of inoculation with five methods to control browning

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