

## IN VITRO SHOOT DIFFERENTIATION IN *EMBLICA OFFICINALIS* GAERTN

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Callus cultures from the hypocotyl regions of the seedling explant of *Emblica officinalis* were established on modified MS-medium. Several shoots differentiated from the callus on MS-medium fortified with a combination of cytokinins (BAP and Kinetin: 1.0-5.0 mg/l) and auxins (NAA/2, 4-D: 0.1-1.0 mg/l) and other additives.

**Keywords :** *Emblica officinalis* ; *In vitro*; Shoot differentiation.

The Indian gooseberry-*Emblica officinalis* Gaertn (Vern. Amla, Fam.Euphorbiaceae) is a fruit tree of the hot arid regions. Keeping in view, its economic importances, we have attempted to induce differentiation in its callus cultures.

The seeds of *E.officinalis* were procured from the Department of Forest, Government of Rajasthan, Jaipur. Decoated seeds were surface sterilized with 0.1% mercuric chloride solution for 5-7 minutes. After thorough washings in chilled sterile anti-oxidant solution (Ascorbic acid (AA : 100 mg/l) + citric acid (CA) : 100 mg/l + polyvinyl pyrrolidone (PVP) : 150 mg/l) the seeds were placed on MS-medium incorporated with BAP (1.0mg/l) and other additives like AA (50 mg/l) + PVP (100 mg/l) + activated charcoal (AC) (500 ng/l). Various experiments were carried out for growth and differentiation using combinations of cytokinins 6- Benzylamino purine (BAP) and kinetin : 1.0-5.0 mg/l and auxins Naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy-acetic-acid (2,4-D) : 0.1-2.0 mg/l and other additives like AA,CA,PVP and AC. The pH of the

medium was adjusted to 5.8 before autoclaving. The cultures were incubated at  $28 \pm 2^{\circ}\text{C}$ , 40-50% relative humidity and 16 hour of light per day (fluorescent tubes and incandescent light, 2500 lx to 3000 lx).

The hypocotyl from 15 day old seedlings were excised and placed on MS-medium with 2,4-D (2.0 mg/l) + NAA (0.5 mg/l) and additives like AA (50 mg/l) + CA (50 mg/l) + PVP (100 mg/l) + AC (500 mg/l). Profuse callus formation was seen after 4 weeks. The callus was subsequently sub-cultured on MS-medium enriched with a high concentration of BAP (3.0 mg/l), Kinetin (2.0 mg/l) and 2,4-D (0.1 mg/l) and additives as above. Shoot buds differentiated from the callus after a period of 20-25 days. These shoots elongated on subsequent sub-cultures Fig.1(b,c). Elongated shoots were placed on to the rooting media to get complete plant.

Neither BAP nor kinetin alone, could induce shoot differentiation in the callus. Two cytokinins viz.BAP and Kinetin were effective in this case. The synergistic effect of BA and 2 : P was observed in wite and black spruce<sup>2</sup> generally high auxin

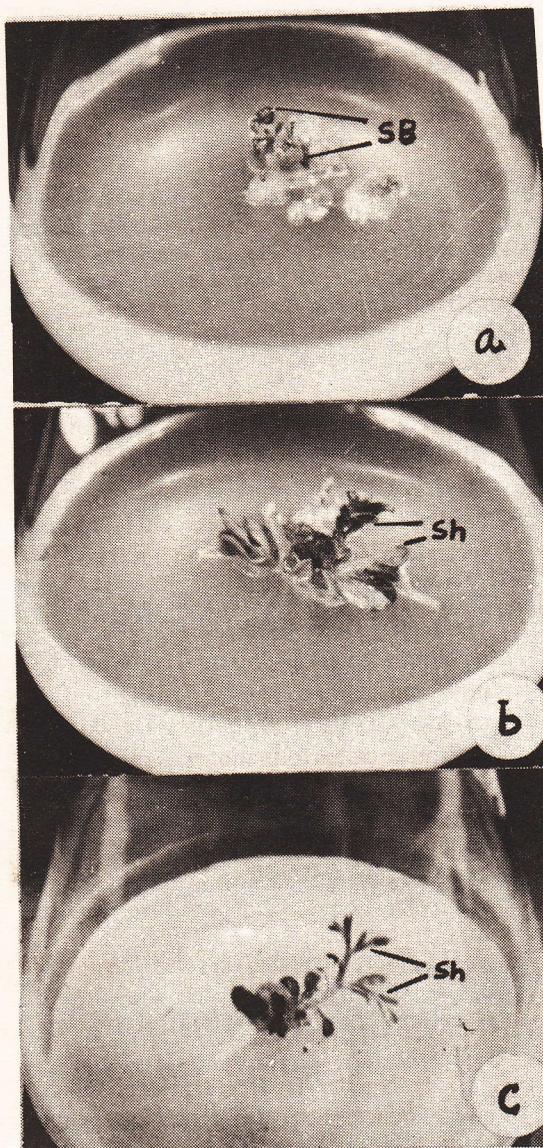


Fig. 1. *In vitro* shoot differentiation in *Emblica officinalis* Gaertn.  
 a : Shoot bud initiation in callus of *E. officinalis*; b : Differentiation of shoot from the callus; c : Shoot elongation.

cytokinin ratio caused cell proliferation and high cytokinin-auxin ratio caused shoot induction on the medium<sup>3,4</sup>. These observations hold true for many of other earlier reports on several plant species like *Acacia* sp., *Albizia lebbek*, *Eucalyptus* sps., *Populus* sps., *Prunus* sps., *Ulmus* sps. and *Citrus* sps.<sup>5-7</sup>. Production of phenolic compounds is a serious problem in tissue cultures of several tree species. Ascorbic acid, activated charcoal and PVP were most commonly used to overcome this problem<sup>8,9</sup>. Similar combination was used to combat the problem of leaching in this taxa.

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