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IN VITRO MICROPROPAGATION OF VARIOUS EXPLANTS OF *SCOPARIA DULCIS* LINN. AND HAEMOGLOBIN ENHANCED SOMATIC EMBRYOGENESIS

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ABSTRACT
India is one of the world's richest sources of medicinal plants comprising nearly 45,000 species including 17,500 flowering plants. Now-a-day people prefer plant based medicines for their primary health care needs because they do not cause side effects. Plant wealth is greatly exploited for its therapeutic potential and medicinal efficacy to cure various human ailments since time immemorial.

INTRODUCTION
India is one of the world's richest sources of medicinal plants comprising nearly 45,000 species including 17,500 flowering plants. Now-a-day people prefer plant based medicines for their primary health care needs because they do not cause side effects. Plant wealth is greatly exploited for its therapeutic potential and medicinal efficacy to cure various human ailments since time immemorial.

Scoparia dulcis L., a medicinal herb, belonging to the family Scophulariaceae is commonly know as Broomweed, Sweetbroom and Vassourinha etc, It is used for the treatment of respiratory problem, fever, external wound, lower blood sugar, blood pressure, urinary tract disease and insect bites. Further, the phytochemicals have anticancerous and antiviral properties. It has been medically used for the treatment of human illness and antitumor activity (Nishino *et al.*, 1993). This plant also reported for the production of several biologically active compounds (Hayashi *et al.*, 1997), Flavonoids (Ramesh *et al.*, 1979) and Triterpenoids (Mahato *et al.*, 1981). Beneficial effects of haemoglobin, observed in several plant species, is due to the trapping of oxygen from air-medium interfaces, facilitating the delivery of this gas to cultured cells (Anthony *et al.*, 1997).

Tissue culture techniques have paved way for rapid multiplication of plants and it is useful where the quality of planting material is required on large scale and in a short period of time. In the present communication, we describe a reproducible protocol for rapid multiplication *of* various explants of *Scoparia dulcis* Linn. and haemoglobin enhanced somatic embryogenesis.

MATERIALS AND METHODS
Scoparia dulcis plants were collected from Anna Medicinal Farm, at Arumbakkam, Chennai, India. After trimming of the larger leaves, the shoot material and flower buds was cut into pieces (2-3 cm). They were washed in running tap water and surface sterilized by immersing in a solution of commercial bleach (4% sodium hypo chlorite) followed by immersion for 2-4 min in a solution of mercuric chloride (0.1%). The explants were finally washed 3-4 times with sterile distilled water and cut into pieces (0.2-2.0 cm long) and aseptically inoculated on Murashige and skoog (1962) medium with 3% sucrose, myo-inositol (100 mg/l) and 0.8% agar (Himedia India). MS solid medium incorporated with various concentration of different auxins such as 3-indol acetic acid (IAA 0.5-2.5 mg/l), 3-indolbutric acid (IBA), α-naphthalene acetic acid (NAA 0.5-2.5 mg/l), 2,4-dichloro phenoxy acetic acid (2,4-D) individually or in combination with cytokinins such as 6-benzylamino purine (BAP 0.5-2.5 mg/l). The pH of the medium was adjusted to 5.8 before adding agar. Culture tubes and conical flasks were sealed using cotton plugs. The medium was sterilized by autoclaving at 120ºC for 20 min. All the culture tubes were maintained at 25 ± 2ºC with culture room provided with 16/8 hr light/dark period [light intensity of 2000 lux, provided by cool white fluorescent tube (Philips India)].

The number of explants producing callus was recorded four weeks after culture initiation. Each explant was repeated five times. Regenerated callus from the above was used for shoot initiation. MS basal medium supplemented with IAA and BAP (0.5-2.5 mg/l each) was used for shoot initiation. The number of shoots produced from the callus was counted after 6-8 weeks culture. Embryogenic leaf calli were transferred to IAA and BAP 1.5 mg/l each with various conc. haemoglobin 100 mg/l to 1000 mg/l after 5-6 weeks results were noted.

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Well developed shoots were transferred to rooting media containing ½ strength MS media with different concentration of naphthalene acetic acid (NAA 0.5-2.5 mg/l) for root induction, in agar- gelled and liquid media with coir. After initiation of roots, rooted plantlets were transferred to plastic cups in a vermiculate and red soil (3:1) mix for two weeks before transferring to the glass house condition.

RESULTS AND DISCUSSION

The leaf, internode and flower buds of explant of *Scoparia dulcis* was tried with MS medium supplemented with auxins and cytokinins for the callus induction. It was observed that 2,4-D and BAP (1.5 mg/l each) induced the highest callus production (88.2%) in leaf explants, internodes (63.6%) and in flower buds (76%) compare to IAA and NAA treatment (Table 1; Fig. a, b & c).

The callus derived from the explants viz. Leaflet, internodes and flower buds were investigated for their multiple shoot formation potential on MS basal salts with BAP alone or combination with auxins (Table.1). Callus, when transferred to MS medium containing BAP, IAA and NAA, the highest percentage of shoot formation (93.3%) was observed with leaf callus in IAA and BAP (1.5 mg/l each), whereas, internodes showed 79.7% of multiple shoots (Fig.1). Although exogenous supply of cytokinin was essential and sufficient to induce multiple shoot formation. Shoots were transferred to the root induction medium containing ½ strength MS medium with NAA (0.5-2.5 mg/l). About 90% of shoots produced well-developed roots within three weeks on half strength MS basal medium with 20% sucrose and NAA (1.0 mg/l).

Further incorporation of haemoglobin at 100 mg/l to 1000 mg/l was tried in all explants. MS medium with 400 mg/l of haemoglobin induced rapid development of somatic embryogenesis in leaf callus (Fig.1 k&l; Fig.2). It clearly indicates the important role played by haemoglobin in micropropagation explants. Beneficial effects of haemoglobin, observed in several plant species, is due to the trapping of oxygen from air-medium interfaces, facilitating the delivery of this gas to cultured cells

The rooted shoots were transferred to plastic cups with vermiculate and red soil (3:1) and 85% of plantlets survived this transfer (Fig. p&q). In conclusion, we have developed an efficient, method for shoot regeneration from callus derived from leaf, inter node and flower buds explants of *S. dulcis*. Development of regeneration protocols for medicinally important species will facilitate access to natural and induced variations.

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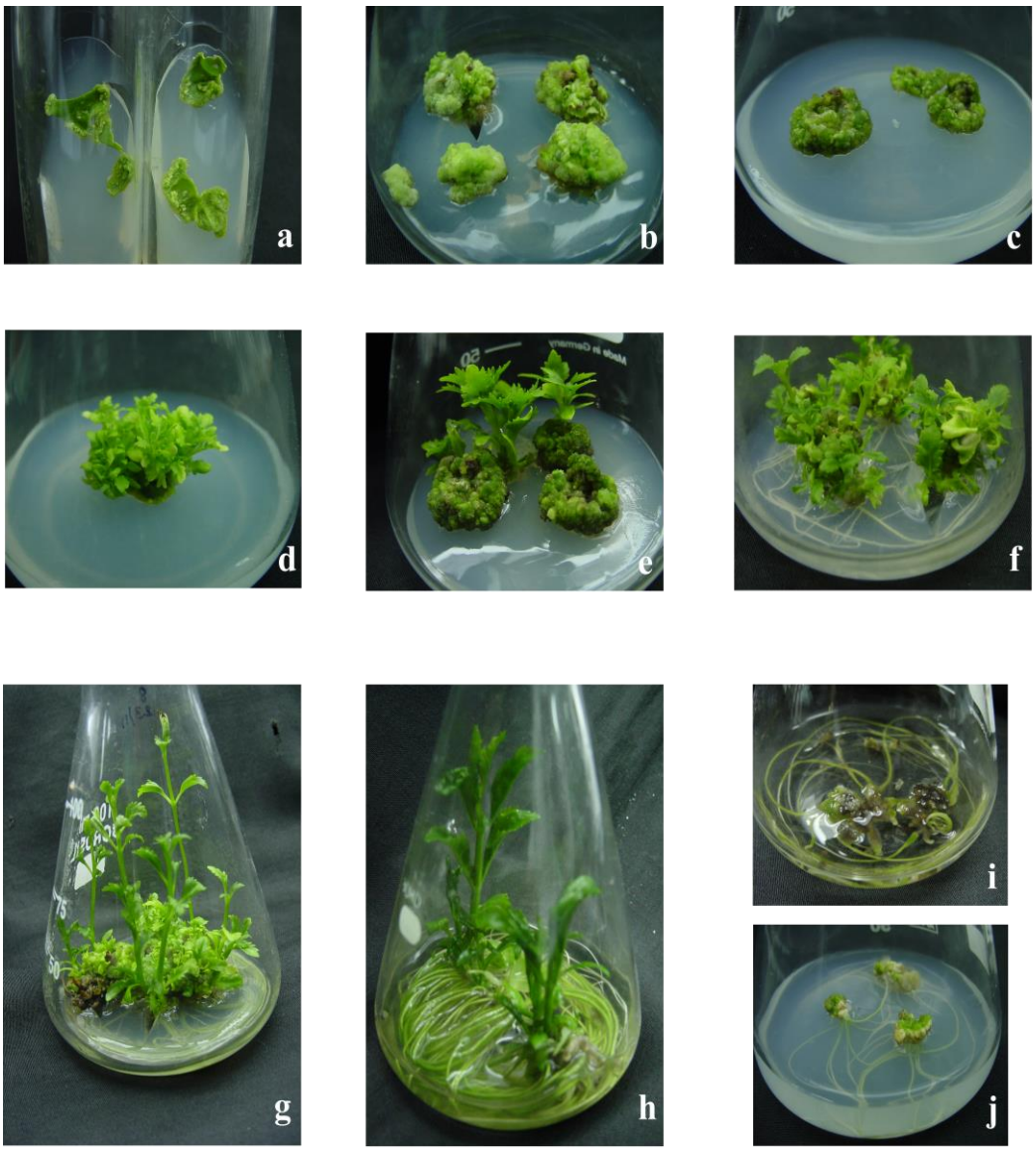

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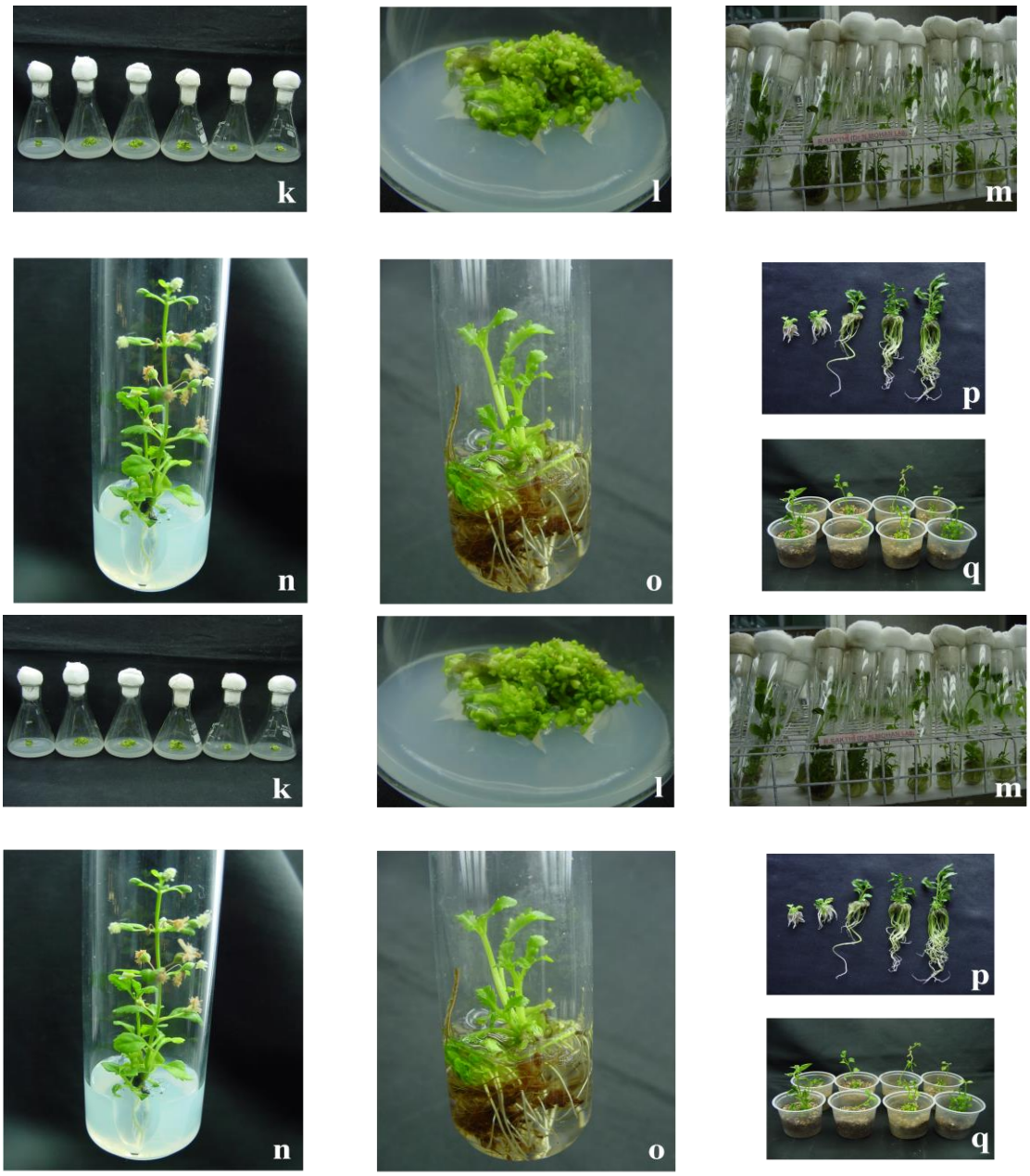

Fig. 1—Micropropagation of *Scoparia dulcis* L. a, Induction of callus from leaf explant; b, Callus from internode explants; c, callus from flower buds explants; d, Multiple shoots formation in leaf callus; e, Shoots formation in stem callus; f, Multiple shoots formation in flower buds callus; g, Shoot elongation; h, Well developed root systems; i, Root culture in liquid media; j, Root culture in semisolid media; k & l, Haemoglobin response somatic embryogenesis; m, well developed plantlets; n, *in vitro* flowering; o, Rooted shoot in liquid media with coir; p & q, Plantlet established in vermiculite and red soil (3:1).

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Table. 1: Effect of different concentration of hormone Supplemented to MS basal medium on callusing, regeneration, multiple shoot formation and rooting in tissue culture of *Scoparia dulcis*

Medium + hormones (mg/l)	Response (%)		
Callus induction	**Leaves**	**internodes**	**flower buds**
MS + 2,4-D (1.0)	80.2	54.6	76.0
MS + 2,4-D + BAP (1.5)	88.2	63.6	71.2
MS + NAA (2.0)	39.6	23.2	27.2
MS + IAA (2.0)	38.2	28.7	32.4
Multiple shoots (from leaf callus only)			
MS + BAP (2.0)	63.0		
MS + BAP + IAA (1.5)	93.3		
MS + BAP + NAA (1.5)	74.2		
Root initiation			
MS + NAA (1.0)	90.0		
MS + IAA (1.0)	62.4		
MS + IBA (1.0)	54.2		

Fig. 2. Effect of haemoglobin concentration on induction of somatic embryos in semi-solid and liquid MS media (IAA + BAP 1.5 mg/l) on 40 d old culture of *Scoparia dulcis*