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# Somatic embryogenesis from sepal explants in *Sapindus trifoliatus*, a plant valuable in herbal soap industry

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ABSTRACT

Somatic embryogenesis and complete plant regeneration were achieved in callus cultures derived from sepal explants of *Sapindus trifoliatus*, an important and valuable tree used in saponin and herbal soap industry. Embryogenic calli were induced on MS medium containing 5.0 mg $l^{-1}$ 2, 4-D and 0.1 mg $l^{-1}$ Kin. Explant orientation markedly influenced on somatic embryogenesis. The calli induced on medium supplemented with 2, 4-D and Kin showed the formation of whitish or greenish nodular embryogenic structure on MS basal medium containing L-glutamine. These nodular embryogenic structures gave rise to somatic embryos or secondary nodular embryogenic structures or both. Nodular embryogenic structures separated from embryogenic calli and transferred to MS medium containing 200 mg $l^{-1}$ L-glutamine produced maximum number of globular, heart and cotyledonary stage somatic embryos. Histological studies have revealed the development of different stages of somatic embryos from nodular embryogenic structure. The embryogenic competence of the culture could be maintained by recurrent production of nodular embryogenic structures and somatic embryos. Somatic embryos were germinated and converted into plantlet on MS basal medium containing 2% sucrose. Plants propagated in vitro from somatic embryos were hardened and successfully established in field condition. The improved and promising regeneration method reported here for the *S. trifoliatus* may be valuable in herbal soap industry.



## 1. Introduction

*Sapindus trifoliatus* Linn., which belongs to the family Sapindaceae and commonly known as soapnut tree, soapberry and Reetha in India, is a medium sized to large, deciduous tree of tropical and sub- tropical regions of Asia. Common names include soapnut and soapberry, both names referring to the use of the crushed seeds to make soap. The fruit contains saponin, a natural detergent which is useful in cleaning clothes and hairs. The plant has very high economic value in saponin industry and become popular as an alternative to laundry detergent and can be opted as an environmental friendly (Anonymous, 1972; Kirtikar and Basu, 2001).

A number of previous studies showed the extraction of saponins from different plant part of *Sapindus* (Kanchanapoom et al., 2001; Grover et al., 2005; Li et al., 2013; Heng et al., 2014). Another bioactive compound Trifolioside II, acyclic sesquiterpene oligoglycoside has also been isolated from pericarps of *S. trifoliatus* along with two known saponins (Kasai et al., 1988). The pericarp of the fruit is well known for its medicinal properties like emetic, tonic, astringent and antihelmintic. Traditionally, it is used in the treatment of asthma, diarrhea, cholera, tubercular glands, colic due to indigestion, and paralysis of limbs and lumbago (Kirtikar and Basu, 2001; Mahar et al., 2013). The fruits of soapnut contain tannic acid, vitamin C and resins that react with hair to stop hair loss, prevent dandruff, scalp and hair follicle damage. The hard, black shell of the seed yields a fair amount of a dye, suitable for dyeing cotton, wool, silk and leather and also used in colouring shoe-creams and polishes and for staining wood (Anonymous, 1972).

Over exploitation due to increased demand in soap industries, slow growth, and poor seed viability restrict its natural regeneration and multiplication (Anonymous, 1972; Asthana et al., 2011). Therefore, there is a need to develop an efficient in vitro regeneration method for fast multiplication of this important tree. During the past years, an in vitro plant regeneration in *S. trifolia-*

*Abbreviations:* BAP, 6-benzylaminopurine; 2, 4-D, 2, 4 dichlorophenoxyacetic acid; Kin, kinetin; IAA, indole-3-acetic acid; MS, Murashige and Skoog (1962); NAA, naphthalene acetic acid; PFD, photon flux density; PGRs, plant growth regulators.

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*tus* through organogenesis has been developed by our group using nodal explants obtained from seedlings (Asthana et al., 2011). However, number of shoots regenerated by organogenesis was very low (six to seven shoots per explant). Hence, another approach i.e. somatic embryogenesis has been attempted for large-scale propagation of this important tree. Owing to high multiplication rates and potential for scale-up via bioreactor, many researchers emphasized somatic embryogenesis as preferred method for multiplication and genetic improvement (Rai and Shekhawat, 2014). Somatic embryogenesis from leaf of *S. trifoliatus* has been reported earlier by Desai et al. (1986). However, most of somatic embryos developed by this protocol were not normal and conversion of somatic embryos into plantlets was not satisfactory. It is well known that selection of appropriate explant is an important factor for the successful establishment of culture and frequency of plant regeneration in many cases. Floral parts like sepals and petals or whole flowers have also been used as explants for shoot regeneration and somatic embryogenesis in some plant species (Torné et al., 1997; Debnath, 2005; Gambino et al., 2007; Mohammadi-Dehcheshmeh et al., 2008; Palmer and Keller, 2011). In the present communication, we report first time somatic embryogenesis in *S. trifoliatus* using sepal of flower buds as explants obtained from mature tree. Efforts have also been made to standardize optimum factors affecting development, maturation and germination of somatic embryos.

## 2. Material and methods

### 2.1. Collection of plant material, preparation of explants and surface sterilization

The whole infloresence bearing bunches of unopened or partially opened flower buds were collected from a 10–15 year old tree of *S. trifoliatus* growing in Ayurvedic garden, Banaras Hindu University, Varanasi, during November-January. Flower buds were separated from the inflorescence and washed thoroughly under running tap water in a conical flask for 5–10 min and then agitated in a 500 ml autoclaved Erlenmeyer flask containing a solution of 2% (v/v) cetrimide, 2–3 drops Tween-20® and 1.0% (v/v) of sodium hypochlorite for 10–15 min followed by a second washing under running tap water to remove the detergent and surfactant. Subsequently, plant material were transferred to laminar air flow hood and agitated with 70% ethanol for 30 s followed by gentle shaking in 0.05% (w/v) mercuric chloride solution for 3–5 min and finally rinsed 3–4 times with autoclaved double distilled water. The sepals excised from flower buds aseptically were used as explant for somatic embryogenesis in *S. trifoliatus*.

### 2.2. Induction and proliferation of callus and formation of nodular embryogenic structure from callus

To evaluate the effect of different plant growth regulators and explant orientation (abaxial or adaxial surface in contact with the medium) on callus induction and formation of nodular embryogenic structure from callus, sepals were excised from the surface sterilized flower buds and cultured either in adaxial or in abaxial orientation on callus initiation medium i.e. 0.8% (w/v) agar solidified full strength MS (Murashige and Skoog, 1962) medium containing 3% (w/v) sucrose and supplemented with different concentrations (0.1, 0.5, 1.0, 3.0, 5.0 or 7.0 mg $l^{-1}$) of auxins (2, 4-D, NAA or IAA) or cytokinins (BAP or Kin) either alone or combination of 2, 4-D (5.0 mg $l^{-1}$) with Kin or BAP (0, 0.01, 0.1 or 0.5 mg $l^{-1}$). Explants inoculated on the medium devoid of any plant growth regulator served as the control. The pH of the medium was adjusted to 5.80 ± 0.02 using 1N NaOH and 0.1N HCl and autoclaved for 15 min at 121 °C temperature. Cultures were incubated at 25 ± 2 °C under a 16 h photoperiod with light intensity of 50–70 μmol $m^{-2}$ $s^{-1}$ PFD provided by cool and white fluorescent tubes (Philips, India).

To study the effect of sucrose and L-glutamine on the formation of nodular embryogenic structure, the calli initiated from sepals inoculated with their abaxial side on optimized medium (MS medium with 5.0 mg $l^{-1}$ 2, 4-D and 0.1 mg $l^{-1}$ Kin) were transferred to full strength MS basal medium containing different concentrations of sucrose (1, 2, 3 or 4%) or L-glutamine (50, 100, 200, 300 or 400 mg $l^{-1}$). In case of L-glutamine experiments, MS basal medium supplemented with 3% sucrose was served as control.

### 2.3. Differentiation of somatic embryos from nodular embryogenic structure

Primary nodular embryogenic structure showed differentiation of either secondary nodular embryogenic structure or different stages of somatic embryos or both on MS basal medium. To study the effect of sucrose concentration on differentiation of somatic embryos, about two week old nodular embryogenic structure were kept for differentiation on full strength MS basal medium supplemented with different concentrations of sucrose (1, 2, 3 or 4%). To study the effect of L-glutamine on differentiation of somatic embryos, two week old nodular embryogenic structures were cultured on full strength MS basal medium supplemented L-glutamine (50, 100, 200, 300 or 400 mg $l^{-1}$). Nodular embryogenic structure cultured on MS medium with 3% sucrose and devoid of L-glutamine was served as the control. For this experiment, five nodular embryogenic structures were inoculated in each test tube and at least 24 test tubes were taken for each treatment. All the experiments were conducted thrice. Data for average number of somatic embryos per nodular embryogenic structure was recorded after eight weeks of culture initiation. The frequency of embryogenesis was calculated as the percentage of explants showing at least one globular somatic embryo out of total number of explants inoculated.

### 2.4. Maturation of somatic embryos

For maturation of somatic embryos, one week old early cotyledonary stage, watery and translucent somatic embryos were selected and transferred to full strength MS medium containing L-glutamine (50, 100, 200, 300 or 400 mg $l^{-1}$). Somatic embryos cultured on MS medium devoid of L-glutamine served as the control. For this experiment, one somatic embryo was cultured in each test tube and at least 24 test tubes were taken for each treatment. Cotyledonary stage somatic embryo with thick and enlarged cotyledons was considered as mature somatic embryo.

### 2.5. Germination of somatic embryos and their conversion to plants

For germination of somatic embryos and their conversion into plants, the mature green cotyledonary stage somatic embryos with well developed cotyledons that separated from mother tissue were transferred to 0.8% agar solidified full-strength MS medium containing different concentrations of sucrose (1, 2, 3 or 4%). For this, one mature cotyledonary stage somatic embryo was cultured in each test tube and at least 24 test tubes were taken for each treatment. Germination frequency (percent germination) for a particular treatment was calculated as the percent of mature somatic embryos showing emergence of both root and shoot out of the total mature somatic embryos inoculated.