

# Study of somatic embryogenesis in leaf explants of *Sapindus emarginatus* vahl

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**Abstract:** Plant regeneration through Somatic embryogenesis has several advantages over other routes to *in vitro* plant production and appears that most promising area of research for large scale production and rapid plant propagation. *Sapindus emarginatus* vahl commonly known as soap nut tree wherein the nut shell contains saponin, which acts like soap when comes in contact with water. It has very high economic and medicinal values in saponin industry and is also being used for important medicinal properties. The present study is aimed on somatic embryogenesis from leaf explants of *Sapindus emarginatus* Vahl employing auxin such as 2, 4-D along with cytokinin BAP/Kn. The present protocol is the first ever reported of its kind and is unique in itself as the latex poses a threat for tissue culture.

**Keywords:** Leaf Explants, *Sapindus Emarginatus*, Somatic Embryogenesis

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## 1. Introduction

Somatic embryos are believed to originate from a single cell and therefore plants derived from these tend to be genetically identical (Tomer and Gupta 1986). Plant regeneration through somatic embryogenesis has several advantages over other routes to *in vitro* plant production and appears the most promising area of research for large scale production and rapid plant propagation. The first report of somatic embryogenesis in carrot tissue cultures *in vitro* was published by Steward *et al.* (1958) and Reinert (1958; 1959). Somatic embryogenesis can be induced directly from a variety of explants or obtained indirectly by manipulating *in vitro* non-embryogenic callus. The totipotency of cells finds best expression in the formation of somatic embryos from single cells and their growth and development to form a complete plantlet (Attree and Fowke 1993; Finer 1994).

*Sapindus emarginatus* Vahl is commonly known as soap nut tree. It has very high economic and medicinal values in saponin industry and is also being used for important medicinal properties. It forms a soft, smooth layer on the skin which protects against infections and insects. Soap nut is a natural exfoliant. It is considered to be second to none and is also very common in the Indian Ayurvedic healing system. The saponin moiety is characterized as hederagenin

group of glycosides. The root bark is also saponaceous and is also used a detergent, in the same way as the fruits. In fact, the skin of the fruit is highly valued by the rural folks as a naturally produced shampoo used for washing the hair, because of which the village women call soapnut as “*Skin Beautifier and Freckle Remover*”.

As soap nut fruit is so rich in iron, it is considered as a hemolytic and is often used to treat anemia, cure chlorosis and epilepsy. It is also used as an expectorant for severe lung congestion and also promote blood circulation in patients with low blood pressure. *Ritha* or Soap nut is a sedative to the uterus and is used to ease childbirth. It can also be prepared as a digestive aid, anti-venom for snake bite, or to treat diarrhea, cholera and paralysis.

## 2. Materials and Methods

### 2.1. Plant Material

Young plants of *Sapindus emarginatus* Vahl were collected and grown under partly shade conditions in the experimental garden of the Aromatic and Medicinal Plants, Department of Botany, Kakatiya University, Warangal. The leaf explants were collected from one year old healthy plants. The leaf explants were washed in a mild non phyto-toxic liquid detergent (2% Labolene) stirred for about 3

min and then washed in tap water. It was followed by a dip in a 0.1% (w/v) mercuric chloride (HgCl<sub>2</sub>) solution for 2 minutes. Finally the leaf explants were washed thoroughly with sterile water before the inoculation onto the sterilized nutrient agar media prepared in culture tubes. All the above operations were performed under aseptic conditions in a laminar air flow cabinet.

## 2.2. Culture Media and Culture Conditions

Leaf explants of different sizes (0.5-10 mm) were cultured with the abaxial surface in contact with induction medium consisting of MS salts, vitamins, 6% sucrose (0.5–3.0 mg/L), BAP/Kn in combination with 2,4-D (0.5-1.0mg/L) maintaining a pH of 5.7- 5.8. The percentage of responding explants was evaluated after 4 weeks of culture. Responses scored were the percentage of explants containing the embryos in the globular stage. All the cultures were incubated under 16/8 h. light/ dark photoperiod at 25 ± 2°C a light intensity of 40 μ mol m<sup>-2</sup> s<sup>-1</sup> and provided with cool-white florescent tubes. The cultures were transferred to fresh medium after an interval of 4 weeks. For germination and plantlet formation somatic embryos were transferred to MS medium supplemented with IAA (0.5-1.0 mg/L) + BAP (0.5–3.0 mg/L) and incubated under the same culture conditions.

## 3. Results

Results on somatic embryogenesis in *Sapindus emarginatus* Vahl are presented in **Tables 1- 2** and the review of somatic embryogenesis in medicinal plants is presented in **Table-1**. Leaf cultured on various concentrations of 2, 4-D (0.5-1.0 mg/L) in combination with BAP/ Kn (0.5-3.0 mg/L) produced swollen and generally de differentiated and developed friable callus after 15- 20 days of culture. Within 25-30 days of culture, globular embryos had formed directly on the surface or primary embryos were cut in to fragments and cultured on the same induction medium and secondary somatic embryos were induced within three weeks. Somatic embryos appeared in this medium on the surface of a callus, 25-30 days after the culture initiation (**Plate I**), a period that was shorter than that observed for induction from zygotic embryo (1.0 mg/L) from explants. The best results were obtained when leaf explants were initially cultured with 2,4-D and Kn (2.0mg/L), with an induction frequency of 83%, a higher value than those obtained from zygotic embryos, which could be expected in a more differentiated tissue, such as that of leaves.

**Table 1.** Effect of Various concentrations of 2,4-D and BAP on Somatic embryogenesis in Leaf explants of *Sapindus emarginatus* Vahl.

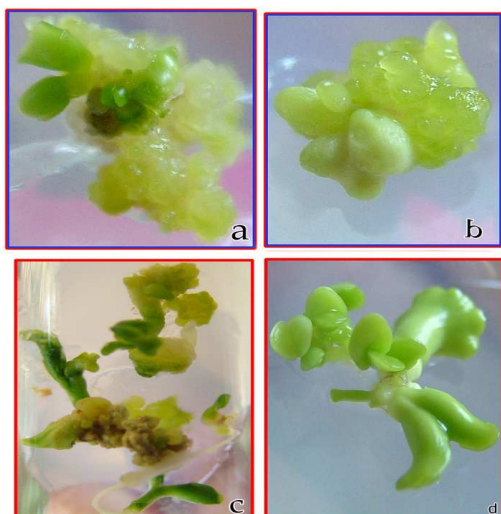
Hormone concentration (mg/L)	% of cultures responding	Average number of somatic embryos/explants ±(S.E.)*
<b>2,4-D+BAP</b>		
0.5+0.5	40	5.3 ± 0.35
0.5+1.0	50	7.3 ± 0.045
0.5+1.5	60	8.3 ± 0.37
0.5+2.0	70	12.3 ± 0.27
0.5+2.5	55	10.2 ± 0.37
0.5+3.0	40	7.5 ± 0.34
1.0+0.5	50	5.0 ± 0.37
1.0+1.0	65	7.0 ± 0.34
1.0+1.5	75	8.5 ± 0.34
1.0+2.0	80	13.5 ± 0.32
1.0+2.5	72	10.3 ± 0.33
1.0+3.0	62	8.5 ± 0.75

\* Mean ± Standard Error

**Table 2.** Effect of Various concentration of 2,4-D and Kn on Somatic embryogenesis in Leaf explants of *Sapindus emarginatus* Vahl.

Hormone concentration (mg/L)	% of cultures responding	Average number of somatic embryos/explants ±(S.E.)*
<b>2,4-D+Kn</b>		
0.5+0.5	45	6.3 ± 0.36
0.5+1.0	53	8.0 ± 0.45
0.5+1.5	65	10.0 ± 0.36
0.5+2.0	73	13.0 ± 0.37
0.5+2.5	58	12.0 ± 0.36
0.5+3.0	42	7.8 ± 0.32
1.0+0.5	54	6.2 ± 0.38
1.0+1.0	68	8.5 ± 0.34
1.0+1.5	76	9.5 ± 0.34
1.0+2.0	83	15.0 ± 0.34
1.0+2.5	73	12.0 ± 0.34
1.0+3.0	65	10.3 ± 0.65

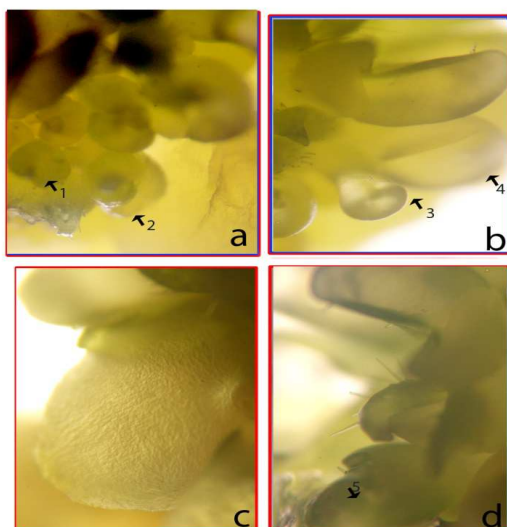
\* Mean ± Standard Error

**PLATE - I**

**Plate -I.** a = somatic embryos (Globular and cotyledonary) formation; b= different stages of somatic embryo formation; c=germination of somatic embryos into planlets; d = germination of somatic embryos into planlets (mature).

### 3.1. Effect of 2,4-D +BAP

Leaf explants were cultured on MS medium containing 30 g/L sucrose amended with various concentration of 2,4-D (1.0 mg/L) in combination with BAP (0.5-3.0 mg/L). Somatic embryogenesis was induced in all combinations and concentrations tested but exhibited varied results (**Table -1**). High percentage of somatic embryogenesis (80%) was observed at 2, 4-D (1.0mg/L) + BAP (2.0mg/L) which produced  $13.5 \pm 0.32$  embryos/explants (**Plate II and III**). As concentration of BAP increased the somatic embryo induction percentage was reduced.

**PLATE - II**

**Plate -II.** a, b, c, d =Stereomicroscopic images of different stages of somatic embryos.

**PLATE - III**

**Plate -III.** a = Proliferation of rooting on MS + 0.5 mg/L IBA; b = Hardened regeneration after 4 weeks; c = hardened regeneration after 6 weeks.

Leaf explants when cultured on MS medium containing 30g/L sucrose amended with various concentration of 2,4-D (0.5mg/L) in combination with BAP (0.5-3.0 mg/L) showed maximum percentage of somatic embryogenesis around 70% at 2,4-D (0.5mg/L) + BAP (2.0mg/L) which produced  $(12.3 \pm 0.27)$  embryos/explants. At 2, 4-D (0.5 mg/L) + BAP (0.5-3.0 mg/L) lesser response was observed than 2,4-D (0.5 mg/L) + BAP (0.5-3.0 mg/L).

### 3.2. Effect of 2, 4-D +Kn

Leaf explants were cultured on MS medium containing (30g/L) sucrose amended with various concentration of BAP (0.5-3.0 mg/L) in combination with 2, 4-D (0.5mg/L). Somatic embryogenesis was induced in all combinations and concentrations tested but varied results (0.5mg/L) (**Table-2**). High percentage of somatic embryogenesis (73%) was observed at 2, 4-D + Kn (2.0mg/L) which produced  $13.0 \pm 0.37$  embryos/explants (**Plate II**). It was further observed that as concentration of Kn increased the somatic embryo induction percentage was reduced.

Leaf explants when cultured on MS medium containing (30g/L) sucrose amended with various concentration of Kn (1.0-3.0mg/L) in combination with Kn (1.0 mg/L) showed maximum percentage of somatic embryogenesis around 83% at 2,4-D (1.0mg/L) + Kn (2.0mg/L) which produced  $15.0 \pm 0.34$  embryos/explants. At concentrations of 2,4-D (0.5 mg/L)+ Kn (0.5-3.0 mg/L) lesser response was observed than the 2,4-D (1.0 mg/L) + Kn (0.5-3.0 mg/L).

Further, the somatic embryos with various developmental stages (heart and Globular) were further sub cultured on fresh MS medium containing various concentration of BAP (0.5- 1.0 mg/L) in combination with IAA (0.5-3.0mg/L) for germination of somatic embryos induced from leaf explants. Of these, media tested MS + IAA (1.0 mg/L) + BAP

(2.0 mg/L) proved to be the best for somatic embryo germination and plantlet formation after 4 weeks of culture (Table 3).

**Table 3.** Effect of IAA in combination with various concentration of BAP on the conversion of Somatic embryoids into Plantlets in *Sapindus emarginatus* Vahl.

Hormone concentration (mg/L)	% of cultures responding	Frequency of Somatic embryos (S.E)*
<b>IAA+BAP</b>		
0.5+0.5	60	07.3 ± 0.36
0.5+1.0	63	09.0 ± 0.45
0.5+1.5	68	12.0 ± 0.36
0.5+2.0	75	14.0 ± 0.37
0.5+2.5	70	10.0 ± 0.36
0.5+3.0	68	08.8 ± 0.32
1.0+0.5	62	09.2 ± 0.38
1.0+1.0	65	10.5 ± 0.34
1.0+1.5	70	12.5 ± 0.34
1.0+2.0	78	15.0 ± 0.34
1.0+2.5	63	11.0 ± 0.34
1.0+3.0	50	10.3 ± 0.65

\* Mean ± Standard Error

## 4. Discussion

In the present investigation, the results on somatic embryogenesis have shown that auxin such as 2,4-D along with cytokinin BAP/Kn are essential for induction of somatic embryogenesis from cotyledon explants of *Sapindus emarginatus* Vahl, where in the growth regulators play a primary role in the medium for induction of somatic embryogenesis in nature. The type of auxin or auxin in combination with cytokinin used in the medium can greatly influence somatic embryo frequency. Proliferated embryogenetic suspension cultures were established in *Sapindus emarginatus* Vahl. This regeneration system may be widely applicable. However the magnitude of several tissues (induction, growth, embryo yield, germination and conversion) essential for efficient plant recovery was different among the *Sapindus emarginatus* Vahl.

Various studies have documented genotype effects on induction of somatic embryo genesis from immature cotyledon explants of soybean using a variety of protocols (Parrott *et al.*, 1989; Shoemaker *et al.*, 1991.) The development stage of cotyledon is known to be critical for induction of somatic embryogenesis (Lazzeri *et al.* 1985; Ranch *et al.* 1985) and hence explants were prepared from immature seeds of 3-5 mm in length. However, selection of explants from uniform seeds many not have ensured uniform deve-

lopmental status among genotypes differing in mature seed size. These genotypic differences for induction capacity might be altered by selection of explants based on criteria other than equal seed length. The selected immature embryos for culture were one half of the length of mature seeds.

Reddy and Reddy (1993) have reported the improved response of auxin like 2, 4-D alone for induction of somatic embryogenesis compared to combination of 2, 4-D and cytokines in *Arachis hypogea*. Somatic embryogenesis is generally believed to be triggered by an auxin and for many plants, 2, 4-D has been widely regarded to be effective for somatic embryogenesis (Ammirato 1983; Finer, 1988). Similarly Binzal *et al.* (1996) reported that the entire process of induction and maturation of the embryos was completed on the same MS medium containing auxin and cytokinins (2, 4-D + TDZ) in *Capsicum annuum* as it was observed the requirement of both the hormones in the investigations. Further, somatic embryo maturation on MS medium containing the combination of auxins (2,4-D) and cytokinins (BAP) was observed in *Cajanus cajan* (Mallikarjuna *et al.* 1996).

### 4.1. Embryo Maturation and Germination

In the present investigation leaf explants showed maximum percentage of somatic embryogenesis and high frequency of somatic embryo induction / explant (15.0 ± 0.34). The calli developed from leaf explants containing globular embryos were transferred to maturation medium containing MS medium supplemented with 2,4-D (0.5 - 1.0 mg/L) + BAP/Kn 2.0 mg/L respectively.

Auxin and auxin like substitutes are mainly used for the *in vitro* induction of somatic embryogenesis in various crops (Litz and Gray 1995). Although Picloram, Dicamba, NAA, 2, 4, 5-T are being used to induce embryogenesis, 2, 4-D is the most commonly used auxin exploited for various crops (Fitch 1990). Cytokinin induced somatic embryogenesis is rare but has also been achieved (Maheshwaran and Williams 1986; Gill and Saxena 1992). Recently, TDZ has also been found to induce somatic embryogenesis (Murty *et al.* 1998). Thus it can be emphatically stated that remarkable progress has been made in the commercialization of somatic embryogenesis since its discovery in carrot by Steward *et al.* (1958). There are also a few recent reports on induction of somatic embryogenesis in presence of cytokinins like Thidiazuron (Saxena *et al.* 1997) and BAP (Malik and Saxena 1992).

## 5. Acclimatization

The embryos turned green with folded cotyledons, which subsequently developed into whole plantlets. Only fully matured embryos when transferred to MS basal medium without growth regulators, produced good shoot and root systems with 20% frequency, but not the heart-shaped ones. This implies that embryos need to mature enough for ger-

mination on basal medium. Complete plantlets were developed on MS basal medium containing BAP (2.0 mg /L) in combination with IAA (1.0mg/L) within three weeks from somatic embryos. Plantlets regenerated via somatic embryos were transferred to half strength MS liquid medium without hormones for two weeks for hardening. A total of 300 regenerants were transferred to polycups containing a mixture of soil and sand in a ratio of 3: 1 with 75% survival rate. A total of 200 regenerated plants were transferred to the pots from the polycups with 80% survival along with seed raised controls. Tissue culture-regenerated plants produced normal flowers and set seeds while no morphological variations were observed.

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