

## *In vitro* Regeneration of Multipurpose Medicinal Tree *Stereospermum Suaveolens* – Factors Controlling the *In Vitro* Regeneration

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### Abstract

*Stereospermum suaveolens* (DC.) is a multipurpose tree endowed with medicinal properties and good timber wood quality. It has analgesic and wound healing property. It is one of the important tree species used in the preparation of *ayurvedic* formulation known as Dashmula, in which roots of Dashmula tree species are used in preparation of *ayurvedic* formulations. Wood is employed for construction work, planks and beam, carts, carriages and wagons, furniture, cabinet work and tool handles. The destruction of this plant species is severe and planting of seedlings is almost negligible. Due to low germination and unsuccessful vegetative propagation, *in vitro* regeneration of *Stereospermum suaveolens* through micropropagation was studied as a tool for large scale propagation. In the present study, shoots were initiated from the nodal segments of *Stereospermum suaveolens* further shoot proliferation was achieved on MS medium supplemented with different concentrations of cytokinins and additives. 100% root initiation was achieved in the MS medium supplemented with IBA. Different substrates were evaluated during hardening phase. The plants of *S. suaveolens* grown on coco peat showed 84.6 percent survival and those on soil mixture showed 76.47 percent survival.

**Keywords:** *Stereospermum suaveolens*; *In vitro*; Dashmula; Additives; Hardening

### Introduction

*Stereospermum suaveolens* (DC.) are among those species, which are endowed with medicinal properties and good timber wood quality. *Stereospermum suaveolens* belongs to family Bignoniaceae. The vernacular name of *Stereospermum suaveolens* are paral/padaria (Hindi), parlu (Bengali), padal (Marathi), padri (Tamil) and godadalipulusu (Telugu). It is one of the important tree species used in the preparation of *ayurvedic* formulation known as Dashmula. In Dashmula tree species, the roots are used in preparation of *ayurvedic* formulations. Every part of this tree has medicinal properties such as decoction of roots is used for fever, inflammatory chest affections, stem bark is used as diuretic and flowers are given with honey to stop hiccup. Other than medicinal properties this tree also has good timber wood quality. Wood of *Stereospermum suaveolens* is employed for construction work, planks and beam, carts, carriages and wagons, furniture, cabinet work and tool handles [1]. It is excellent firewood and makes good quality charcoal. Leaves are lopped for fodder [2].

Exploitation of medicinal plants for commercial use coupled with the destruction of underground parts, slow reproduction, slow growing and habitat-specific nature, are the crucial factors in meeting the goal of sustainability [3,4]. In Dashmula species, the destruction is severe due to total uprooting of plants since the roots are the source of active components. The destruction of these plants is severe and planting of seedlings is almost negligible. The application of *in vitro* culture for propagation of Dashmula tree species will help in sustainable availability of propagules and plant products [5]. The tissue culture of forest trees has shown promise in obtaining regenerants and clonal multiplication for domestication of wild populations, afforestation and economically important trees that have been cultivated for generations. Considering the importance of this medicinal tree for medicinal uses, pharmaceuticals and industries, it can be exploited at the commercial level. The medicinal value indicates that the use of various parts (leaves, roots, bark, etc.) of this tree species as plant extracts may be helpful in overcoming the disorders/disease predominant in the many rural areas of the country [6].

Furthermore, rising demand with shrinking habitats may lead to the local extinction of many medicinal plant species. Hence keeping the importance of this tree in view, it was selected for present investigation. The present study is focused on the effect of additives on *in vitro* shoot proliferation of *Stereospermum suaveolens*.

### Materials and Methods

Nodal segments from field grown seedlings and terminal twigs of one and two-year-old tree were used as explants. The nodes were cut from healthy seedlings, surface sterilized with 0.2% (w/v) aqueous mercuric chloride for 5 minutes and washed 3-4 times with double distill water. The sterilized nodes were inoculated on different plant tissue culture medium such as MS [7], WPM [8] and SH [9]. The best result was observed in MS medium and hence the experiment was carry forwarded with different concentrations of plant growth regulators and additives. Nodes of developed shoots were placed on MS medium supplemented with different concentrations of BAP and Kinetin to study the effect of plant growth regulators on shoot proliferation. The effect of different concentration of PVP, citric acid, casein hydrolysate, adenine sulphate and activated charcoal in MS medium supplemented with 0.44  $\mu$ M on *in vitro* shoot proliferation was investigated by inoculating the *in vitro* nodes grown in standardized medium at 25°C under 16 h light and 8 h dark regime.

### Result and Discussion

Three types of basal media with 4.44  $\mu$ M BAP induced shoot

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elongation from cotyledonary nodes of seedlings. The MS, WPM and SH media induced bud break in 80%, 40% and 20% nodes, respectively. The nutritional requirements vary from species to species and the type of explant taken; this can be explained as has been reported *in vitro* shoot organogenesis from excised immature cotyledons of Stone Pine [10]. They took different concentrations of BA (0, 5, 25 or 50  $\mu\text{M}$ ) to different culture media (MS, SH or GD) and obtained highest positive response in SH and GD medium supplemented with 5  $\mu\text{M}$  BA. When the nodes of *Stereospermum suaveolens* placed on MS medium supplemented with 4.44  $\mu\text{M}$  BAP showed maximum number of shoots and nodes. Thus, MS medium supplemented with 4.44  $\mu\text{M}$  BAP was found to be the most suitable medium for establishment of nodal explants of *S. suaveolens*. In earlier studies also MS medium was found suitable for *in vitro* establishment of explants from hardwood trees such as *Terminalia arjuna* Roxb [11], *Tectona grandis* [12], *Gmelina arborea* [13], woody climber *Bauhinia vahlii* [14] and *Stereospermum personatum* [15]. Shoot bud elongation occurred when nodes were placed on MS medium supplemented with 0.0, 0.04, 0.44 or 4.44  $\mu\text{M}$  BAP [16]. The nodes *S. suaveolens* were placed on MS medium supplemented with 0.44  $\mu\text{M}$  BAP showed maximum number of shoots (Figure 1). Shoot bud elongation occurred in the nodes placed on MS medium supplemented with kinetin (0.0, 0.04, 0.46 or 4.65  $\mu\text{M}$ ). The nodes placed on MS medium supplemented with 0.46  $\mu\text{M}$  kinetin showed maximum number of shoots per node  $1.13 \pm 0.09$ , mean shoot length (mm)  $21.8 \pm 0.42$  and number of nodes per shoot  $3.0 \pm 0.16$ . Hence BAP found to be more suitable as compared to Kinetin. The cytokinin concentrations play the most important factor affecting shoot multiplication. Multiple shoots were obtained from cotyledonary nodes derived from one week old axenic seedlings of *Dalbergia sisoo* on MS medium containing either BA, kinetin, 2-iP or TDZ while BA was the most effective growth regulator [17]. Multiple shoots of *Quercus leucotrichopora* and *Q. glauca* were obtained from the intact embryos as well as from the cotyledonary node of three week old *in vitro* grown seedlings on WP and MS media supplemented with BA either alone or in combination with GA3 / IBA [18]. They found that BA (22.19  $\mu\text{M}$ ) was effective for induction of multiple shoots and addition of GA3 to the medium further enhanced number and shoot height but resulted in shoot thinness. They achieved high frequency shoot multiplication using nodal explants.

The tissue culture medium turns black or brown due to the release of polyphenolic compounds from the explants. This problem is more severe in woody plants. Therefore various additives with different concentrations were added in the established medium to check there



Figure 1: Established culture of nodes of *S. suaveolens* on MS medium supplemented with 0.44  $\mu\text{M}$  BAP.



Figure 2: *In vitro* shoot proliferation of *S. suaveolens* on MS Supplemented with 0.44  $\mu\text{M}$ +25 mg/l AS.

| MS Medium +0.44 $\mu\text{M}$ BAP + AS (mg l <sup>-1</sup> ) | Shoots(node <sup>-1</sup> ) | Length (mm) (shoot <sup>-1</sup> ) | Nodes(shoot <sup>-1</sup> ) |
|--|-----------------------------|------------------------------------|-----------------------------|
| 0.0  | $1.0 \pm 0.0^{*b}$          | $12.0 \pm 0.65^d$                  | $2.2 \pm 0.11^b$            |
| 25.0   | $2.6 \pm 0.21^a$            | $28.4 \pm 0.39^a$                  | $4.1 \pm 0.21^a$            |
| 50.0   | $1.13 \pm 0.09^b$           | $22.4 \pm 0.55^b$                  | $2.6 \pm 0.13^b$            |
| 100.0  | $1.0 \pm 0.0^b$             | $15.0 \pm 0.84^c$                  | $2.2 \pm 0.11^b$            |

\*Mean  $\pm$  1 SEM; Each experiment consisted of five replicates and repeated three times. The means separated using DMR (Costat; Version 4.02). Similar alphabets within a column do not differ significantly at  $P \leq 0.05$ .

Table 1: Effect of adenine sulphate (AS) on shoot proliferation of *S. suaveolens*.

| MS Medium +0.44 $\mu\text{M}$ BAP + AS (mg l <sup>-1</sup> ) | Shoots(node <sup>-1</sup> ) | Length (mm) (shoot <sup>-1</sup> ) | Nodes(shoot <sup>-1</sup> ) |
|--|-----------------------------|------------------------------------|-----------------------------|
| 0.0  | $1.0 \pm 0.0^{*a}$          | $12.0 \pm 0.65^b$                  | $2.2 \pm 0.11^a$            |
| 250.0  | $1.13 \pm 0.09^a$           | $18.2 \pm 0.71^a$                  | $2.4 \pm 0.13^a$            |
| 500.0  | $1.0 \pm 0.0^a$             | $13.6 \pm 0.59^b$                  | $2.4 \pm 0.13^a$            |
| 1000   | $1.0 \pm 0.0^a$             | $6.5 \pm 0.42^c$                   | $1.4 \pm 0.13^b$            |

\*Mean  $\pm$  1 SEM; Each experiment consisted of five replicates and repeated three times. The means separated using DMR (Costat; Version 4.02). Similar alphabets within a column do not differ significantly at  $P \leq 0.05$ .

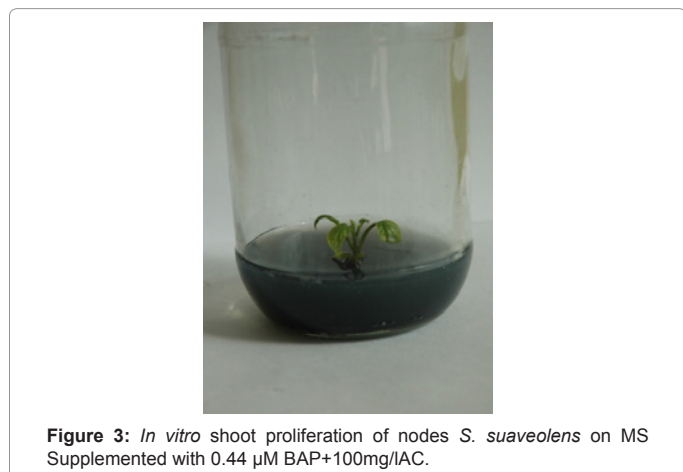
Table 2: Effect of PVP on shoot proliferation of *S. suaveolens*.

effect on the shoot proliferation and the release of phenolic compounds. Pierik [19] reported that the addition of activated charcoal, PVP, some antioxidants and some amino acids to the medium can alleviate the problem of browning in the medium and also enhance the shoot proliferation. The nodes placed on MS medium supplemented with 0.44  $\mu\text{M}$  BAP and 25.0 mg/l AS showed shoot number  $2.6 \pm 0.21$ , shoot length (mm)  $28.4 \pm 0.39$  and number of nodes per micro shoot  $4.1 \pm 0.21$ (Table 1, Figure 2). According to Quraishi et al. [20] addition of adenine sulphate appears to supplement cytokinin requirement of the nodes that is not fulfilled by the BAP or kinetin. The nodes placed on MS supplemented with 0.44  $\mu\text{M}$  BAP and 25.0 mg/l CH showed shoot number  $1.2 \pm 0.0$ , shoot length (mm)  $12.6 \pm 0.81$  and number of nodes per micro shoot  $2.0 \pm 0.16$ . Howell et al. [21] controlled the leaching of brown inhibitory substances into the established culture of *Cleistanthus collinus*. They also showed the best axillary shoot proliferation on MS medium supplemented with citric acid (101.1  $\mu\text{M}$ ) and PVP 40 (12.5 or 25  $\mu\text{M}$ ) supplemented with 0.44  $\mu\text{M}$  BAP. The *S. suaveolens* were placed on MS supplemented with 0.44  $\mu\text{M}$  BAP and 50.0 mg/l CA showed shoot number  $1.2 \pm 0.11$ , shoot length (mm)  $22.9 \pm 0.58$  and number of nodes per micro shoot  $3.06 \pm 0.22$  and when placed on MS medium supplemented with 0.44  $\mu\text{M}$  BAP and 250.0 mg/l PVP showed shoot number  $1.13 \pm 0.09$ , maximum shoot length

| MS Medium<br>+0.44 $\mu\text{M}$ BAP<br>+ AS ( $\text{mg l}^{-1}$ ) | Shoots(node $^{-1}$ )        | Length (mm)<br>(shoot $^{-1}$ ) | Nodes(shoot $^{-1}$ )       |
|---|------------------------------|---------------------------------|-----------------------------|
| 0.0   | 1.0 $\pm$ 0.0 <sup>a</sup>   | 12.0 $\pm$ 0.65 <sup>c</sup>    | 2.2 $\pm$ 0.11 <sup>b</sup> |
| 100.0   | 1.26 $\pm$ 0.18 <sup>a</sup> | 22.3 $\pm$ 0.66 <sup>a</sup>    | 3.6 $\pm$ 0.13 <sup>a</sup> |
| 200.0   | 1.0 $\pm$ 0.0 <sup>a</sup>   | 18.2 $\pm$ 0.71 <sup>b</sup>    | 2.4 $\pm$ 0.13 <sup>b</sup> |
| 400.0   | 1.0 $\pm$ 0.0 <sup>a</sup>   | 11.0 $\pm$ 0.98 <sup>c</sup>    | 2.0 $\pm$ 0.16 <sup>b</sup> |

\*Mean  $\pm$  1 SEM; Each experiment consisted of five replicates and repeated three times. The means separated using DMR (Costat; Version 4.02). Similar alphabets within a column do not differ significantly at  $P \leq 0.05$ .

**Table 3:** Effect of activated charcoal (AC) on shoot proliferation of *S. suaveolens*.



**Figure 3:** *In vitro* shoot proliferation of nodes *S. suaveolens* on MS Supplemented with 0.44  $\mu\text{M}$  BAP+100mg/l AC.

(mm)  $18.2 \pm 0.71$  and number of nodes per micro shoot  $2.4 \pm 0.13$  (Table 2). Similarly [22] reported that when PVP is incorporated in the medium as an adsorbent and used to remove the toxic metabolites from the medium. The subcultured stem nodes from *in vitro* grown plants of *Acacia catechu* on MS medium adjuvanted with  $\text{N}^6$ -benzyladenine and augmented with  $1.5 \text{ g l}^{-1}$  PVP to control browning [23]. The nodes of *Stereospermum suaveolens* when placed on MS supplemented with  $0.44 \mu\text{M}$  BAP and  $100.0 \text{ mg/l}$  AC showed shoot number  $1.26 \pm 0.18$ , shoot length (mm)  $22.3 \pm 0.66$  and number of nodes per micro shoot  $3.6 \pm 0.13$  (Table 3, Figure 3). In the present study the effect of additives were checked for influence on shoot proliferation and concluded with the result that the nodes of *S. suaveolens* when placed on MS medium supplemented with BAP and AS showed maximum shoot proliferation. Then the shoots were transferred in the rooting medium and finally acclimatized following the primary and secondary hardening in coco peat and sand, soil, and manure. The similar results were observed when the shoots were transferred in the substrate for hardening [24].

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