



Effect of cytokinin and silver nitrate on *in vitro* regeneration of *Stereospermum calais* L.: An important medicinal forest tree

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Abstract

Stereospermum calais L. is a well-known pharmacologically powerful herb reported in convention medical systems. Micropropagation of *Stereospermum calais* was based on nodal explants grown on a MS medium supplemented by various combinations of BAP. Induction of multiple shoots with a maximum of 6 shoots and a shoot length of 2 cm was observed on the MS medium supplemented by 3 mg L⁻¹ BAP and AgNO₃ (1 mg L⁻¹). For root induction, *in vitro* generated shoots were treated with various IBA concentrations on a basal MS medium of ¼ concentration, supplemented by AgNO₃ (1 mg L⁻¹). The maximum number of roots (10) was found on ¼ MS level with 1 mg L⁻¹ IBA and AgNO₃ (1 mg L⁻¹). The seedlings produced were treated with the hardening procedure for acclimatization and transferred to the soil.

Keywords: Forest tree, micropropagation, medicinal plant, *Stereospermum*

Introduction

Forests are the treasure of the natural medicines. A valuable root drug extensively used in herbal medicine systems is *Stereospermum calais* L. It is a vulnerable forest tree, distributed in Shri Lanka, Myanmar and India, particularly in the forests of Western-South Ghats, Central and Maharashtra Sahydris. *S. calais* belongs to family Bignoniaceae^[1]. It is a large straight stemmed deciduous forest tree about 18-30 m in height and 2.8 m in girth found throughout in the moist regions of India up to an elevation of approximately 1200 m, chiefly in deciduous woods. It is known as a “Yellow snake” in English, “Padeli” in Gujarati and “Patala” in Sanskrit^[10]. Leaves are helpful in otitis, pain relief, rheumatism, malaria, fever and wounds. Leaf juice blended with lime juice is used in manic cases^[11]. A decoction of the leaves is used for the treatment of chronic dyspepsia and has anti-pyretic properties as well. The roots are acerbic, acrid, anodyne, appetizer, constipating, diuretic, lithotropic, ex-pectorant, cardio-tonic, aphrodisiac, anti-inflammatory, anti-bacterial, restorative and anti-emetic. Root decoction is used to treat asthma and cough^[6].

S. calais are propagated by seeds. However, seeding is poor and seed sustainability is low. Problem related to its natural propagation and indiscriminate exploitation for medicinal purpose (mostly roots) has pushed *S. calais* to the list of endangered plant species of India. Yet, with the growing need for medicinal plants, especially those that are rare and threatened in nature, the multiplication of these species in large numbers is required. *In vitro* culture techniques provide a fast way of generating such elite and rare germplasms^[2] and another method for preserving forests^[8]. Very few reports have been found concerning micropropagation in this woody tree. This study has developed a protocol for micropropagation based on nodal explants.

Materials and Methods

Mature fruits of *S. calais* were harvested in July from 15-year-old tree growing in the forest of Jambughoda in the Panchmahal district, Gujarat, India. The woody capsules, about 15 cm in length, were filled with numerous winged

seeds. Each capsule contains approximately 20-30 seeds. Mature seeds were separated and selected for *in vitro* germination. The wings were severed with scissors. Then, the seeds were treated with 0.5% Bavistin (5 minutes) under aseptic conditions in a laminar airflow cabinet, followed by washing the seeds thoroughly with sterile distilled water for two times. Subsequently, the seeds were surface sterilised with 0.05% mercuric chloride (HgCl₂) for 30 seconds. Reagent residues were removed by rinsing the seeds four times with sterile distilled water. Sterile seeds have been inoculated on MS medium. Seeds germinated within 4 to 5 weeks.

Nodal explants (2-3 cm long) from shoots grown *in vitro* (15-18 days) were used to induce multiple shoots. The nodal explants were implanted on agar (0.8%), based MS media supplemented with BAP in varied combinations alone and in combination with AgNO₃ (1 mg L⁻¹) (Table 1). The pH of the medium was adjusted to 5.8 before being autoclaved to 121°C (15 minutes). All culture vessels were stored in the culture room (at 25±1°C) under fluorescent light with 16-hour photoperiod. A cluster of shoots was separated and transferred to rooting media. For root induction, *in vitro* generated shoots were transferred on ¼ strength of MS media supplemented with various concentrations of IBA and AgNO₃ (1 mg L⁻¹) (Table 2).

For acclimatization the plantlets were removed carefully, washed thoroughly with distilled water to remove agar, dipped in a solution of fungicides (0.1% Bavistin) to take care of contamination and were transferred to thermocol glasses (3.2 cm diameter) containing the mixture of sand, soil and manure (1:1:1). The plantlets were irrigated with ¼ strength MS medium and processed for hardening as reported earlier^[9]. The hardened seedlings were transferred into the field.

Result and discussions

The germination rate was observed at approximately 73%. Initial sprouting of the axillary bud was observed on day 8 of inoculation. All media tested for propagation have shown germination of buds. Depending on the strength of the supplements, within 12 to 15 days germinated buds developed in 2 to 4 multiple shoots per node (Fig- 1).

Table 1: Multiple shoots formation on nodal explants of *S. colais* with BAP alone and in combination with AgNO₃

Medium No.	BAP (mg/l)	AgNO ₃ (mg/l)	No. of shoots Mean ± S.E.	Shoot length (cm) Mean ± S.E.
1	-	-	0.4 ^{ab} ± 0.16	0.23 ^{ab} ± 0.06
2	0.5	0	3 ^{ab} ± 0.36	0.37 ^{ab} ± 0.06
3	1	0	2 ^{ab} ± 0.36	1.2 ^b ± 0.12
4	2	0	0.7 ^{ab} ± 0.29	2.5 ^{cd} ± 0.07
5	3	0	2.4 ^{ab} ± 0.48	2.5 ^d ± 0.18
6	0	1	2.20 ^{ab} ± 0.16	0.9 ^{bc} ± 0.13
7	1	1	4.10 ^{bc} ± 0.16	4.06 ± 0.56
8	2	1	5.30 ^{cd} ± 0.44	5.20 ± 0.26
9	3	1	6.60 ^d ± 0.16	2.36 ^{cd} ± 0.11

*- Indicates no response. Mean bearing the same superscripts letter within the column are not significantly ($P \leq 0.05$) different according to Tukey-HSD test. Mean ± SE: standard error of the mean.

The present study reveals that multiple shoot induction with maximum 6-7 shoots (Table 1) and shoot length of 2 cm was seen on MS medium supplemented with 3 mg L⁻¹ BAP and AgNO₃ (1 mg L⁻¹) (Fig- 2I). BAP alone has shown an increase in shoots with a maximum of 1 to 2 multiples. BAP in combination with AgNO₃ (1mg L⁻¹) showed better shoot multiplication than BAP alone (Table 1). *S. calais* is rich in

phenolics which influence the growth of *in vitro* cultures. Results of the present study reveal that cytokinin (BAP) in combination with antioxidants (AgNO₃) prevent the impact of phenols of explants and showed better growth of regenerated shoots than the use of cytokines alone in the media.

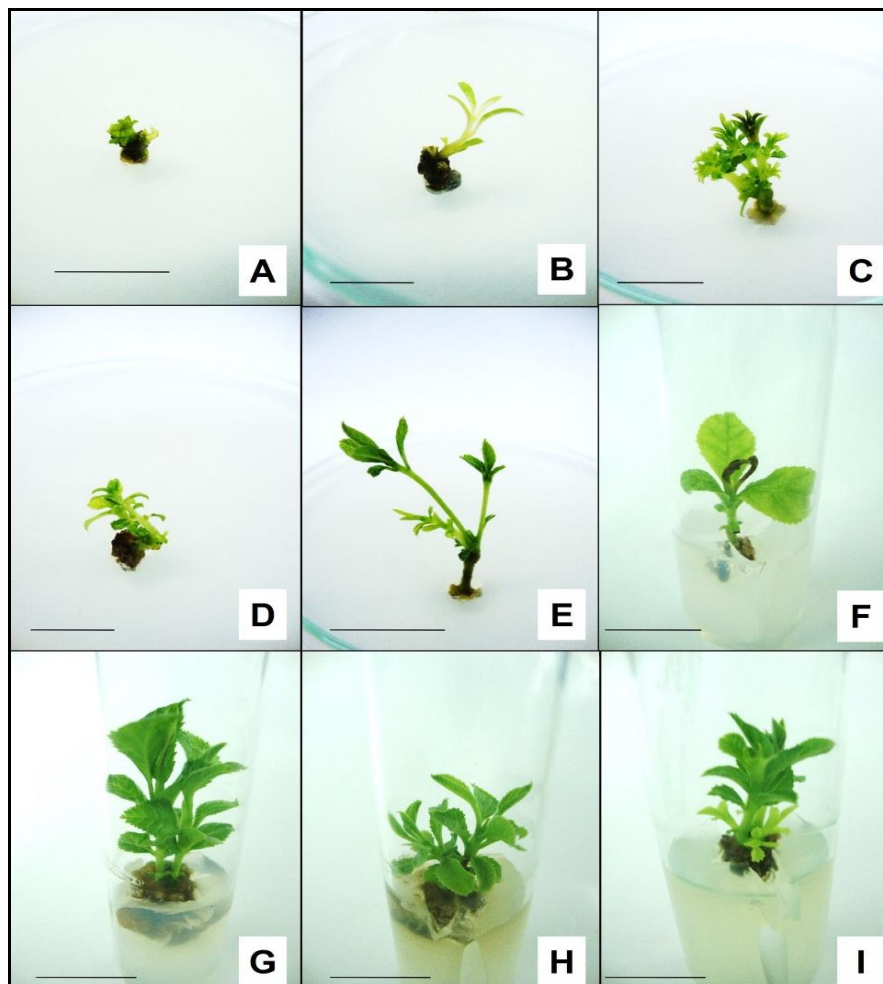


Fig 1: Effect of hormones on shoot proliferation of *Stereospermum colais* L. A, Nodal explant implanted on plain MS. B-E, Multiples regenerated on MS medium supplemented with various concentration of BAP (0.5, 1, 2 and 3 mg/l subsequently); F-I, Multiples regenerated on MS medium supplemented with various concentration of BAP (0, 1, 2 and 3 mg/l subsequently) in combination with AgNO₃ (1 mg/l).

Horizontal bar in each photograph is equal to 1 cm

Well grown *in vitro* generated micro-shoots were transferred for root induction on ¼ strength MS media supplemented with various concentrations of IBA in combination with AgNO₃ (1 mg L⁻¹). Poor root induction was observed on micro-shoots implanted on plain MS medium. The roots

were extremely thin and sponge-like (Fig- 3). The maximum number of roots formed was 10.5 with 1 mg L⁻¹ IBA combination with AgNO₃ (1 mg L⁻¹) (Table 2). Induced root growth was quite slow and 1-2 cm in length was reached after 30 days of incubation.

In vitro propagation of *S. calais* has been reported earlier on MS medium supplemented with BAP (0.44 μM) and adenine sulphate (25 mg L^{-1}) [14]. It is reported that the effect of various additives such as casein hydrolysate, activated charcoal, coconut milk and silver nitrate on the regeneration of *Oroxylum indicum* [3]. Silver nitrate has been shown to be the best additive for regenerating *Oroxylum*. Earlier reports on tropical trees belonging to the family Bignoniaceae exemplified the shoot proliferation on MS medium supplemented with BA and rooting on MS medium supplemented with IBA [11, 13, 16].

Table 2: Root induction on *in vitro* generated shoots of *S. calais* implanted on $\frac{1}{4}$ strength of MS media supplemented with IBA and AgNO_3 (1 mg/l).

IBA (mg/l)	No. of roots Mean \pm S.E.	Root length (cm) Mean \pm S.E.
0	0.8 ^a \pm 0.22	1.20 ^a \pm 0.59
0.5	3.1 ^a \pm 0.16	2.10 ^a \pm 0.35
1	10.5 ^b \pm 1.35	1.25 ^b \pm 0.12
2	4.2 ^a \pm 0.24	2.3 ^b \pm 0.21

Mean bearing the same superscripts letter within the column are not significantly ($P \leq 0.05$) different according to Tukey-HSD test. \pm SE: standard error of the mean.

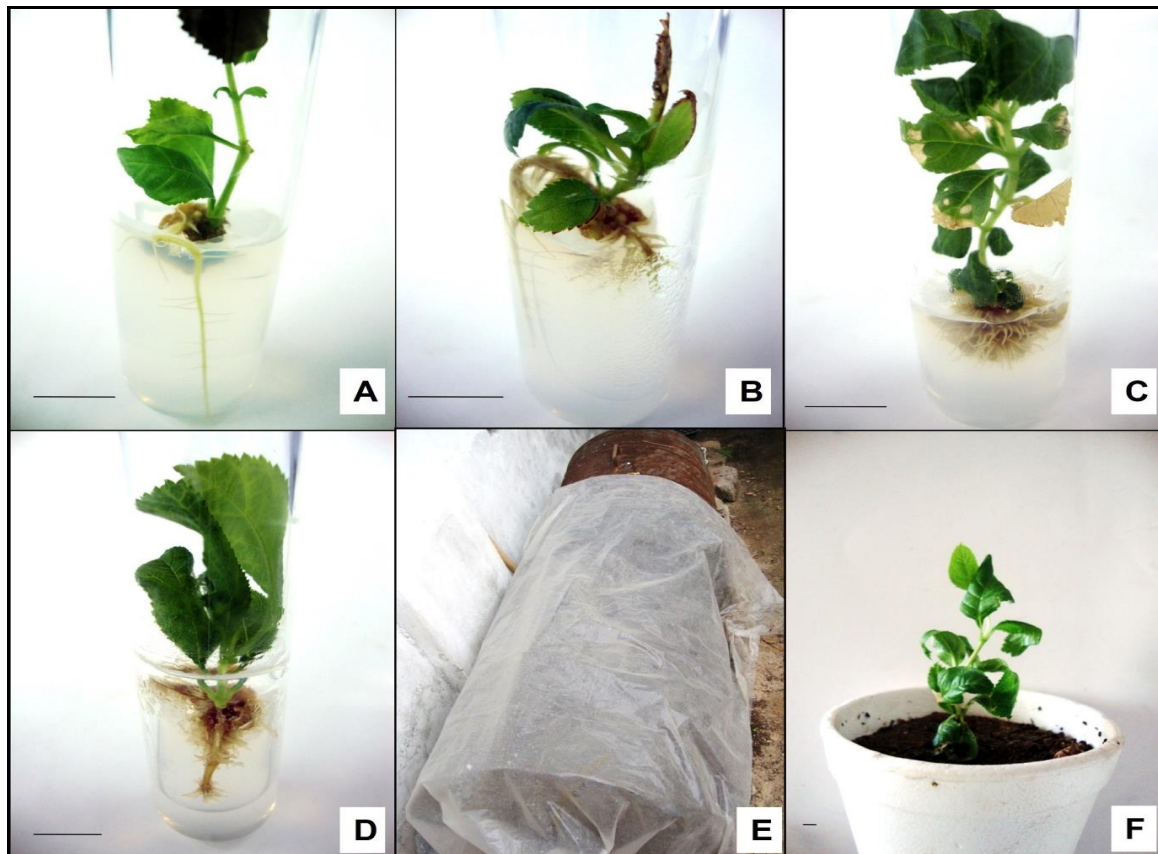


Fig 2: Root induction on *in vitro* generated shoots of *Stereospermum colais* L. A-D, Root induction on *in vitro* generated shoots implanted on 4 MS medium supplemented with various concentration of IBA (0, 0.5, 1 and 2 mg/l subsequently) in combination with AgNO_3 (1 mg/l). Horizontal bar in each photograph is equal to 1 cm.

S. calais plant are rich in phenolic compounds that affect the growth of *in vitro* cultures and causes browning of the explant and the surrounding medium. Browning, especially during the preparation of explants, is the major limiting factor. The exudation of phenolics (browning) prevents the growth of cultures. To prevent phenol exudation, antioxidants like ascorbic acid and PVP have been added to the culture medium. In the present study, AgNO_3 (1 mg L^{-1}) was added to the medium to reduce the effect of browning on *S. calais* cultures. Under the influence of this chemical, *S. calais* explants rarely turned brown. Previously, similar supplementation had been reported in *Aloe barbadensis* cultures [12, 7, 4]. In many other plants such as *Madhuka latifolia* [15] and *Anacardium occidentale* [5], addition of antioxidants has been reported to alleviate the browning problem.

In vitro cultivated seedlings were well acclimated without greenhouse conditions (Fig- 3E). The resulting seedlings were transferred to thermocol glasses containing the mixture

of sand, soil and manure (1:1:1) and stored in the tunnel. The humidity was maintained around transplanted plants by covering the tunnel with polyethylene sheet for two weeks and regularly spray with water. After four weeks, the seedlings were moved into a shed (covered with burlap) where moisture was maintained by keeping the burlap moist with water. This technique has been successful in acclimatizing seedlings without mist/greenhouse installation. Potted plants were similar in morphological characteristics to the parent stock. Development and hardened facilities (Fig- 3F) were transferred to the area. There was a mortality rate of 40 percent.

Conclusion

The protocol established in this study may be used for mass selection for large-scale cultivation of this important and highly sought-after medicinal plant. Through back-planning, the highest participation rate can be reached within a given timeframe.

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