TISSUE CULTURE STUDIES OF TWO MEDICINALLY IMPORTANT TREE SPECIES: *OROXYLUM INDICUM* (V.) AND *STEREOSPERMUM SUAVEOLENS* DC

Synopsis

To be submitted to

The Maharaja Sayajirao University of Baroda

For the Degree of

Doctor of Philosophy

In

Botany

Submitted by

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August, 2016

INTRODUCTION

Forests play a major role in maintaining climatic stability conserving water and soil, biological diversity and serve as a valuable source for various timber and non timber products. The natural regeneration of forest trees is poor, as it is difficult to propagate by simple vegetative methods due to various reasons like slow growth, long life cycle, loss of seed viability and poor germination of seeds (Naik 1998). The medicinally important forests trees are being heavily exploited for various reasons leading to reduction of forest cover and plant density(Dhawan and Saxena 2004).Therefore steps should be taken to propagate them on mass scale (Chaturvedi et al 2007). Plant tissue culture techniques have been useful to overcome this problem through micropropagation methods. Many endangered and threatened tree species have been regenerated through in vitro methods using juvenile and mature explants for their large scale multiplication (Purohit et al., 1996, Hussain et al., 2008). In vitro propagation methods, allows conservation and large-scale multiplication of species throughout the year irrespective of the season.

Another important method for conservation of endangered and commercially important tree species is synthetic seed technology (Danso and Ford Lloyd 2003). It is an effective technique for the propagation of plant species which produce non-viable seeds or seeds with low germination rates (Saiprasad 2001).

In the present work tissue culture studies like in vitro regeneration and synthetic seed with respect to propagation and conservation has been carried out in two tree species of Bignoniaceae: *Oroxylum indicum and Stereospermum suaveolens*. Indiscriminately the species are harvested for roots which are used in the preparation of dashmoolarishta and chywanprash has now pushed them under threatened category (Yashoda et al 2004).

Oroxylum indicum (L.) *Vent.*, is medicinally as well as an economically important tree. The stem and root bark contains 3 – flavons – Oroxylin A, Chrysin and Baicalein having antimicrobial, anticancerous, antifungal and anti-inflammatory properties. It is useful in many diseases like diarrhoea, dysentery, rheumatism, cough, inflammations, fever, etc. (Anonymous, 2001).

Stereospermum suaveolens DC. is another medicinally important tree with pinnately compound leaves which are 1-2 ft long. The leaflets are broadly elliptic with serrate margin,

being rough above and pubescent beneath. Fruits are capsules and seeds are with long membranous wings. Roots are used in ayurvedic preparation of Dashmoolarisht and Chyawanprash (Yashoda et al 2004). They roots constituents are reported to contain p coumaric acid, triacontanol,3-cetyl alcohol, oleic, palmitic, stearic acid, lapachol, dehydroalpha-lapachone and dehydrotectol in root heartwood; β-sitosterol and n-triacontal in root bark.Flowers, roots and leaves are used in vomiting, diarrhoea and other fevers (Anonymous 1998). Gradual erosion of natural populations has reduced seed production in this plant (Baul 2009).

The studies were carried out with the following objectives for both species:

OBJECTIVES OF THE STUDY

- **Establishing shoot cultures utilizing suitable explants**
- **Multiplication of shoot cultures in MS and WPM media**
- **Rooting of in vitro shoots**
- **Hardening of plantlets**
- **Regeneration of plantlet from synthetic seed**

MATERIALS AND METHODS

PLANT MATERIAL:

Plants: *Oroxylum indicum* and *Stereospermum suaveolens*

Seeds of both the plants (C plus trees) were brought from seed technology Lab., Rajpipla Forest division and 1-2 year old of saplings of *Stereospermum suaveolens* were also collected from the same.

Methodology

I. **Seed germination**

Seed were germinated in different substrates to develop seedlings explants.

In *Oroxylum indicum* following substrates were used:

- Cocopeat
- Cocopeat:soil (1:1)
- Cocopeat:sand (1:1)
- Filter paper with distil water
- MS medium
- Soil

- In *Stereospermum suaveolens* following substrates were used:
  - Cocopeat,

- Cocopeat:soil (1:1)
- Ccocopeat:sand (1:1)
- Filter paper with distil water
- MS and WPM medium
- Sand and soil

- All the substrates were sterilized in autoclave except soil and sand, the seeds were soaked overnight before placing them in each substrate/s.
- A known quantity of cocopeat (dry weight) was soaked in distil water (4 times) overnight and then used for sowing the seeds.

II. Regeneration studies

❖ Explant preparation for both species:

The 20 days old seedlings (cotyledonary leaf, cotyledonary node, hypocotyl) and nodal explants (1-2 year old) were harvested and were given the following treatments:

- initially placed in running water for 1 hour (seedling) and 2 hours (nodal explants)
- washed with 1-2 drops of labolene for 5-10 minutes.
- seedling explants were treated with100 mg/l PVP (3 minutes), 200 mg/l bavistin (3 minutes) and then 0.1 % $HgCl_2$ (3 minutes)
- nodal explants were treated with 100 mg/l PVP (3 minutes), 500 mg/l of bavistin ,500 mg/l streptomycin (5 minutes each) and 0.1 % $HgCl_2$ (5 minutes)
- both explants were rinsed with sterile water for three times after each treatments
- explants were cut in appropriate sizes and transferred to respective media

❖ Establishment of cultures

Cultures were established in MS and WPM basal medium, and media fortified with following PGR's:

O.indicum

- Individual cytokinin (for all the explants)
  - BAP(2-30µM) ; Kn(2-30µM) ;TDZ(0.1-2µM)
- Combination of cytokinins (Cotyledonary node and nodal)
  - BAP(8,16,20µM) + Kn(2,4,8,16µM),

- BAP(8,16,20µM)+TDZ(0.1,0.2,0.25,0.5µM) and
- Kn(2,4,8µM) + TDZ(0.1,0.2,0.25,0.5µM)

➢ Combination of cytokinins and auxins (Cotyledonary node and nodal)

- Optimized concentrations of individual cytokinin (BAP/Kn/TDZ) + IAA/IBA/NAA(0.1,0.5,1 µM)

S.suaveolens

➢ individual cytokinin (for all the explants)

- BAP(2-30µM) ; Kn(2-30µM) ; TDZ(0.1-2µM)

➢ Combination of cytokinins

Cotyledonary node

- BAP(8,16,20µM) + Kn(2,4,8µM),
- BAP(8,16,20µM)+TDZ(0.1,0.2,0.25µM) and
- Kn(2,4,8µM) + TDZ(0.1,0.2,0.25µM)

Nodal explant

- BAP(2,4,8µM) + Kn(2,4,8µM),
- BAP(2,4,8µM)+TDZ(0.1,0.2,0.25µM) and
- Kn(2,4,8µM) + TDZ(0.1,0.2,0.25µM)

➢ Combination of cytokinins and auxins (Cotyledonary node and nodal)

- Optimized concentrations of BAP, Kn and TDZ + IAA/NAA(0.1,0.5,1 µM)

❖ **Multiplication of shoots**

In both the species in vitro nodes derived from in vitro shoots were subcultured on fresh MS and WPM media fortified with respective PGRs and assessed for further formation of multiples.

❖ **Rooting**

Elongated shoots (4-6 cm) of both the species were placed for in vitro rooting in MS and WPM liquid and static media (half strength and full strength).

In liquid medium filter paper bridge was used as a support for the microshoots.

The shoots were washed with distil water for one minute and then dipped in bavistin for one minute and then placed vertically in the following media.

O.indicum

- 1/2MS + IBA & NAA(0,1,2,2.5,5,10 µM)
- MS+ IBA & NAA(0,1,2,2.5,5,10 µM)

- 1/2 WPM+ IBA & NAA(0,1,2,2.5,5,10 µM)
- WPM+ IBA & NAA(0,1,2,2.5,5,10 µM)

S.suaveolens

- 1/2MS + IBA & NAA(0,1,2,2.5,5 µM)
- MS+ IBA & NAA(0,1,2,2.5,5 µM)
- 1/2 WPM+ IBA & NAA(0,1,2,2.5,5µM)
- WPM+ IBA & NAA(0,1,2,2.5,5 µM)

III. **Hardening of plantlets**

- *Oroxylum indicum and Stereospermum suaveolens* plantlets were hardened in different planting substrates like cocopeat, sand, soil individually and their combinations filled in thermocol cups.
- The growth was assessed for parameters like shoot length, root length, number of roots and plant height before transferring the plantlets to respective substrate. After 2-3 months again the same parameters were recorded.

IV. Synthetic seed studies

In *O.indicum* in vivo and in vitro buds and in *S. suaveolens* in vitro buds were selected as explants for encapsulation.

❖ **Bead preparation in both species:**

- Beads were prepared by dropping sodium alginate (2%, 3%, 4%) with explants into calcium chloride (50, 75,100mM) solution
- Incubated for 30 minutes
- Calcium chloride solution was drained and the beads were washed with distil water (3 times).
- Beads were placed on respective substrate, stored at 4ºC and then transferred regenerative media.

❖ **Matrix**:

The matrix of the beads consists of sodium alginate prepared in medium with or without PGRs for both species as follows:

O.indicum

- ½ WPM +S (1%) basal medium
- ½WPM + S(1%) + BAP(16 µM);TDZ(0.5 µM) and
- WPM+S(3%) basal medium

- WPM +S(3%) + BAP (16 µM); BAP(16 µM)+IBA(0.1 µM)

In *S.suaveolens*

- ½ MS +S(1%) basal medium
- ½MS+S(1%)+BAP(20µM);Kn(8µM);TDZ(0.2µM);BAP(8µM)+TDZ(0.2 µM)
- MS+S(3%) basal medium
- MS+S(3%)+ BAP(20µM) ;Kn(8µM);TDZ(0.2µM);BAP(8µM)+TDZ(0.2 µM)

- **Substrate for storage:**

  All the encapsulated beads were stored in substrates as follows:

  In *O. indicum* filter paper containing half and full strength liquid basal WPM medium and while in *S. Suaveolens* petri plates with filter paper containing half and full strength liquid basal MS medium were used as substrates.

- **Storage period**: In both species the encapsulated beads were stored at 4ºC for 0,7,15, 30 days.

❖ **Regenerative media**:

The beads were transferred at regular time interval to following regenerative media. In *O. indicum* only static regenerative media and in *S. suaveolens* both liquid and static media were taken.

O. indicum

A. in vitro buds
   - ½ WPM+BM;IBA(5µM); NAA(5 µM)
   - ½ WPM+BM;NAA(5 µM); BAP (16µM) +Kn(4 µM)
   - ½ WPM+BM; NAA(5 µM)
   - WPM+BM; BAP (16µM) +Kn(4 µM);BAP(16 µM)+IAA(1 µM)
   - WPM +BM; NAA(5 µM)
   - WPM+BM; NAA(5 µM); BAP (16µM) +Kn(4 µM)

B. in vivo buds
   - ½ WPM+BM; NAA(5 µM)
   - ½ WPM+BM; NAA(5 µM); IBA(5 µM)
   - ½ WPM+BM; IBA(5 µM)
   - WPM+BM;NAA(5 µM)
   - WPM +BM;NAA(5 µM); BAP (16µM) +Kn(4 µM),
   - WPM+BM; TDZ(0.5 µM); BAP (16µM) +Kn(4 µM),

With additives (invivo and invitro buds)
- ½ WPM+ CW(10%);GA(10 µM)
- WPM+ CW(10%);GA(10 µM)

S.suaveolens

- in vitro buds
- ½ MS+BM; IBA(2 µM); NAA(2µM)

- ½ MS+BM; IBA(2 µM); NAA(2 µM);TDZ(0.2 µM) +IBA(2 µM);TDZ(0.2 µM) +NAA(2 µM)
- ½ MS+BM; IBA(2 µM);NAA(2 µM);BAP(4 µM)+Kn(8µM)
- ½ MS+ Kn(8µM)+IBA(2 µM)
- ½ MS+BM; IBA(2 µM); NAA(2 µM);Kn(8µM)
- MS+BM; Kn(8µM) +TDZ(0.2 µM); TDZ(0.2µM);IBA(2 µM)
- MS+BM; BAP(20 µM); BAP(20 µM)+IBA(2 µM)
- MS+BM;IBA(2µM);NAA(2µM);BAP(20µM)+TDZ(0.2µM)+IBA(2 µM);BAP(8 µM)+TDZ(0.2 µM)+NAA(2 µM)
- MS+BM; IBA(2 µM); NAA(2µM); Kn(8µM)+IBA(2 µM)
- MS+BM; IBA(2 µM); NAA(2µM);BAP(8µM)+TDZ(0.2 µM)

With additives

- ½ MS+ CW(10%);GA(10 µM);$AgNO_3$(50mg/l)
- MS+ CW(10%);GA(10 µM);$AgNO_3$(50mg/l)

Results

Oroxylum indicum

- **Seed germination studies:** The results from seed germination revealed that cocopeat was the optimized substrate for generating maximum number of seedlings. The seedlings were used as juvenile explants for establishing cultures.

Regeneration studies

- In *O .indicum* the cotyledonary leaf and hypocotyl explants failed to regenerate into shoots in MS and WPM media fortified with individual cytokinins (BAP/Kn/TDZ) and only callus was observed from the cut ends.
- The cotyledonary node and nodal explants were responsive in terms of shoot formation in MS and WPM medium fortified with PGRs but the later explants induced healthy shoots.
- Multiple shoots developed from nodal explant in WPM medium fortified with BAP (16 and 20 µM) individually, BAP (16µM) + Kn (4 µM) and BAP (16µM) + IBA (0.1 µM).
- In few combinations, explants differentiated mophogenic callus at its base.
- The mophogenic callus was multiplied on fresh medium with same combination and it regenerated shoots.
- The stunted shoots were elongated with the help of additives like coconut water and GA_3.
- Root induction was observed in only liquid (MS and WPM) medium and optimum response was observed in full strength WPM medium fortified with IBA (10µM).

Hardening:

- Plantlets which were transferred from culture room to greenhouse failed to survive. In vitro plantlets were hardened under greenhouse conditions and growth and development was observed in the plantlets placed in sand and soil substrate individually.

Synthetic seed studies:

- In *O.indicum* the encapsulated in vitro buds and in vivo buds had the potency to regenerate on WPM media with different PGRs and a varied response was observed in terms of percent germination of synseed.

Stereospermum suaveolens

- **Seed germination studies:** Cocopeat was the optimized substrate for generating maximum number of seedlings, from which juvenile explants were used for establishing cultures.

Regeneration studies:

- In *S.suaveolens* cotyledonary leaf and hypocotyl explants failed to grow into shoots in MS and WPM media fortified with individual cytokinins (BAP/Kn/TDZ).
- The cotyledonary node and nodal explants responded in culture as shoot formation was observed in MS and WPM medium.
- Both the media were helpful in formation of shoots.
- The number of shoots enhanced in several concentrations of individual and synergistic combinations of cytokinins. MS medium fortified with BAP (8µM) +TDZ (0.2µM) resulted in highest number of multiples shoots.
- This elongated shoots were rooted in both MS and WPM liquid and static medium with half strength and full strength fortified with IBA /NAA concentrations.

Hardening:

- Plantlets which were transferred from culture room to greenhouse failed to survive. Therefore, in vitro plantlets were placed in different substrates and hardened under greenhouse. Optimum growth and development was observed in the plantlets growing in cocopeat: soil (1:1) substrate.

Synthetic seeds studies:

- The encapsulated in vitro buds in sodium alginate (3%) matrix prepared in MS medium fortified with different PGRs could germinate in both liquid and static media.

- 100 % conversion into shoot and root was observed in few combinations.

Summary

Oroxylum indicum and *Stereospermum suaveolens* are medicinally important trees which are exploited for their roots for preparation of ayurvedic formulations and are now placed under threatened category. In the present studies tissue culture techniques were adopted to develop protocols for regeneration of these species utilizing suitable explants.

In both the species nodal explants proved to be better in comparison to cotyledonary nodes for establishing shoot cultures. MS and WPM medium fortified with different PGRs individually or in combinations obtained varied response. In *S.suaveolens* MS medium was optimum for shoot multiplication and in *O.indicum* WPM medium was effective. In both the species liquid medium was optimum compared to static for root induction. The plantlets of both species were hardened successfully under greenhouse conditions.

Synthetic seed studies revealed that in both species matrix composition, substrate for storage and regenerative medium affected the germination of the same. Explants retained the viability to germinate till 30 days only.

Acknowledgment: I m thankful to UGC for providing financial assistance as a JRF in major project on *Stereospermum suaveolens.*

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