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

A Summary of the thesis
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SUMMARY OF THE THESIS

One of the most interesting areas of biotechnology is tissue culture and micropropagation. Tissue culture is the ability to establish and maintain plant organs(embryos,shoots,roots and flowers) and plant tissues(cells,callus and protoplasts) in aseptic culture. Hence the present thesis entitiled 'Tissue culture studies on two important medicinally important tree species,*Oroxylum indicum* (V.) and *Stereospermum suaveolens* DC.' was carried out.

These trees face problems in natural regeneration as they have low percent of seed germination and are exploited for their plant parts. The roots of both the plants are used for preparation of Dashmoola and Chywanprash and hence indiscrminately harvested from wild, which has now placed them under threatened category.Hence there is an urgent need to conserve this valuable plants.Therfore the studies on seed germination,in vitro regeneration and synthetic seed very carried out in these species.

Section I : Seed germination studies

Trees use seeds as a principal means of establishing their next generation in the natural world. Seeds serve as a delivery system for the transfer of genetic material from one generation to the next. In most tree species, seeds are critical for production of seedlings. Some trees can easily be grown from seed but, for some trees, it may be much quicker and easier to propagate them from cuttings. Seed propagation can be a tricky process for a number of tree species.

The *Oroxylum indicum* and *Stereospermum suaveolens* species possess low percent seed germination under natural conditions.Thereofore the seeds were placed in different substrates for germination.

In *O.indicum* cocopeat was best substrate in terms of seed germination but if cocopeat is used in combination with soil or sand can produce good quality seedlings as it can give better results in terms of collar diameter, seedling length, biomass. Thus cocopeat can be ideal substrate if used individually and in combination.

In *S.suaveolens* also cocopeat resulted in highest germination generating maximum seedlings.Also cocopeat possess maximum germination rate and maximum mean daily germination and germination index was observed in cocopeat and MS medium followed by

filter paper substrate.Thus overall results depict that cocopeat was the ideal substrate for *S.suaveolens* seeds having maximum percent and speed of germination.

Section II: Regeneration studies

In-vitro propagation of plants holds tremendous potential for the production of high-quality plant-based medicines. The clonal propagation of several plant species may be achieved through the establishment of explants *in vitro* followed by rapid shoot multiplication and development and finally hardening and establishment of the plantlets in vivo(Murashige,1974). *In vitro* clonal propagation has been extensively used for large scale multiplication of many important forest tree species (Ahuja, 1993; Bonga and von Aderkas, 1992) Reports says that success of in vitro shoot formation depends on the type of explants used (Zobayed and Saxena, 2003;Leng *et al,* 2004;Hong *et al,* 2004,Koroch *et al,* 2002). The type of cytokinins and its concentration depends on plant species for micropropagation of woody plants and various cytokinins differ in their activity (Nikolic *et al,* 2006).

In the present studies *in vitro* regeneration of *O.indicum* and *S.suaveolens* were carried out using different explants in MS and WPM medium for developing a rapid regeneration protocol.

In both the species the cotyledonary leaf and hypocotyl explants failed to establish shoot cultures in MS and WPM medium fortified with the cytokinins tried. Cotyledonary node and nodal explants revealed their potency to regenerate shoot in presence of individual cytokinins and synergistic combinations of PGRs.In both the species the nodal explants was proved to be optimum explants in comparison to cotyledonary node for inducing healthy shoots.

In *O.indicum* and *S.suaveolens* both the medium were able to establish cultures but overall WPM medium was effective for regeneration of *O.indicum* and MS medium for *S.suaveolens*.

In *O.indicum* maximum shoot were induced in MS medium fortified with BAP (8µM) with TDZ (0.25µM) and in WPM supplemented with BAP(16µM)+IAA(1µM) from nodal explants. Half strength WPM liquid medium fortified with IBA at 10µM was optimum for root induction and hardening of plantlets was successfully done in sand substrate, and these plants were transferred to soil and grown in botanical garden.

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In S.suaveolens also cocopeat was a suitable substrate in which maximum percent of seed germination was recorded. MS medium supplemented with a combination of BAP(8µM) and TDZ(0.2µM) resulted in forming optimum number of healthy multiples through nodal explants

and half strength MS liquid medium supplemented with IBA(2.5µM) induced maximum number of roots.The plants were hardened successfully with 100% survival in cocopeat:soil substrate under greenhouse conditions.

Section III:Synthetic seed studies

Alginate encapsulation has become a viable technique for *in vitro* germplasm conservation and synthetic seed production from nodal segments can be used for cost-effective mass clonal propagation and delivery of tissue-cultured plants (Ara *et al,* 2000; Nyende *et al,* 2003). The concentration of sodium alginate and calcium chloride is one of the important factors for the successful regeneration of plants through encapsulation technology as it affects the gel matrix and capsule quality. The optimal ion exchange of sodium and calcium controls the capsule hardiness, and it varies with propagules type and plant species (Rai *et al,* 2009 and Singh *et al,* 2010).

In the present studies the 3% sodium alginate with 75mM $CaCl_2$ solution resulted in formation of round and firm beads which was the optimum gel matrix for preparation of synseed in *O.indicum* and *S.suaveolens*.Therefore all the matrices were prepared in the same.

In O.indcium in vitro nodes were suitable propagule for synseed formation and the development of shoot and root (plantlet) was observed from in vitro nodes encapsulated in ½ WPM+BAP(16µM)+IBA(0.1µM) matrix, when placed on ½ WPM regenerative medium fortified with NAA (5µM) after 15 days of storage

In *S.suaveolens* the development of shoot and root (plantlet) was observed maximum from *in vitro* nodes encapsulated in 1/2 MS medium supplemented with Kn(8µM) matrix and placed on MS regenerative medium containing NAA (2µM) after 15 days of storage.

In both the species *O.indicum* and *S.suaveolens* the regenerating ability of synseeds was lost with increase in storage period and was completely nil after 30 days of storage

OUTCOME OF THE STUDY

- From the present work a substrate for seed germination was standardised to develop large number of seedlings.
- A rapid regeneration protocol was developed in *O.indicum* and *S.suaveolens* for establishment of shoot cultures.
- Hardening of these plants were successfully done under greenhouse conditions
- Development of synthetic seed were done utilising ideal gel matrix and synseeds germinated and remain viable upto 15 days ofstorage.