7. CONCLUSIONS

Some of the salient conclusions of the current investigations are as follows:

- The seed germination potential of *Solanum xanthocarpum* was assessed at different developmental stages (immature green, mature yellow and mature brown berry); it was found to vary with the physiological age of the berry, the given nutritional recipe, sucrose concentration, and the presence of AC.

- The type and potential of morphogenetic response of the various explants (hypocotyls, cotyledonary leaves, roots, leaves, and shoots) varied with the explant type, nutrient medium and chemical treatment. The best proliferation response was achieved using shoot explants whereas roots showed poor regeneration potential.

- The frequency, nature and morphology of callus varied with the nutrient regime and explant source. Organogenesis could be induced in the callus cultures by varying the growth stimulus in the nutrient pool.

- The regeneration pathway varied with the quality and quantity of growth adjuncts employed and their combinations in the nutrient medium.

- Rooting was induced in *in vitro* raised shoots and these tissue culture raised plantlets were successfully transferred to greenhouse conditions after acclimatization and fruits were obtained.

- The fungal requirements of both immature and mature seeds of *Malaxis acuminata* could be successfully bypassed using an appropriate nutrient medium *in vitro*.

- Intermediary top shaped structure, the protocorm is typical of orchid seed germination and has a high proliferation potential; however, its further growth and differentiation is subjective to the availability of suitable nutrient medium and culture environment. In *M. acuminata*, the protocorms generally proliferated before root formation.
Conclusions

• *In vitro* pseudobulbs can be successfully regenerated under selective nutritional Protocorms and PLB segments were efficiently utilized for culture multiplication in apt nutrient medium and growth adjuncts and sucrose concentrations.

• The asymbiotically raised seedlings of *M. acuminata* require an intricate acclimatization procedure prior to their transfer to the green house surroundings and their survival rate was low.

• The susceptibility of the two species to infection with *Agrobacterium rhizogenes* A4 strain was found to depend on the species infected and the type of explant.

• Hairy roots were successfully induced with an increased frequency in *S. xanthocarpum* using *A. rhizogenes* A4 on the additional treatment of acetosyringone.

• The capacity to produce solasodine in the *in vivo* sourced *S. xanthocarpum* plants varied with the plant part tested and the maturity of the fruits.

• Tissue cultures (callus, multiple shoots, roots, and seedlings) established utilizing various explants of *S. xanthocarpum* showed the potential to synthesize solasodine in various amounts.

• A negative correlation of solasodine profile with morphological differentiation was indicative of solasodine synthesis was tissue specific being remarkably influenced by organogenetic changes there in.

• The production of solasodine in the *in vitro* tissues was also subjective to the chemical composition of the nutrient pool and the concentrations of the growth regulators.

• The *in vitro* raised callus and roots exhibited the production of solasodine in comparable amounts to the *in vivo* plant parts indicating that tissue cultures possess the genetic information of the donor plant, which is fully expressed in the tissue cultured organs.

• The incompetence of the hairy roots to produce solasodine in amounts approaching the *in vivo* or *in vitro* cultures points to the fact that the full biosynthetic capacity may not always be expressed in culture.
Conclusions

The generalizations stated above are based on the results achieved in the present study of medicinally important plants- *Malaxis acuminata* and *Solanum xanthocarpum*. There have been, however, some spillovers which need further studies on the following lines:

- To direct efforts for developing shake flask and bioreactor cultivation strategies, scaling-up for large scale cost effective propagation of the above mentioned species.
- To work on the enhancement of the biosynthetic competence of the hairy roots of *S. xanthocarpum*.
- To develop a protocol for the successful transformation of recalcitrant *M. acuminata* using *A. rhizogenes* and enable hairy root formation.
- To assess the biosynthetic capacity of the field grown plant parts and tissue cultured protocorms/PLBs/seedlings in *M. acuminata*.
- To check the solasodine and malaxin production capability of the different *in vivo* plant parts at different ages and seasons in *S. xanthocarpum* and *M. acuminata*, respectively.