6. SUMMARY

*Adenia hondala* (Gaertn.) de Wilde and *Baliospermum montanum* Muell.-Arg. are two endangered species of potential medicinal value. They are red listed as vulnerable and rare in south India. Considering the importance of both these plants and the threat faced by them the present investigation was carried out to establish efficient and easily reproducible protocol for large-scale clonal propagation through shoot induction and somatic embryogenesis. Conventional tissue culture procedures were carried out using a wide variety and combination of plant growth regulator to clone plants of exact genotype. The highlights of the tissue culture studies are:

In *Adenia hondala*, the maximum number of shoots was produced in the basal MS medium containing $0.5 \text{ mgL}^{-1}$ BAP and $0.5 \text{ mgL}^{-1}$ Kn (6.25±0.5 shoots/culture) after 30 days. The addition of 10% coconut water further enhanced the number of shoot (8.25±0.9 shoots/culture). $2.5 \text{ mgL}^{-1}$ GA$_3$ supported the elongation of the regenerated shoots (7.95±1.1 cms). Using this combination an approximate 4,000 plants could be produced in three subcultures.

Callus via regeneration in MS medium + 1 mgL$^{-1}$ BAP + 0.5 mgL$^{-1}$ Kn + 10% CW offered a still better regeneration pathway in terms of the number of shoots (14.25±1.5 shoots/culture) and the number of plants produced could be double the number of direct multiplication using axillary buds. However, somatic embryogenesis both direct and indirect could not yield sufficient number of plantlets as obtained in the above protocols.

In *Baliospermum montanum*, direct regeneration from leaf explants when cultured in MS + 1 mgL$^{-1}$ Kn + 0.5 mgL$^{-1}$ NAA+10% cw produced a maximum of 8.2±0.8 shoots/culture. Ga$_3$ at 1.0 mgL$^{-1}$ enhanced the shoot length. Using this protocols a maximum of 2500 plants could be produced in three subcultures.
Nodal culture of *B. montanum* produced a greater number of shoots that from leaf cultures in the standardized combination of 1 mgL$^{-1}$ BAP + 0.5 mgL$^{-1}$ Kn. The nodal cultures by three subsequent subculture within a combination produced about 5000 plants.

To augment the existing findings of morphogenesis in tissue culture, the samples of material from tissue cultures have been subjected to periodical histological screening and biochemical estimations of primary metabolites and enzymes.

Histological screening helped to evaluate the cultures as organogenic or non-organogenic. Shoot buds and somatic embryos could be easily located at an earlier stage of development. In *Baliospermum mortanum* several nodular structures with thick dermal covering was observed and these nodules are believed to be the structures, which could have been developed as shoot buds. Presence of lignified vascular elements in the non-organogenic callus was a marker in identifying the callus as organogenic or non-organogenic. The quantitative estimations of starch, protein and soluble sugars distinguished organogenic and non-organogenic cultures. There was always an accumulation of metabolites in the organogenic cultures up to the 10-15 days and when the organs or somatic embryos matured there was a decline in the quantity of metabolites. In a non-organogenic culture a steady accumulation of metabolites was observed. In any clonal multiplication protocol, fidelity of genotype is to be tested because artificial environmental conditions in the tissue culture may alter the capacity to synthesize phytoconstituents. In the present study steroids, saponins, tannins, triterpenoids, alkaloids, phenols, tannin, catechin were tested in the regenerated plants and found that they are retained in the regenerated plants also. Similarly, electrophoretic pattern also indicated that the quality of the regenerated plants is in par with the stock plants.