GENERAL SUMMARY AND CONCLUSION:

In the past few years, there has been an increase in awareness about the utility of plants and their products. This trend has resulted in a growing need for plants with genetic stability and short, smart life cycles. Tissue culture generated varieties have successfully met such parameters and have enabled industries to derive the required products without threatening the biodiversity. Furthermore the development of horticulturally elite stocks through tissue culture has also become common practice.

Micropropagation has gradually established itself as one of the most viable technique for generation in vitro. In the present study attempts have been made to regenerate plants in vitro, through axillary shoot bud multiplication which ensures maximum genetic stability, through the development of somatic embryogenic systems for rapid mass propagation and finally via regeneration from callus via caulogenesis and rhizogenesis.

Two varieties of *Mussaenda erythrophylla* (namely 'scarlet' and 'rosea') and one variety of *M. phillipica* ('aurorae') were studied. Apart from the general objectives of development of efficient systems of micropropagation, biochemical studies were performed with the aim to detect biochemical / molecular markers which would enable an easy demarcation of the varieties.

Axillary shoot bud multiplication was achieved in all three varieties of *Mussaenda* on MS and B5 medium fortified with BAP and AdS Rooting was achieved on half strength MS medium supplemented with IBA (0.5 mg/l) and thiamin HCl (800 mg/l) for 'rosea' and 'scarlet' varieties while for 'aurorae'
variety, the optimum concentrations was 0.5 mg/l IBA and 500 mg/l thiamin HCl. A high frequency of shoot regeneration (9-12 shoots per explant) was obtained in MS medium supplemented with BAP (2.25mg/l) and AdS (40 mg/l).

The effects of BAP, NAA, L-glutamine and CH in the induction of somatic embryogenesis from calli in Mussaenda were studied. Greatest amount of embryogenic calli was obtained on MS medium supplemented with BAP (2 mg/l), NAA (0.5 mg/l) and L-glutamine (100 mg/l). When these calli were transferred on medium containing low concentrations of auxin and cytokinin along with 50 mg/l CH and 100 mg/l L-glutamine, cotyledonary stage embryoids were produced. These embryoids developed into whole plantlets following their transfer on MS medium lacking growth regulators.

Biochemical studies such as total protein content showed uniformity in all three varieties. However isozyme analysis revealed that acid phosphatase, peroxidase and esterase could be used as molecular markers to demarcate the species as well as varieties.

In case of acid phosphatase 'scarlet' variety showed bands at 0.23, 0.32 and 0.46, 'rosea' variety showed extra bands at 0.56 and 0.66 Rf values while 'aurorae' had the extra band at Rf value 0.56.

Peroxidase profile showed uniform pattern of banding but the band intensities were different. In case of the esterase profile 'scarlet' variety
exhibited bands at Rf values 0.264, 0.34 and 0.48, 'rosea' variety showed one band at 0.272 and 'aurorae' also showed one band at 0.076.

Acid phosphatase proved to be the most successful marker for discriminating the varieties. There were no detectable differences between the donor and the in vitro regenerated plants through axillary bud multiplication. Cytological studies also showed uniformity.

In Wedelia chinensis multiple shoot formation from axillary bud explants was achieved best on MS medium fortified with BAP (2.5mg/l) and AdS (40mg/l). Roots were induced to form from these shoots on MS medium containing IBA (1mg/l). Acclimatization in field conditions was also successful.

Calli were obtained from leaf explants on MS medium containing BAP and NAA. The calli proliferated 2-3 times as well as nodular in appearance when they were subcultured in MS medium containing BAP and L-glutamine. These nodular light green friable calli, when subcultured on MS basal medium, responded with the production of shoots. Rhizogenesis was observed seven days after reinoculation of the shoots in MS basal medium.

As expected, no differences between the donor and the in vitro generated plantlets through axillary bud multiplication were recorded either in the isozyme profiles (peroxidase and acid phosphatase) or in cytological studies.

Eupatorium ayapana is a plant with immense medicinal value. Successful development of multiple shoots from axillary buds was best recorded on MS
medium augmented with BAP (2mg/l), AdS (40mg/l) and thiamine HCl (4mg/l) after 60 days in culture. Excess BAP concentrations exerted an inhibitory effect on the rate of multiplication. Half strength MS medium augmented with 1mg/l IBA was found to induce rooting 15-20 days after inoculation.

Extensive development of green nodular calli was recorded on MS medium supplemented with BAP (1mg/l) and NAA (0.5mg/l). Shoot regeneration from these calli was achieved on MS medium containing BAP (0.5mg/l). When these shoots were transferred to MS basal medium free of growth regulators, rooting was recorded.

Similar banding patterns and intensities were recorded for isozyme profiles (peroxidase and acid phosphatase) of both the donor and in vitro regenerated plants through axillary bud multiplication. Cytological studies also did not reveal any karyotypic abnormalities.

Keeping the growing demand of taxol (an anticancerous alkaloid) in mind, efficient micropropagation systems for *Taxus wallichiana* was developed.

Adventitious shoots were directly produced from nodal segments on MMS medium. Preconditioning of the explants on MMS medium supplemented with PVP (2 mg/l) and BAP (0.5 mg/l) for 21 days and subsequent culture on MMS medium containing BAP (0.5 mg/l), AdS (10 mg/l), NAA (0.5 mg/l) and PVP (1 mg/l), facilitated axillary bud initiation and proliferation. Rooting was achieved in two stages, at first the microshoots cultured on MMS medium
supplemented with 5 mg/l NAA for 15 days, produced a small amount of callus at the base. Thereafter, in the second stage these shoots with basal callus were transferred to MMS medium containing 0.5 mg/l NAA, 1 mg/l IBA and 800 mg/l thiamin HCl, producing a large number of roots from the callus. It is worth mentioning that if the shoots were cultured in MMS fortified with 0.5 mg/l NAA, 1 mg/l IBA and 800 mg/l thiamin HCl exclusively from the beginning, they formed neither callus nor roots.

The effects of 6-benzyl amino purine (BAP), kinetin 2,4-dichlorophenoxyacetic acid (2,4 D), α-naphthalene acetic acid (NAA), indole butyric acid (IBA), L-glutamine, casein hydrolysate (CH), activated charcoal, Polyvinyl pyrrolidone (PVP) and ascorbic acid were tested in B5 medium with respect to callus initiation and subsequent growth from shoot bud, leaf and internode explants of T. wallichiana. Preconditioning of the explants in B5 medium augmented with PVP for 15 days and subsequent addition of 1 gm/l activated charcoal to the phytohormone containing B5 medium is essential for the callus induction of all the explants types. Although Taxus callus culture exhibited extremely slow growth rate, shoot bud derived callus grow more rapidly than others. B5 medium supplemented with 0.5 mg/l BAP, 0.5 mg/l NAA and 1 gm/l activated charcoal proved to be the best for callus culture. Attempts to induce plantlets regeneration in the callus via organogenesis and / or somatic embryogenesis were unsuccessful.

In conclusion it may be mentioned that efficient systems of micropropagation was successfully developed in all four genera investigated. The results indicate that a high degree of genetic stability was maintained and
true to type plants were generated in vitro. Further studies need to be undertaken to develop easy and viable systems of acclimatization for habitat sensitive genera.