Summary

and

Conclusion
CHAPTER - 6

SUMMARY AND CONCLUSION

*Bauhinia tomentosa* is a woody erect ornamental shrub or small tree, up to 4-5 m in height, extensively grown in garden for its attractive flowers. Dried leaves, flower buds, decoction of roots and bark are used in medicine. Decoction of the root bark is used for abdominal troubles and as an anthelmintic, also used as fibre, timber, tannin, dye and fodder etc.

Propagation of *Bauhinia tomentosa* through seed is unreliable due to seed dormancy and poor germination.

Plant tissue culture technique provides a viable alternative for managing these valuable resources in a sustainable manner. Most importantly, micropropagation provides an efficient method for ex-situ conservation of plant biodiversity and mass multiplication of various plant species from a minimum of available plant material which could meet the demand.

Direct multiple shoot regeneration was achieved from cotyledonary node (CN), aseptic and field grown nodal explants, cultured on MS medium containing various cytokinins (BA, Kn and 2-iP) solely or in combination with auxins (IAA and NAA). Among the different concentration of cytokinins tested, BA (5.0µM) showed the highest regeneration frequency (84%) and number (9.0 ± 0.63) of shoots from CN after 8 weeks of culture. However, addition of auxin at lower concentration enhanced the shoot multiplication rate as well as shoots number. Highest number of axillary shoots (16.2 ± 0.20) was achieved on MS medium containing BA (5.0µM) and NAA (0.5µM).
From aseptic nodal explants, the maximum regeneration frequency (71%) with highest number (9.4 ± 1.02) of shoots per explant and shoot length (4.2 ± 0.53 cm.) was obtained on MS medium augmented with BA (7.5 μM) and NAA (0.5 μM).

Nodal segments from field grown plant were also tested for in vitro multiplication in different treatments, representing various combinations of auxin and cytokinins. BA (5.0 μM) + NAA (0.5 μM) + Ads (50 mg/L−1) was found to be the optimum for greatest number (10.0 ± 1.37) of shoots per explant.

The effect of thidiazuron (TDZ) was investigated on multiple shoot induction from CN explants. The highest shoot regeneration frequency (62%) and maximum number (6.8 ± 0.58) of shoots per explant was recorded on MS medium amended with 0.8 μM TDZ. However, the cultures grown continuously on TDZ containing medium formed fasciated and distorted shoots. However, the problem was overcome by subculturing such cultures on TDZ free MS medium.

Nodal segments obtained from in vitro culture were encapsulated in calcium alginate hydrogel containing MS medium. 3% sodium alginate and 100 mM CaCl₂·2H₂O were found most suitable for synthetic seed production. MS medium supplemented with BA (5.0 μM) and NAA (0.5 μM) gave the maximum frequency (63%) of conversion of encapsulated nodal segments into plantalets with a maximum of (4.6 ± 0.33) shoots after 8 weeks of culture.

Cold storage of synthetic seeds was also carried out at various time period (0, 1, 2, 3, 4 and 5 weeks) and it was found that after 4 weeks of cold storage (4°C), the percent conversion frequency decreased considerably.
The regenerated shoots were rooted satisfactorily on MS liquid medium supplemented with 2.5 μM IBA and 5.0 μM chlorogenic acid. Maximum frequency (70%) of root formation and the highest root length (2.3 ± 0.35) has achieved after 4 weeks.

The *in vitro* raised plantlets with well developed shoots and roots were successfully acclimatized in sterile soilrite and showed 60% survival ability.

**Pioneering steps to establish protocol for micropropagation in *B. tomentosa*, in vitro leads to the following conclusions:**

1. Seed germination can be enhanced by using *in vitro* method by using \( \frac{1}{2} \) medium.

2. Direct multiple shoot regeneration could be obtained from CN, aseptic and field grown nodal explants using cytokinins (BA, Kn, 2-iP) either solely or in combination with auxins (IAA and NAA) and an additive (Ads).

3. Direct multiple shoot formation was achieved from CN explants using TDZ alone.

4. Maximum shoot multiplication was achieved on MS medium containing BA (5.0 μM) + NAA (0.5 μM) in CN and BA (7.5 μM) + NAA (0.5 μM) in aseptic nodal explants.

5. BA (5.0 μM) + NAA (0.5 μM) gave maximum response in nodal segments obtained from field grown plant.

6. Best rooting was achieved in liquid culture on filter paper bridge with IBA (2.5 μM) and chlorogenic acid (5.0 μM).
7. The *in vitro* regenerated plantlets were successfully hardened off in soilrite followed by their transfer to garden soil.

8. Encapsulated nodal segments showed maximum conversion frequency on MS medium containing BA (5.0μM) and NAA (0.5μM) and retained their viability even after 4 weeks of storage at 4°C.

The present study describes the successful production of *B. tomentosa* in sufficient number, highlighting the role of tissue culture in multiplication of this economically important multipurpose plant.