Chapter 6
Summary
&
Conclusions
SUMMARY AND CONCLUSIONS

In recent years there has been an increased interest in plant tissue culture technique which offers a viable tool for mass multiplication and conservation of species especially those having medicinal or pharmacological value. Most of the medicinal plants are collected from wild population which involve destructive harvesting because of the use of plant parts like root, stem, wood, bark and the whole plant in case of herbs and only a few medicinal plants are commercially cultivated, thus challenging their existence in the nature. Therefore, to meet the future demands and conservation of valuable medicinal plant species, development of *in vitro* regeneration protocols, exploiting techniques of plant tissue culture is mandatory. In the present study two medicinally important species of genus *Cassia* i.e. *C. angustifolia* Vahl. and *C. sophera* Linn. have been selected to develop reproducible and efficient *in vitro* protocols via different modes of regeneration for mass propagation and conservation of these plant species.

6.1 *Cassia angustifolia* Vahl.

Seeds of *C. angustifolia* germinated under *in vitro* culture conditions to raise aseptic seedlings for the collection of different explants for shoot morphogenesis via direct or indirect organogenesis. Maximum seed germination (77.00 ± 2.31%) was obtained on medium comprised of half strength MS supplemented with 5.0 \( \mu \text{M GA}_3 \) after 4 weeks of inoculation, while, germination started within 3.20 ± 0.24 days of inoculation. Three different explants viz.: CN, NS and ST excised from axenic seedlings at different age were cultured on various concentrations of cytokinins (BA, Kn and 2iP) or auxins (IAA, IBA and NAA) singly or in combinations. CN explants excised from 14 days old seedlings showed early and enhanced regeneration compared to those from 7 and 21 days old seedlings, similarly NS and ST explants taken from 21 days old seedlings provided better response than explants of 14 and 28 days old seedlings.
CN explant proved to be the best explant amongst all the three explants tested for direct shoot regeneration in *C. angustifolia* on various hormonal treatments. Amongst three cytokinins tested, 5.0 µM BA found to be the optimal concentration producing the highest 25.33 ± 0.20 shoots/explant attaining a maximum shoot length of 4.33 ± 0.20 cm in 75.40 ± 0.30% cultures through CN explants after 6 weeks of inoculation. Explants cultured on Kn supplemented media, provided optimal response at higher concentration i.e. 7.5 µM, producing an average of 8.96 ± 0.20 shoots/explant with shoot length of 4.23 ± 0.14 cm in 54.60 ± 0.23% cultures. While, 2iP proved to be the least effective cytokinin in *C. angustifolia* providing merely 6.10 ± 0.20 shoots/explant having shoot length of 3.26 ± 0.17 cm in 42.40 ± 0.30% cultures. Thus, BA proved to be the best cytokinin followed by Kn and 2iP (BA > Kn > 2iP).

Addition of lower concentrations of auxins with cytokinin enhanced the shoot multiplication rate and the explants cultured on BA (5.0 µM) + NAA (0.6 µM) combination produced a maximum of 39.16 ± 0.14 shoots/explant with shoot length of 5.63 ± 0.20 cm in 85.26 ± 0.17% cultures after 6 weeks. Cultures were maintained and transferred to fresh medium of the same composition up to 6 subculture passages of 6 weeks interval. Addition of adenine sulphate (AdS) at various concentrations to the regeneration medium after first subculturing, found effective to overcome the adverse effects of leaf abscission and shoot tip necrosis. Hence, the medium comprised of MS + BA (5.0 µM) + NAA (0.6 µM) + AdS (30.0 µM) proved to be the best regeneration medium for multiplication and proliferation of shoots in *C. angustifolia*. Differentiation and multiplication of shoots continued up to 4th subculture passage, where the highest 64.90 ± 0.32 shoots/explant having shoot length of 6.56 ± 0.23 cm were obtained. The emergence of shoots became constant at 5th subculture passage and thereafter a decline in regeneration potential was observed.

The CN explants cultured on a wide range of TDZ concentrations responded similar as the single BA treatment on shoot morphogenesis. Optimal response was recorded at 2.5 µM TDZ with the production of maximum 28.53 ± 0.99 shoots/explant having shoot length of 4.03 ± 0.14 cm in 94.33 ± 2.33% cultures after 6 weeks. However, prolonged exposure of TDZ resulted in certain anomalies and retarded the elongation in shoots. Therefore, a two-step culture
procedure was adopted in which the shoots regenerated on 2.5 µM TDZ were further transferred to MS medium devoid of TDZ as well as to the medium containing lower concentrations of BA after 6 weeks. The regenerated shoots showed growth and development of new shoots on BA containing medium and the highest 45.50 ± 0.51 shoots/explant having shoot length of 6.20 ± 0.11 cm were obtained at 2.5 µM BA after 6 weeks. The regenerative tissue was regularly subcultured on to the fresh medium containing 2.5 µM BA at an interval of 6 weeks but the number of shoots remained constant and 45.50 ± 0.51 shoots/explant were obtained at the end of each subculture passage up to second subculturing and beyond that a decline in regeneration efficacy was recorded. Thus, TDZ induced cultures could not be maintained for long term multiplication and proliferation of shoots.

The augmentation of 5.0 µM BA proved to be optimal for nodal explants, where a maximum of 20.20 ± 0.11 shoots/explant with shoot length of 4.20 ± 0.11 cm was obtained in 66.00 ± 1.15% cultures after 6 weeks of inoculation. Regeneration efficacy of the explant further enhanced on cytokinin-auxin combination treatments and reached to the maximum 30.33 ± 0.24 shoots/explant having shoot length of 5.26 ± 0.14 cm in 79.33 ± 1.79% cultures on MS + BA (5.0 µM) + NAA (0.6 µM) in 6 weeks of incubation. TDZ at an optimal concentration of 2.5 µM exhibited maximum regeneration efficiency (86.00 ± 2.30%), producing an average of 21.70 ± 0.62 shoots/explant with shoot length of 3.80 ± 0.17 cm after 6 weeks. TDZ induced cultures were further transferred to BA containing medium for growth and proliferation. The medium comprised of 2.5 µM BA enhanced the number of shoots to 32.03 ± 0.31 shoots/explant attaining maximum shoot length of 5.66 ± 0.17 cm after 6 weeks.

Like CN and NS explants, ST explants also exhibited best regeneration on cytokinin-auxin combinations, producing the maximum 22.46 ± 0.29 shoots/explant with shoot length of 4.00 ± 0.11 cm on medium comprised of MS + BA (5.0 µM) + NAA (0.6 µM) after 6 weeks. ST explants were also cultured on different concentrations of TDZ and the same strategy of two-step culture procedure was adopted in which TDZ (2.5 µM) induced cultures (15.06 ± 0.1 shoots/explant) were transferred to medium containing lower concentrations of BA. Medium containing 2.5 µM BA improved the regeneration efficacy and the
shoot number increased to $25.90 \pm 0.20$ shoots/explant with shoot length of $4.56 \pm 0.23$ cm after 6 weeks.

Various factors like composition of culture medium, pH of the medium and concentration of sucrose affected the shoot morphogenesis and vary from species to species. In the present study all these factors were optimized for different explants (CN, NS and ST) for maximum shoot regeneration and it was found that among different media tested (B$_5$, L$_2$, MS and WPM), MS medium at pH value of 5.8 with 3% sucrose provided highest response for shoot multiplication and proliferation in *C. angustifolia*.

For indirect organogenesis, three different explants namely cotyledonary leaf (CL), leaf (L) and root (R) were taken from seedlings of different age group and cultured on different concentrations of cytokinins and auxins. Among these three explants, CL explants (14 days old) and root (30 days old) explants found to be efficient for the production of multiple shoots via callus formation, while leaf derived callus failed to induce shoot differentiation. Root explant produced compact and nodular organogenic callus on medium supplemented with TDZ (1.0 µM) with $90.66 \pm 1.45\%$ regeneration response, while the cotyledonary leaf explants produced regenerative callus on 2,4-D (5.0 µM) containing medium suggesting that the efficiency of callus induction may vary within the same plant species depending on the type of explants used.

Callus derived through root explants on TDZ (1.0 µM) was transferred to different concentrations of cytokinins and the optimal response was obtained at 2.5 µM BA with the production of $24.56 \pm 1.97$ shoots/explant having shoot length of $4.70 \pm 0.26$ cm after 6 weeks. Incorporation of auxins with optimal BA concentration facilitated better shoot differentiation from the callus and a maximum of $35.63 \pm 0.75$ shoots/explant having shoot length of $5.43 \pm 0.20$ cm were produced in $90.33 \pm 1.45\%$ cultures on MS + BA (2.5 µM) + NAA (0.6 µM) after 6 weeks. Similarly, cotyledonary leaf derived callus showed maximum shoot differentiation ($23.16 \pm 1.44$ shoots/explant) with an average shoot length of $5.00 \pm 0.26$ cm in $96.33 \pm 1.45\%$ cultures on medium comprised of MS + BA (5.0 µM) + NAA (0.4 µM), although the number of shoots was less compared to root explant.
Cotyledonary leaf derived callus could not be used for long term maintenance and proliferation of shoots, as a sudden decline in the regeneration potential of the callus was observed just after first subculture passage. However, on the other hand, root derived callus was regularly subcultured on to the fresh medium of optimal hormonal composition [MS + BA (2.5 µM) + NAA (0.6 µM)] at an interval of 6 weeks. Elongated shoots were isolated and transferred to rooting media at the end of each subculture passage. Differentiation of shoot buds with multiplication and proliferation of shoots continued up to 4th subculture passage where the highest 42.66 ± 1.47 shoots/explant with shoot length of 6.46 ± 0.12 cm were obtained and thereafter decline in regeneration potential was observed.

Immature cotyledons excised from green seeds of semi mature pods found ideal for the production of embryogenic callus in *C. angustifolia*. A number of treatments containing different auxins were tested for the induction of somatic embryogenesis. Best response (83.90 ± 1.70%) was obtained at 10.0 µM 2,4-D with the production of 9.23 ± 0.67 embryoids/explant after 6 weeks of culture. The embryogenic response further enhanced (90.56 ± 1.88%) with the addition of 1.0 µM BA along with optimal 10.0 µM 2,4-D, wherein a maximum 22.80 ± 1.59 embryoids/explant was obtained with 35.33 ± 2.90% germination on the same medium.

Regenerated microshoots of 3-4 cm length were excised from the cultures and rooted either *in vitro* or *ex vitro* for the development of complete plantlets. The gelling substance used to solidify the rooting medium had great impact on *in vitro* rooting in *C. angustifolia*. Best rooting response was obtained in liquid rooting medium (without agar) compared to agar or phytagel gelled rooting medium. Half strength liquid MS medium supplemented with IBA (1.0 µM) found to be optimal for *in vitro* root induction in 67.33 ± 2.60% microshoots with maximum of 3.93 ± 0.29 roots/shoot having root length of 3.00 ± 0.28 cm after 4 weeks. Rooting percentage further enhanced by the incorporation of phloroglucinol (PG) at 5.0 µM with optimal rooting medium and produced maximum 5.56 ± 0.23 roots/shoot having root length of 6.23 ± 0.14 cm after 4 weeks.

*Ex vitro* rooting provided an alternative way for the induction of roots in sterile soilrite through pulse treatment with an auxin. In the present study, microshoots
were best rooted through pulse treatment with 200 µM IBA for 30 min, producing an average of 4.83 ± 0.24 roots/shoot having 5.80 ± 0.23 cm of root length after 4 weeks. Ex vitro rooting proved to be beneficial, cost effective and required less time compared to in vitro rooting as hardening and acclimatization of plantlets took place simultaneously.

In vitro rooted plantlets were hardened and acclimatized under controlled conditions on three different planting substrates namely garden soil, soilrite and vermiculite. Among all the three planting substrates tested, maximum survival percentage was recorded in soilrite (89.33 ± 2.33%) followed by vermiculite (64.66 ± 2.60%) and garden soil (54.66 ± 2.90%).

Acclimatized plantlets were successfully transferred to the green house under shade and then finally to the field conditions with 90% survival. No detectable phenotypic variations were observed in the in vitro raised plantlets when compared with in vivo grown plants.

For the production of synthetic or artificial seeds, the in vitro derived nodal segments excised from one month old microshoots were encapsulated for short term storage and conservation of propagules. Encapsulation was best achieved with 3% sodium alginate as gelling matrix and 100 mM calcium chloride as complexing agent for the production of ideal beads with uniform texture. Conversion or regrowth of the shoots from the beads was obtained on optimal medium comprised of MS + BA (2.5 µM) + NAA (0.4 µM) with highest 94.06 ± 1.56% conversion response after 6 weeks. Microshoots were isolated from the cultures and rooted either in vitro or ex vitro through optimized protocols. However, direct sowing of synseeds in sterilized soilrite or ex vitro sowing resulted in simultaneous production of shoots as well as roots in 20% beads within 6 weeks.

Encapsulated as well as non-encapsulated nodal segments were stored at 4°C for different time periods and then germinated on optimal medium to assess the reproducibility of the explant after cold storage. After 4 weeks of storage at 4°C encapsulated nodal explants showed better conversion response (72.30 ± 1.21%) compared to non-encapsulated nodal explants (33.33 ± 1.75%) on medium containing MS + BA (2.5 µM) + NAA (0.4 µM). However, further
increase in the storage duration reduced the reproducibility of the explant and after 8 weeks of storage, only 43.90 ± 1.79% and 12.80 ± 1.32% conversion was recorded in encapsulated and non-encapsulated explants.

Different physiological parameters like content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were evaluated at different acclimatization periods of regenerated plantlets. All the pigments showed an initial drop in the content during first week of acclimatization but during subsequent weeks a linear increase in pigments content was recorded. Net photosynthetic rate (P_N ratio) was also evaluated and it showed the same trend, decreased during the first week of acclimatization and as soon as the plantlets get acclimatized to the external environment, P_N ratio increased linearly.

Histological observations at different developmental stages of regenerative tissue obtained through CN explants revealed the origin of shoot buds directly from the mother explant without any intervening callus tissue, while the CL and root derived callus revealed the mode of differentiation of shoot buds in callus tissue, confirming indirect organogenesis. Histology of embryogenic callus at various developmental stages confirmed indirect origin of somatic embryos through immature cotyledons.

6.2 Cassia sophera Linn.

In C. sophera also, seeds were germinated in vitro to collect various explants for shoot morphogenesis via different modes of regeneration. Maximum seed germination (99.33 ± 0.66%) was achieved on half strength MS medium containing 1.0 µM GA_3 after 4 weeks of inoculation and the germination started within 4.46 ± 0.14 days of inoculation. Different explants namely cotyledonal node (CN), nodal segment (NS) and shoot tip (ST) were taken from aseptic seedlings of different age group (14, 21 and 28 days old) for direct shoot regeneration. All the three different types of explants collected from 21 days old seedlings found to be more responsive compared to 14 and 28 days old seedlings. Among three explants tested, NS proved to be the best explant for shoot morphogenesis in this plant species.

Nodal explants were cultured on different concentrations of three cytokinins (BA, Kn and 2iP) singly or in combination with various auxins at lower concentrations
for direct shoot induction. Amongst various concentrations of cytokinins, optimal response (90.33 ± 3.17%) was induced at 5.0 µM BA with the production of an average 16.46 ± 1.21 shoots/explant having shoot length of 4.80 ± 0.37 cm after 6 weeks. Kn and 2iP provided optimal regeneration at higher concentration i.e. 7.5 µM. The explants cultured on 7.5 µM Kn produced 7.60 ± 0.34 shoots/explant with the shoot length of 3.66 ± 0.20 cm in 62.33 ± 1.45% cultures. While, 2iP provided least number of shoots (5.16 ± 0.49 shoots/explant) having an average shoot length of 3.10 ± 0.20 cm in 53.33 ± 2.02% cultures. Thus, amongst three cytokinins tested, BA proved to be the best cytokinin in *C. sophera* also.

The regeneration potential of the explant further increased when cultured on cytokinin-auxin combination treatments and the highest frequency (97.33 ± 1.45%) of shoot production (25.36 ± 0.34 shoots/explant) having maximum shoot length (6.23 ± 0.24 cm) was obtained on MS + BA (5.0 µM) + NAA (1.0 µM) after 6 weeks. Regenerative tissues were subcultured on to the fresh medium of same composition and maintained up to 6 subculture passages of 6 weeks interval. At the end of each subculture passage elongated shoots were harvested and transferred to rooting medium. The regeneration medium was supplemented with AdS at different concentrations to prevent shoot tip necrosis and yellowing and abscission of leaves. In this regard the medium comprised of MS + BA (5.0 µM) + NAA (1.0 µM) + AdS (20.0 µM) proved to be the best for culture multiplication and proliferation on which differentiation of shoots continued up to 4<sup>th</sup> subculture passage and the highest 42.33 ± 0.32 shoots/explant having shoot length of 7.76 ± 0.14 cm were obtained. Beyond 4<sup>th</sup> subculture passage reduction in regeneration frequency was observed and the number shoots declined afterwards.

The treatment of TDZ at various concentrations, although induced multiple shoots through nodal segments, but the shoots were stunted and showed abnormal features on prolonged culture incubation. Optimal response (95.00 ± 1.15%) was obtained at 2.5 µM TDZ with the production of an average 13.76 ± 0.38 shoots/explant having shoot length of 3.26 ± 0.14 cm after 6 weeks. To avoid the deleterious effects of TDZ, regenerating cultures were subsequently transferred on to fresh medium devoid of TDZ as well as to medium containing
lower concentrations of BA. The number of shoots increased to the maximum 19.83 ± 0.02 shoots/explant with shoot length of 6.00 ± 0.11 cm when the medium was supplemented with 1.0 µM BA. Thus, the two-step culture procedure proved effective in this plant species also but the overall production of shoots and regeneration potential of the explant was better in cytokinin-auxin combination medium compared to TDZ supplemented medium.

CN explants cultured on different concentrations of cytokinins also provided optimal response at 5.0 µM BA with the production of an average 12.43 ± 0.29 shoots/explant with shoot length of 4.23 ± 0.26 cm in 78.66 ± 2.33% cultures after 6 weeks. The combination of cytokinin-auxin treatments showed synergistic effect on shoot multiplication and the highest number of shoots (19.50 ± 0.51 shoots/explant) having shoot length of 5.23 ± 0.14 cm were obtained in 86.00 ± 2.08% cultures on MS + BA (5.0 µM) + NAA (1.0 µM) through CN explant after 6 weeks.

The CN explants cultured on TDZ supplemented media showed a poor response with the production of only 6.73 ± 0.17 shoots/explant having shoot length of 2.26 ± 0.14 cm in 93.00 ± 1.52% of cultures on MS + TDZ (2.5 µM) after 6 weeks. Again it was required to transfer the regenerative tissue to TDZ free medium to retrieve the regenerability of the tissue and for that lower concentrations of BA (1.0 µM) proved to be effective and improved the number of shoots to 14.90 ± 1.35 shoots/explant with shoot length of 5.76 ± 0.23 cm after 6 weeks.

Similar to C. angustifolia, ST explant of C. sophera also exhibited least response on various hormonal supplements. Maximum regeneration was observed in cytokinin-auxin combination with the production of 10.56 ± 0.23 shoots/explant having shoot length of 4.80 ± 0.11 cm in 69.66 ± 1.45% cultures on MS + BA (5.0 µM) + NAA (1.0 µM) after 6 weeks. The cultures induced at 2.5 µM TDZ showed an increase in shoot regeneration from initial 4.70 ± 0.20 shoots/explant to a maximum of 8.56 ± 0.23 shoots/explant when transferred to MS + BA (1.0 µM) with shoot length of 5.20 ± 0.11 cm in 6 weeks of incubation.

Different factors like nutrient composition of the culture medium, pH of the medium and concentration of sucrose were optimized through all the three explants for maximum regeneration. Amongst all the four media tested (B5, L2,
MS and WPM), MS medium supplemented with 3% sucrose at pH 5.8 provided optimal response through all the explants and thus used throughout the study.

For the production of callus, three different explants viz.: cotyledonary leaf (CL), leaf (L) and root (R) excised from axenic seedlings of different age were cultured on various concentrations of cytokinins and auxins. Among these three explants, CL explants excised from 14 days old seedlings provided best regenerative callus compared to leaf explants (30 days old), while, root explant failed to induce callus on all the concentrations of hormones. The optimal growth of callus (73.40 ± 0.45%) was obtained through CL explants on medium containing 5.0 µM 2,4-D with the production of green compact and nodular callus. Whereas, leaf explants showed an average growth (53.53 ± 0.81%) of green compact and nodular callus on 7.5 µM of 2,4-D.

Multiple shoots were differentiated from cotyledonary leaf derived callus on MS medium containing cytokinin and auxins singly or in combination, while leaf derived callus failed to differentiate shoots. The highest 14.63 ± 0.23 shoots/explant was produced from CL derived callus on MS + BA (2.5 µM) + NAA (0.5 µM) with shoot length of 5.73 ± 0.20 cm in 74.83 ± 0.32% cultures after 6 weeks of transfer. The regenerative tissue of CL explants regularly subcultured on to the fresh medium after 6 weeks interval on optimal medium comprised of MS + BA (2.5 µM) + NAA (0.5 µM). Multiple shoots continued to differentiate up to 2nd subculture passages where a maximum of 20.60 ± 0.45 shoots/explant with shoot length of 6.43 ± 0.23 cm were obtained and thereafter decline in the regeneration potential was observed.

Microshoots were rooted through both in vitro and ex vitro methods. In vitro rooting was best achieved on agar gelled half strength MS medium supplemented with 1.0 µM IBA producing a maximum of 5.70 ± 0.47 roots/shoot having root length of 5.63 ± 0.49 cm after 4 weeks. Phytagel gelled rooting medium although provided more number of roots/shoot (7.63 ± 0.23) on optimal rooting medium, but, the average root length (4.66 ± 0.35 cm) was less than agar gelled medium. Ex vitro rooting was obtained through pulse treatment with 200 µM IBA for 30 min and subsequently transferring the microshoots to sterile
soilrite with the production of maximum 5.06 ± 0.17 roots/shoot having root length of 5.93 ± 0.17 cm in 90.66 ± 2.96% microshoots after 6 weeks.

Rooted plantlets were hardened and acclimatized in three different planting substrates before transplanting in the external environment. Maximum survival of plantlets was observed in soilrite (97.66 ± 1.45%) followed by vermiculite (90.66 ± 2.33%) and garden soil (70.66 ± 2.96%). After acclimatization period of 4 weeks under controlled conditions, plantlets were successfully transferred to field conditions with 90% survival rate.

Synthetic seeds were prepared through encapsulation of in vitro derived nodal segments of one month old microshoots. Ideal beads with uniform texture were obtained through 3% sodium alginate and 100 mM calcium chloride. Maximum conversion of beads (92.33 ± 1.45%) into shoots was observed on optimal medium comprised of MS + BA (2.5 µM) + NAA (0.5 µM) after 6 weeks. The microshoots were rooted either in vitro or ex vitro on optimized rooting media. Ex vitro sowing of synseeds in sterilised soilrite produced shoots as well as roots simultaneously in 30% beads after 6 weeks of transfer.

The effect of cold storage for short term conservation and reproducibility of the explant was tested by storing encapsulated and non-encapsulated nodal segments at 4°C for different time period. Encapsulated explants exhibited an average of 64.00 ± 3.05% conversion on optimal regeneration medium after a storage period of 4 weeks on 4°C, while, the non-encapsulated explants showed only 25.00 ± 2.88% conversion on the same concentration after 4 weeks of cold storage. The conversion percentage further decreased on increasing the storage duration of both encapsulated and non-encapsulated explants.

Estimation of different pigment contents was made during different acclimatization periods of regenerated plantlets. Contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids decreased during the first week of acclimatization but later on increased linearly in subsequent weeks of acclimatization. Net photosynthetic rate (P_N ratio) also showed the same trend and decreased during first week while later on as the plantlets get adapted to the ex vitro conditions, P_N ratio increased linearly.
Histology of the regenerative tissue obtained through NS explant confirmed the direct origin of shoot buds from the explant without callus formation, while CL derived callus revealed the formation of numerous meristemoids which lead to the development of shoot buds and thus confirming indirect mode of organogenesis.

**Conclusions**

The following conclusions have been drawn from the present study:

1. *In vitro* raised aseptic seedlings were used for the collection of various explants in both *C. angustifolia* and *C. sophera*.

2. In *C. angustifolia* maximum seed germination was recorded on half strength MS medium supplemented with 5.0 µM GA$_3$, whereas in *C. sophera* highest seed germination was obtained on half strength MS medium containing 1.0 µM GA$_3$.

3. MS medium supplemented with 3% sucrose and pH adjusted at 5.8 was found optimum for shoot morphogenesis in both the plant species.

4. Direct shoot regeneration was achieved through cotyledonary node (CN), nodal segment (NS) and shoot tip (ST) explants on various cytokinins and auxins singly or in combination in both the plants.

5. Age of the explant showed a great impact on shoot multiplication rate, thus different explant were excised from most responsive stage of the seedlings.

6. In *C. angustifolia*, CN explant excised from 14 days old axenic seedlings was found most responsive compared to NS and ST explants. While, in *C. sophera*, NS (21 days old) proved to be the best explant in comparison to CN and ST explants.

7. BA at 5.0 µM found to be the best concentration of cytokinin for induction of multiple shoots in both the plants irrespective of the explant type.
8. Augmentation of lower concentrations of auxins (IAA, IBA and NAA) with optimal BA concentration (5.0 µM) found to be highly efficient for the production of maximum shoots in both the plant species.

9. In *C. angustifolia* maximum shoots/explant were produced on MS medium supplemented with BA (5.0 µM) + NAA (0.6 µM) through CN explants, whereas, in *C. sophera*, NS explants produced maximum shoots/explant on medium comprised of MS + BA (5.0 µM) + NAA (1.0 µM).

10. Adenine sulphate (AdS) at a concentration of 30 µM and 20 µM was added to the optimal regeneration medium in *C. angustifolia* and *C. sophera* respectively, to prevent yellowing or abscission of leaves and shoot tip necrosis during subculture passages.

11. TDZ at 2.5 µM proved optimal for maximum shoot regeneration through CN and NS explants in *C. angustifolia* and *C. sophera* respectively.

12. Indirect organogenesis has also been achieved in both the plant species through various explants viz.: cotyledonary leaf (CL), leaf (L) and root (R) explants.

13. In *C. angustifolia*, compact and nodular callus was induced through root explant (30 days old) at 1.0 µM TDZ, while, CL explant (14 days old) produced compact and nodular callus at 5.0 µM 2,4-D in both the plants.

14. Medium comprised of MS + BA (2.5 µM) + NAA (0.6 µM) proved efficient for maximum shoot differentiation from root derived callus in *C. angustifolia*. Whereas, in *C. sophera*, maximum differentiation of shoots was achieved through CL derived callus on MS + BA (2.5 µM) + NAA (0.5 µM).

15. Embryogenesis was induced in *C. angustifolia* through immature cotyledons excised from semi mature seeds, while in *C. sophera* no embryogenic response was obtained on any treatment from various explants tested.

16. Best embryogenic response was obtained on MS medium supplemented with 2,4-D (10.0 µM) and BA (1.0 µM).
17. Microshoots were rooted through both *in vitro* and *ex vitro* modes of rooting in both the plants.

18. *In vitro* rooting was best achieved on half strength liquid MS medium supplemented with 1.0 µM IBA and 5.0 µM PG in *C. angustifolia*. Whereas in *C. sophera*, agar gelled rooting medium comprised of half strength MS + 1.0 µM IBA found effective for maximum root induction.

19. *Ex vitro* rooting through pulse treatment with 200 µM IBA for 30 min found beneficial in both the plants as hardening and acclimatization took place simultaneously with rooting.

20. Synseed production with 3% sodium alginate and 100 mM calcium chloride proved to be ideal for the formation of uniform beads in both the plants.

21. Maximum conversion of beads was recorded on MS + BA (2.5 µM) + NAA (0.4 µM) in *C. angustifolia*, while, in *C. sophera* medium comprised of MS + BA (2.5 µM) + NAA (0.5 µM) provided maximum conversion of synseeds.

22. Synseeds were successfully stored at 4ºC for 8 weeks for short term conservation and provided satisfactory conversion into microshoots after 4 weeks of storage.

23. Amongst different planting substrates tested (garden soil, soilrite and vermiculite), soilrite was found to be the best planting substrate for maximum survival of regenerated plantlets in both the plants.

24. During acclimatization of regenerated plantlets increase in pigment contents and net photosynthetic rate was observed from an initial decline during first week of acclimatization in both the plants.

25. Histological sections clearly revealed the mode of regeneration via direct or indirect organogenesis as well as somatic embryogenesis.