CHAPTER 6: CONCLUSION

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The purpose of this investigation or study is to validate *Rheum emodi's* clonal propagation., which is an endangered herb, through the callus inducing propagation from the leaf explant. Plant tissue culture is one of the most important methods that is regularly used for efficient preservation of several species of plants that are endangered or threatened. In *Rheum* species, natural or vegetative propagation is not that much satisfactory and even less successful through the common regeneration methods. Therefore, regeneration through *invitro* propagation of *Rheum* species is an effective substitute for the mass multiplication to solve the environmental concerns or problems caused by a lack of planting material.

Sterilization procedure was optimized. Less exposure or no sterilant contribute to the more contamination and no germination while the increasing time contributes to reduced contamination but also effects the growth as they prove lethal to the leaves. For the induction of callus, different auxin concentration was tested, from which the best percentage of callus formation was observed in the nutrient media that is MS medium "Murashige and Skoog medium" perforated with the plant growth regulators such as 2,4-dichlorophenoxyacetic acid (2,4-D) along with 6- Benzylaminopurine (BAP). Result showed that this combination can proved to be the most appropriate combination of hormones for inducing callus from the leaves explants of *Rheum emodi*. Further, the decrease in callusing was detected in the leaves explants that were cultured in the MS basal medium without any plant growth regulators or hormones.

The maximum and best *in vitro* shoot proliferation with significant growth was obtained on BAP (6- Benzylaminopurine) in combination with Kn (Kinetin). This combination worked best for producing the most shoots per explant and a substantial mean length for the development of adventitious shoots.

The frequency of shoot regeneration was decreased when treated with TDZ (Thidiazuron) alone. TDZ was found to be a weak cytokinin as compared to BAP and Kn for *in vitro* shooting when cultured on MS media. Sucrose at 3% and 4% in the MS medium gave the best results with 5-6 folds *invitro* shoot multiplication with maximum shoots number and with considerable mean number of shoots. At lower concentration of sucrose (1-2 %), 1-2-fold *in vitro* shoot multiplication was recorded with deprived growth of shoots. Increased percentage of sucrose ranges (5-6%) leads to retarded growth of shoots along with the yellowing of leaves and basal callusing of shoots. The shooted cultures were then transferred to fresh media, MS basal medium (full strength) with IAA "Indole-3-acetic acid" along with BAP is considered for apt rooting. The seven weeks old plantlets that were directly transfer to polybags without acclimatization have very poor survival rate even when transplanted in rainy season. In contrast, a survival percentage of 85–90% was attained when plantlets were hardened and acclimated prior to being planted in the field.

Synthetic seed technology offers great potential in micropropagation. For synthetic seed production, the MS media with 3-4 % of sodium alginate that were treated with the 50 mM $CaCl_2$ produced the circular shaped, coat firm enough for handling beads. The survival and germination probabilities of the encapsulated embryos decreased from 46% to 27% after storage at 4°C; as compared to encapsulated embryos without storage.

In present study, the survival and the germination percentage of non-encapsulated versus encapsulated embryos are compared. The storage signifies the reduction in both the survival and germination rate of naked and the encapsulated embryos in comparison to the embryos which have not been stored. After 30 days of storage, encapsulated embryos had a lower germination rate than after 20 days.

The somaclonal variation in many therapeutic plants has been successfully detected using molecular markers. If the desired final outcome is true to type plants, genetic stability of *in vitro* regenerants must be assessed. The total genomic DNA was isolated using the CTAB technique from randomly chosen regenerants and the mother plant in order to determine the clonal fidelity. 20 ISSR markers and 15 RAPD markers were

assayed. Out of which, 7 RAPD and 15 ISSR primer sets generate 98 distinct amplicons that give rise to the distinct, clear, monomorphic patterns among all the randomly selected regenerated plants that were examined.

Results shows that there is no polymorphism within the RAPD and ISSR analysis among the *invitro* raised plantlets and the mother plant, all the markers produce the monomorphic bands and no polymorphism was detected among the micropropagated plants which confirms the genetic homogeneity of *invitro* raised plantlets. These markers could be proved to be an ideal tool for schedule analysis of genetic stability within the micropropagated plantlets prior to commercialization.