CHAPTER 7: SUMMARY

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In the world map, India belongs to one of the 12th mega diversity countries having 10 bio geographic provinces. Among the world's 8 biodiversity hotspots, India alone includes two since it has extremely high levels of endemicity, richness in species. The altitudinal and climatic discrepancies with different ecological habitats of this country contributes to the development of an incredibly diverse and rich flora in herbal plants, which proves to be a valuable source of raw materials for the pharmaceutical industries as well as in traditional medicinal systems. For medicinal purpose, WHO has recorded over 21000 plant species and about 2500 plant species in India. It was estimated that about 32% of plants in India are endemic and not found elsewhere. The Northern Himalayas are booming with a wide range of highly demanded medicinal plants.

Rheum constitutes around 60 species around the world and among them 10 species are belong to Indian Himalayan regions. *Rheum emodi* a highly recognized medicinal plant in Uttarakhand, locally known by the name rhubarb or Dolu, is a robust; firm perennial herb belongs to the family 'polygonaceae' and it was cultivated over 5000 years for their medicinal properties. This herb is found throughout the world, particularly in Asia, in temperate, tropical, and subtropical climates. In Himalayas of Garhwal, this species is generally found in Alpine zone having rocky soil in 2800 to 3600 m altitudes. This genus is mainly present in Asian countries like India (northern state Kashmir to Assam state in east), Nepal, China, Bhutan, Pakistan, Tibet, Korea, and Russia.

Its morphological characteristics is described as, a height ranging from 1.5-3.0 m, with broad leaves and thick stem. This perennial herb has small flowers of purple to pale red colour with 3 mm diameter. Roots are dark brown, strong and thick. Rhizomes are dull orange to yellowish brown coloured with a length of 6-12 inches. This medicinal herb covers the wide range of medicinal properties specifically antimicrobial, anticancerous, antifungal, antioxidant, hepatoprotective, wound healing, purgative,

laxative, stomachic, immune enhancing activity, this all gave a name of “wondrous drug” to this world wide used medicinal plant.

Rheum emodi contains a variety of compounds like anthraquinones (chrysophanol, rhein, emodin, aloe emodin, physicon and their glycosides) and stilbenes (picetannol, resveratrol and their glycosides), volatile oils, tannins and saponins. Rhubarb roots powder has a natural healer against wounds. *Rheum emodi* a highly medicinal plant in Uttarakhand has now in endangered list. Local communities use its rhizome for many medicinal properties, its leaves were used as eating purposes, all this leads to its decline in its natural habitat and thus this herb species demands conservation.

In nature, the conventional propagation of medicinal plants through vegetative propagation or seed propagation is slow and this process is very much hampered by the environmental conditions as well as poor germination power of seed even the death of seedlings at 2- leaved stage. Seeds of *Rheum emodi* have a very low viability. After 6 months their germination potential reduced to half. Due to limited seed viability and germination rates, excellent planting material cannot be obtained only by seed multiplication.

Therefore, present study has an aim to establish the clonal propagation protocol for *Rheum emodi* herb through the callus inducing propagation from leaf explant and to assess the *in-vitro* propagated *Rheum emodi* through molecular marker for the pure germplasm and somaclonal variation. The study's findings may show that the molecular markers chosen are the best option for routinely evaluating the genetic integrity of micropropagated plantlets before they are sold, opening the door to more effective conservation methods for the endangered *Rheum emodi* plant. The process for producing synthetic or artificial seeds is also mentioned. Additionally, it was seen how storage affected the encapsulated embryos. These findings might help develop a better conservation plan for the *Rheum emodi* plant, which is in danger of extinction.
One of the most important strategies for effectively conserving and micropropagating many rare and endangered plant species is the use of plant tissue culture techniques. Plant-regeneration through the aseptic tissue culture techniques proves to be a better option for its propagation.

Regeneration through the *in vitro* propagation enables the immense proliferation of many plants, which also includes endangered species as well as species whose efficiency is compromised by unsatisfactory, constrained, or labour-intensive propagation methods. In *Rheum* species, natural or vegetative propagation is not that much satisfactory and even less successful through the common regeneration methods. Therefore, regeneration through *in vitro* propagation of *Rheum* species is an effective substitute for the mass multiplication to solve the problems related and created by the lack of planting material or the environmental issue.

Synthetic seed technology offers great potential in micropropagation. It deals with the storage and conservation of endangered or rare plant species along with their easy handling and transportation. Its synthetic seed production can also lead to its conservation and can be implemented for the mass propagation in forestry.

Thus, the current study focuses on the complete plant regeneration of *Rheum emodi* with assessing the genetic fidelity among the regenerants and synthetic seed production. Sterilization procedure was optimized and After 15 days of monitoring the inoculated explants for growth and contamination, it was found that the mercuric chloride (1 min.) along with 70% ethanol (1 min.) and Sodium Hypochlorite (30 secs) proves to be the best combination for 100% germination and 0% contamination. For the callus induction, different auxin concentration was tested, from which the Murashige and Skoog (MS) medium showed the highest rate of callus development (84.44%) perforated with 8 mg/L of "2,4-D" (2.4-dichlorophenoxyacetic acid) along with the "BAP" (6- benzyl-aminopurine) having concentration of 2.5 mg/L. later, afterward 6 weeks, a fragile yellowish-brown callus was observed. Thus, the result showed that the combination of auxin with cytokinin to be most appropriate combination of hormones for inducing callus from the leaves explants of *Rheum emodi*.

The greatest *in vitro* shoot multiplication with significant growth and development was discovered after 4-5 weeks of subculturing on MS media with 6.0 mg/L BAP (6-Benzylaminopurine) in combination with the 2.5 mg/L of Kn (Kinetin). The highest number of shoots per explant was 3.67 ± 0.27, while the greatest frequency of

adventitious shoot generation was found to be 75.56 ± 0.27% with a mean length of 19.00 ± 1.70 mm. The percentage 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 of 12.22 and maximum percentage recorded as 82.22 ± 0.72 with mean number of shoots were recorded as 20.33 ± 0.72mm. After 15 days, the shooted cultures showing rooting were transferred to the fresh media and then maintained upto 4 weeks.

The MS basal medium (full strength) with IAA (Indole-3-acetic acid) of concentration 5.0 mg/L in combination with BAP (2.0 mg/L) was found to have a high frequency of rooting, with the highest frequency of roots per shoot being 5.0 ± 0.47 with an average root length of 11 ± 1.25mm.

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 conversion of the artificial seeds into plantlets was made practicable by the above-mentioned calcium chloride concentration and sodium alginate concentration of 3%. The propagules showed proliferative potential by survival percentage as 60.0 ± 1.2 on basal MS media with 3% alginate. Encapsulated embryos were stored to 30 days and 60 days to test their survival and the germination rate. When compared to encapsulated embryos that were not stored, after being kept at 4°C, the encapsulated embryos' survival and germination rates dropped from 46% to 27%.

The survival and germination rates of unencapsulated versus encapsulated embryos are contrasted in experiments. Observed values of survival and germination frequencies are obtained after the storage of 20 days to 30 days after the 5 weeks culture. The storage signifies the reduction in both the survival and germination frequencies of naked and the encapsulated embryos in comparison to the embryos which have not been stored. The percentage of encapsulated embryos that germinate after 30 days of storage (22.2%) was lower than the storage of 20 days (44.4%).

Plantlets germinates through this were morphological identical to the mother plant and further cultured in *invitro* condition.

This method is useful for those plants in which production of seeds is very less or the viability of seeds is low or the seeds are very expensive. These approaches could be used in the future for mass propagation of *Rheum emodi*. More studies and experiments should be performed for improved and better output. Development of medicinal plant sector should be done and dissemination of synthetic seeds should be promoted.

The somaclonal variation or genetic variation may appear in micropropagated plantlets due to many reasons and ins and outs associated with the *in vitro* techniques. True- to-type plants are the essential requirement thus the assessing genetic fidelity becomes important. In recent years, molecular approaches are being considered as alternatives for conservation and improvement of various plants through genetic profiling. Different markers have been developed for the authentication of plant species. PCR based markers like RAPD/ISSR involves *in-vitro* amplification of particular sequences by using thermostable DNA polymerase enzyme and specific oligonucleotide primers.

Due to many advantages such as low input cost and low technology required and especially for the research purpose, RAPD become one of the most useful markers for assessing the somaclonal variation. To evaluate genetic integrity of the regenerants, two molecular markers like RAPD and the ISSR are used, as these markers use only a tiny amount of DNA sample, don't entail any radioactive labels, and are additionally quick, easy to handle, cost-effective, and very reliable procedures. The somaclonal variation in many therapeutic plants has been successfully detected using these markers.

Total genomic DNA was recovered by the CTAB technique from the randomly chosen regenerants and the mother plant in order to determine the clonal fidelity. PCR components and conditions are optimized simultaneously and then 20 ISSR markers and 15 RAPD markers were assayed. Out of 15 RAPD and 20 ISSR markers, seven RAPD and fifteen ISSR primer sets generate 98 distinct amplicons that gives 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 regenerated plantlets that were raised by aseptic method and the mother plant. Since all molecular markers results in monomorphic bands, no variation between the micropropagated plants was found, which confirms the genetic homogeneity of *in vitro* raised plantlets. These DNA based molecular markers have acted as versatile tools in different field of biology. These markers could be proved to be an ideal tool for schedule analysis of genetic stability within the micropropagated plantlets prior to commercialization.

As per our knowledge, this could be the primary report in state Uttarakhand that shows the employ of molecular markers to conduct the clonal fidelity of the regenerated plantlets of *Rheum emodi* using the molecular marker RAPD and ISSR molecular markers. The present study also reported the successful assessment of clonal fidelity of *in vitro* generated micropropagated plantlets by using the molecular marker, RAPD and ISSR markers.