4. Problems, prospects and objectives

Reddy and Srivasuki (1990) reported various limitations involved in propagation of Red sanders by natural and conventional means. Natural propagation has some constraints such as prolonged dormancy and poor seed germination (Kalimuthu and Lakshmanan, 1995). Owing to the hardness of the pods, germination is difficult and the percentage of success is comparatively low. Poor pod set was noticed under natural self pollination and pod maturity takes about eleven months after flower opening (Dayanand, 1988). Past experiments on air layering yielded less promising results but the improved techniques with modifications has given 100% success (Reddy and Srinivasuki, 1990).

Earlier studies using conventional clonal propagation methods like grafting and rooted cuttings were not very successful (Dayanand and Lohidas, 1988). Though the tree coppices well, producing root suckers freely, the regeneration and growth is very slow (Ahmed and Nayar, 1984). Vegetative propagation using IBA has been standardized for *P. santalinus* with a view to establish clonal seed orchards, which was not effective (Reddy, 1991). Studies on the development and maturity of the pods in Red sanders revealed that the pods harvested and sown in March gave better results with respect to germination and establishment of seedlings (Dayanand, 1988).

Preliminary investigations on micropropagation of Red sanders was done using nodes and shoot tips derived from *in vitro* regenerated seedlings as explants and shoots differentiated from cotyledonary callus on ¼ MS medium with BAP (3x10⁻⁶ M) and adenine (4x10⁻⁴ M), which was better than other media combinations.
Adventitious shoot formation from the cotyledonary callus occurred on MS medium with BAP (3x10⁻⁶ M) at 28±2°C (Patri et al., 1988). Studies on micropropagation of P. santalinus revealed best response on MS medium with shoot multiplication and leaf differentiation (Mithila and Srivasuki, 1992). Rapid multiplication of “Red sanders” was achieved by culturing mesocotyl explants on B5 medium fortified with 3.0 mgL⁻¹ BAP and 1.0 mgL⁻¹ NAA within a six-week culture period. Shoot tip necrosis expressed in regenerated shoots was controlled. Shoots treated with IAA, NAA and IBA (1mgL⁻¹) each, prior to transferring them to the rooting medium, exhibited better rooting than those with no prior treatment (Anuradha and Pullaiah, 1999).

Successful development of plantlets of “Red sanders” by induction of multiple shoots from shoot tips and transfer of micropropagated plants to soil has been reported on MS medium with 0.2 mgL⁻¹ BAP and 0.1 mgL⁻¹ KN (Lakshmisita et al., 1992). In vitro regeneration in P. santalinus was also achieved when detached cotyledons from in vitro germinated seedlings were cultured on MS medium containing NAA (0.1mgL⁻¹), KN (1mgL⁻¹) and BAP (1mgL⁻¹). The regenerated shoots rooted on ½ strength MS medium with IAA (1mgL⁻¹) and the fully developed plantlets were successfully established in the soil (Arockiasamy et al, 2000). Studies on ex situ conservation of P. santalinus revealed that tissue culture can be used as one of the tools for the conservation of this endangered tree species (Murughesh et al., 1999).

In P. santalinus another problem to be investigated is regarding seed dormancy. The causes for seed dormancy may be due to the exo, endo or combined
factors has still not been reported. In previous reports a dormancy period of one year was stated, hence in order to enhance the germination capacity, seed germination studies were planned to carry out both in in vivo and in vitro conditions.

Germplasm in the form of seed storage was also considered as one of the strategy for conservation for all the selected species i.e, *P. santalinus, R. serpentina* and *R. tetraphylla*.

Conservation of biodiversity on planet earth is a priority, and so is molecular diversity. Polymorphism, a vital component of genetic diversity is considered as an important factor for the survival of a species. It is important not only in the evolutionary point of view, but also for the need of applied biological research. In fact polymorphism patterns of many species with large effective population sizes have been shaped during millions of years by the action of very small selection intensities. Mutations will be lost if such populations dwindle. Therefore, conservation of endangered plant species is a topic of priority area of research.

Analysis and characterization of genetic variation is fundamental to any conservation strategy, whether in situ or ex situ. In the past the genetic diversity analyses of morphological variants was detected by biometrical approaches or protein isoenzyme profiles. The complimentary DNA sequencing and whole array of molecular marker techniques are relevant and helpful for investigating molecular diversity. Hence, the accessions of *P. santalinus* genetic diversity was detected using morphological and molecular markers. In case of *R. serpentina* and *R. tetraphylla*, investigations on genetic diversity and similarity are relevant. *R. serpentina* is a conservation dependent species in various locations. In contrast *R. tetraphylla* is a
weed. Therefore, information on genetic identity between R. serpentina and R. tetraphylla RAPD analysis would be helpful, possibly for genetic improvement of R. tetraphylla. Molecular markers are now routinely used in the management of genetic resources. The objectives of the present investigation are:

a) Extensive field survey and collection of germplasm of P. santalinus, R. serpentina and R. tetraphylla from various locations in different districts of Andhra Pradesh.

b) Ex situ conservation of collected germplasm in the experimental site, field gene bank and seed bank.

c) To examine the viability of the seeds of P. santalinus collected from various locations.

d) To study the morphological and molecular variations among the collected accessions of P. santalinus.

e) To analyse the molecular variations in interpopulations from 6 locations from A.P (India) and intrapopulations (Dulapally, Medak District) of R. serpentina collected from various locations.

f) To study the molecular variations in interpopulations of R. tetraphylla collected from various locations.

g) To understand the genetic similarity between R. serpentina and R. tetraphylla using RAPD markers.