SUMMARY

R. serpentina is vegetatively propagated by stem and root cuttings and has a poor seed viability and low germination percentage. Tissue culture methods for propagation offer an effective and quicker way to overcome the obstacles for production of a large number of propagules. Tissue culture techniques are becoming increasingly popular as an alternative means of plant vegetative propagation. Significant advantage is offered by the aseptic methods of clonal propagation over the conventional methods and a large number of plants can be produced from a single individual.

Tissue culture methods would be a valuable alternative for rapid propagation and conservation of this valuable threatened plant species.

In the present investigation various *in vitro* aspects of tissue culture have been tried with different explants of *R. serpentina*. Young branches of field grown plants were used for primary establishment of cultures. For shoot proliferation, shoot tips and nodal explants from 8-9 months old field grown plants were used as explant sources.

Callus formation at the basal portion of the nodal explants and shoot tips inhibited growth of the axillary buds in a high percentage of cultures. It was observed that the frequency of callus formation was greater in cultures of shoot tips than nodal explants. Formation of the base callus significantly decreased the frequency of multiple shoot formation, number of shoots per explant and also shoot length.

Increasing the BA concentration increased the percentage of explant produced shoots and the optimum concentration was 2.0 mg/ l irrespective of concentrations of NAA used.

Using GA_3 with BA and Kn did not increase the number of shoots per explant like BA with NAA and Kn with NAA. Optimal concentration of GA_3 was 0.1 mg/ l with 2.0 mg/ l BA and 0.5 mg/ l with Kn.

In the present investigation, MS medium supplemented with different auxin-cytokinin combinations was used to study the callusing response. Field grown plants and *in vitro* grown plants were used for this purpose. It was found that callus proliferation strictly depended on exogenous hormone supplementation. In absence of exogenous hormone, explants failed to induce callus and became necrotic and died within a few days.

Among the four auxins used, 2,4-D was found to be the best in respect of callusing response. On the other hand, BA was superior to Kn for callus growth when supplemented with 2,4-D.

The combination of 2,4-D and BA was the most effective formulation for both explants (internode and leaves) used in the induction of callus and growth of callus, whereas IBA alone or either with BA or Kn was the least effective to induce callus formation.

Field grown explants failed to induce root formation when placed in a culture medium. However, shoots obtained from the first and the second subcultures failed to induce root formation. Rooting took place only when explants were taken from third subcultures. NAA was superior to other auxins when used singly or combinedly with

the other auxins for rooting. Further, the percentage of cultures responded to rooting was always higher in shoot tip than nodal explants having one axillary bud.

The results clearly show that both the shoot tips and nodal explants of *R. serpentina* plant are capable of producing multiple shoots *in vitro* and subsequently root to form complete plantlets.

In order to know and compare reserpine content in shoots and leaves of *in vitro* grown plants, reserpine content of field grown plants were also studied as a control. It was noted that *in vitro* grown plant parts possessed greater amount of reserpine compared to those in field grown plant parts. Almost 4 times reserpine were found to be present in roots compared to shoot and leaves. Culture media also possessed some amount of reserpine.