(B) Summary

Title: Clonal Multiplication of *Pericopsis mooniana* by tissue culture.

Research Institute: Department of Forestry & Environmental Science, University of Sri Jayewardenepura, Nugegoda

Chief Scientific Investigator: Prof. Hemanthi Ranasinghe

Period of Contract: 18th March, 1998 to 7th October, 2001

Scientific background and Scope/Objectives of the Project:

Perocopsis mooniana which belongs to the family Fabaceae is a tree found in the lowland rainforests of Sri Lanka on sandy or clayey soils. Due to its high carpentary value, especially for cabinet work it is being overexploited and now has become greatly threatened and included in the IUCN Red Data Book. Therefore, there is an urgent need for *insitu* or *exsitu* conservation of this species.

Due to its long gestation period, and the rapidly deteriorating forest conditions, traditional methods of propagation are not suitable to increase the abundance of this species for the conservation of germplasm. Therefore, there is a need to propagate this species by modern methods. Therefore, the main objective of the research project was to develop a suitable method/methods for successful clonal propagation of *Pericopsis mooniana* by tissue culture.

Materials & Methods

Shoot proliferation and organogenesis through callus cultures were tried for *Pericopsis mooniana* for a period of 2 years commencing from 1998. *In vitro* grown seedlings, nursery grown 6 months old seedlings and shoot meristems taken from plus trees in forest reserves in Yagirala and Deraniyagala were used as parent material for shoot proliferation and callus cultures. In shoot proliferation, explants were sterilized using three chemical sterilizers; 10/- v/v Chlorox, 70% v/v Ethanol and 0.15% v/v $HgCl_2$. The surface sterilized juvenile shoot tips and current year shoot tips were placed individually on MS media supplemented with cytokinins (BAP, Kinetin) and auxins (IBA, NAA) for shoot proliferation.The culture tubes were sealed and incubated in the culture room at 25°C for 16 hrs. /day light source with 100 $MEm^{-2}s^{-1}$ to allow proliferation. A fully randomized design with 20 replicates were used. The multiple shoots were subcultured every two weeks. Shoots obtained from explants were grown on MS medium with various concentrations of Gibberelic Acid.

In generating a callus, leaf petioles, leaf lamina and stem cuttings were used from the sources mentioned earlier. These were placed in MS media supplemented with BAP and 24 D at various concentrations. Callus once formed, was subcultured on a regular basis and then some of it was cut to small pieces and placed on media with cytokinins only and cytokinins in combination with auxins for bud formation. Inoculated cultures were incubated at 25°C with a light source of 100 $MEm^{-2}s^{-1}$ for 16 hrs. /day photoperiod. A randomized block design with 20 replicates were used.

For root initiation, multiplied shoots which were about 3-4 cm height or had about 2-3 pairs of leaves were placed on a variety of growth media containing ½ strength MS medium with varied concentrations of IBA (0.3 mg/l to 5 mg/l) alone and also in combination with BAP ranging from 0.1 – 0.5 mg/l. Half of these cultures were incubated at 25°C without light source and other half were incubated at 25°C with a light source of 100 $MEm^{-2}s^{-1}$ for 16 hrs/day photoperiod. A fully randomized design with 20 replicates were used.

Results and Discussion

In vitro grown seedlings showed the highest potential to be used for shoot proliferation followed by 6 months old seedlings and then the current year apical meristems from Plus trees. For the initiation of callus, leaf lamina, leaf petiole and stem from *in vitro* grown seedlings showed the highest potential.

With regard to surface sterilization, 83.5% of survival rate was obtained with negligible amount of contamination. using 10% v/v Chlorox (8 min), 70% v/v Ethanol (2 min) 0.15% v/v $HgCl_2$ (2 min). Seeds germinated on all the growth media tried, however, the best rate of germination (90%) was seen in Full strength Ms medium + 3 mg/l BAP.

Statistically significant differences ($P<0.05$) were obtained between culture media for shoot proliferation.The best culture medium for shoot proliferation was MS medium + 5mg/l BAP + 0.5 mg/l NAA where % shoot proliferation was 90%, no. of shoots after one month was 5 and the height to the tip was 19mm. The corresponding values for the medium MS medium + 0.3 mg/l IBA + 1.0 mg/l BAP were 80%, 6 and 22mm respectively.

Type of explant showed significant differences in the success of callus formation where the leaf lamina and leaf petiole proved superior to stem cuttings. The best culture medium for callus induction was MS medium + 2mg/ l BAP + 5 mg/l 2,4-D where the % of callus formation after one month was 100% using leaf lamina, 98% using leaf petiole. Stem did not show any callus formation. Average diameter of callus after one month was 22.3 mm in leaf petiole while it was 17.5mm in leaf lamina.

The pieces of callus transferred into media for bud formation did not show significant success as the callus turned dark green with no development of buds. When they were left on same medium for a longer period, it led to browning of tissues. It may be due to the exudation of oxidized polyphenolic compounds from callus to the medium. Therefore, attempts should be made to remove polyphenolic compounds from the media in the future attempts.

28

Rooting did not occur in any of the rooting media tried in the present experiment even after leaving the explants on the rooting media for more than a month. There are no recorded events where rooting had been achieved successfully on this plant. The difficulty in rooting could be due to the tree being a hardwood. More work should be done on this in the future.

Conclusions and Recommendations

- *Pericopsis mooniana* (Nedun) can be effectively multiplied using shoot proliferation using juvenile tissues ie. Shoot tips of *in vitro* grown seedlings, shoot meristems of juvenile seedlings etc.

- With regard to surface sterilization, 83.5% of survival rate was obtained with negligible amount of contamination. using 10% v/v Chlorox (8 min), 70% v/v Ethanol (2 min) 0.15% v/v $HgCl_2$ (2 min).

- Seeds germinated on all the growth media tried, however, the best rate of germination (90%) was seen in Full strength Ms medium + 3 mg/l BAP.

- Best culture medium for shoot proliferation was MS medium + 5mg/l BAP + 0.5 mg/l NAA where % shoot proliferation was 90%, no. of shoots after one month was 5 and the height to the tip was 19mm. The corresponding values for the medium MS medium + 0.3 mg/l IBA + 1.0 mg/l BAP were 80%, 6 and 22mm respectively.

- The best culture medium for callus induction was MS medium + 2mg/ l BAP + 5 mg/l 2,4-D where the % of callus formation after one month was 100% using leaf lamina, 98% using leaf petiole. Stem did not show any callus formation. Average diameter of callus after one month was 22.3 mm in leaf petiole while it was 17.5mm in leaf lamina.

- Bud growth was not obtained from callus cultures

- Rooting induction was not successful despite many attempt at varying the growth media and the environmental conditions.

Recommendations

The following recommendations can be put forward based on the experience of the present study;

- The possibility of obtaining a greater number of multiple shoots using juvenile tissues
- Formation of buds from callus
- Formation of adventitious roots
- Somatic embryogenesis
- Field trials using *invitro* grown seedlings

Papers published on work done under the contract

Ranasinghe, D.M.S.H.K. and Wijesinghe, S (1999) In vitro propagation of *Pericopsis mooniana* (Nedun) by shoot tip culture, 2nd Annual Research Session, Faculty of Graduate Studies, University of Sri Jayewardenepura.

(C) Use of the research grant for registration of a higher degree

The work carried out in the research project had been directed towards a M.Phil. Degree in the Faculty of Graduate Studies, University of Sri Jayewardenepura. Bulk of the work had been completed and currently the work is being written up for submission. Once it is submitted and approved by the Board, a copy of the theses can be submitted to the Library of the National Science Foundation.