PROJECT PROFILE

Project Title: **Development of protoplast regeneration system in Eucalyptus**

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Start and Completion dates : 2002 to 2005

Objectives:

1. Determination of suitable source material for protoplast isolation including, embryogenic callus, suspension cultures, *in vitro* grown shoots, cotyledons, leaves and seedling material.
2. Identification of suitable cell wall degrading enzyme treatments, isolation mixtures and time duration for enzyme incubation conditions.
3. Development of suitable method for purification of protoplasts fraction.
4. Standardization methods for the determination of protoplast viability
5. Standardization of suitable medium components such as basal salts, osmoticum phytohormones, plating density and culture conditions

Funding Agency: Indian Council of Forestry Research and Education (ICFRE)

Summary:

Callus obtained from cotyledons of *E.camaldulensis* was used for suspension culture establishment, which was found to be the best source of protoplasts. The enzymatic isolation procedure was very efficient for releasing protoplasts from *in vitro* leaves or suspension cultures. Cellulase 1.0 % with Macerozyme 0.17 % and Mannitol 0.4 M with incubation period of 17 hrs yielded high number of viable protoplasts. The protoplasts obtained from *in vitro* leaves were varied in size but suspension cultures yielded

physiologically homogenous uniform size of protoplasts, which were ideal for protoplast culture. Protoplasts obtained from cotyledons and embryogenic callus were also uniform in size with high viability. Yield of protoplasts from suspension culture was 2.95 $X10^6$ protoplast/g.f.wt and *in vitro* leaves produced 1.2 X 10^6 protoplast/g.f.wt. Protoplast viability immediately after isolation was 65 ± 7% in suspension cultures and 47 ± 4% in *in vitro* leaves. Cell wall formation was noticed as florescent layer around the protoplasts after 15-18 hrs of culture in liquid medium containing mannitol with the plating density of 2 $X10^5$ protoplast/ml. After 3 days of culture as suspension in KM8p medium, based on the maintenance of the spherical shape and cell wall regeneration of 45 and 20% viability was recorded in the case of suspension culture and *in vitro* leaves derived protoplasts respectively. No cell wall regeneration was recorded in alginate beads. Plating efficiency (micro calli formation) of the two protoplasts sources was determined as 14 and 7%. The micro calli when transferred to medium containing Benzyl aminopurine (BAP) and Kinetin showed pink-colored callus and the morphological features were very similar to embryogenic callus.