6. SUMMARY

Cycads are conventionally propagated through seeds. The erratic seed germination, rapid loss of seed viability, slow growth and low morphogenic potential has brought down their distribution to a limited area. IUCN has classified more than half of the 132 species of Cycadales as endangered, vulnerable or rare. Micropropagation is an alternative means of mass multiplication of those valuable species. But cycads are known for their recalcitrance to tissue culture. In the present study, three valuable cycads, *Zamia furfuracea* L., *Cycas revoluta* Thunb., *Cycas circinalis* L. have been subjected to extensive tissue culture experimentation with a wide category, range and combination of plant growth regulators to get an efficient protocol for their *in vitro* propagation. The objective was also focused at elucidating the process of poor morphogenic response of Cycad genotype in tissue culture. A histological screening of cultured cell, tissues and organs at different intervals was also carried out to have a better understanding of the developmental stages of morphogenesis. Histochemistry and histoenzymology were used to locate sites of activities and characterize the events of morphogenesis. The metabolite pathways operating in a cell are well connected with cell differentiation process. Therefore primary metabolites like protein, Starch, Soluble sugars and secondary metabolites like resin were periodically quantified to underline the physiological events taking place during morphogenesis. Electrophoretic techniques have been used to analyse isozyme patterns of different enzymes at different phases of tissue growth in the medium.

The important results obtained are
1. Out of the different media tried, the medium containing a combination of B5 and MS salts was ideal for getting maximum response.

2. Young rachis of *Zamia furfuracea*, *Cycas revoluta* and megagametophyte of *Cycas circinalis* are the explants, which showed maximum response in tissue culture.

3. In *Z. furfuracea* maximum callusing was obtained in a hormone combination of 3 mg L\(^{-1}\) NAA + 1 mg L\(^{-1}\) Kin.

4. A cytokinin support to auxin was always found to be necessary for optimum callogenesis.

5. The calli produced were friable in texture and creamy white, light yellow or green in colour.

6. In *Cycas revoluta* optimum callusing was obtained from rachis explant in 5 mg L\(^{-1}\) NAA + 0.5 mg L\(^{-1}\) BAP.

7. In *Cycas circinalis*, optimum callusing was at 5 mg L\(^{-1}\) NAA + 1 mg L\(^{-1}\) BAP from rachis and 3 mg L\(^{-1}\) NAA + 0.5 mg L\(^{-1}\) BAP from megagametophyte explants.

8. Juvenile calli were showing no resin synthesis but, as the callus aged in culture, they developed intense deposition of resin which made the callus dark brown.

9. During callogenesis starch and soluble sugar gradually increased in the cells as the callus cells matured. Protein content was also initially higher for 15 – 20 days, but later reduced.

10. For obtaining organogenesis in *Zamia*, juvenile calli were experimented with 325 different combinations of plant growth regulators. Induction of somatic embryos
was obtained in only 40 treatments. Plant growth regulator combinations including Kin + 2iP (0.5 -1 mg L⁻¹) along with either NAA or 2,4-D (0.5-1 mg L⁻¹) were found to be optimum. 10% coconut water showed an enhancing effect on embryo induction. Induced embryos matured with only low frequency. Though attempts were made with wide range of supplements and conditions, mature embryos did not develop into plantlets. Induction of shoot and root from the callus was obtained in 15 hormonal combinations from among 325 treatments. Combinations including Kin + 2iP+ IAA (1 mg L⁻¹) was found to be optimum. As in the case of somatic embryos complete plant recover was not achieved.

11. In *Cycas circinalis*, somatic embryo induction was observed only from the callus obtained from megagametophyte. Though 288 combinations were tried, induction was noticed, only in 12 combinations. The optimum hormonal combination was 1 mg L⁻¹ BAP + 0.5 mg L⁻¹ NAA+ 20 % megagametophyte extract. Induced embryos were not matured beyond globular stageas the component cells were eventually lignified. Multiple shoot buds were also obtained from megagametophyte, which also did not continue growth.

12. In *Cycas revoluta*, except the development of shoots buds from scale leaves, organogenic response was poor.

13. Quantification of metabolites during various phases of cell morphogenesis revealed that primary metabolites are utilized at the time of embryogenesis / organogenesis.

14. Resin deposition occurred in the cells at a higher level, which reduced the morphogenetic potential.
15. PAGE showed that isozyme patterns of enzymes vary during tissue differentiation in culture. The isozyme patterns of esterases and peroxidases helped to distinguish compact embryogenic calli from friable non-embryogenic one in *Zamia furfuracea* and *Cycas circinalis*.

The important conclusion derived from the present investigation is that organogenesis and subsequent growth is not as easy as inducing callus in Cycads. Hormonal regime alone could not steer the sole morphogenesis. Induced embryos / organs failed to grow into complete plantlets. Mitotic activity was stopped with the untimely lignification of the component cells involved in the morphogenesis. The accumulation of the resin in the cells in culture would be the most probable hindrance in achieving the cell differentiation and plantlet recovery.