CHAPTER 6

Conclusion

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This preliminary study to develop a system for micropropagation of *Gracilaria changii* (Rhodophyta) was aimed at formulating a culture medium for successful tissue culture and optimizing the conditions required for protoplast isolation. The establishment of a plant sterilisation protocol is a prerequisite for the success of both tissue culture and protoplast isolation.

Of the six forms of treatment investigated, only one generated axenic and healthy explants. This treatment involved washing and sonicating the seaweed thalli in UHQ (ultra high quality) water followed by a 30 seconds soaking in 1% w/v povidone-iodine. The thalli were later incubated for 24 hours in seawater solution containing the following antibiotics; 0.1 $g.L^{-1}$ kanamycin, 0.3 $g.L^{-1}$ penicillin G, 0.02 $g.L^{-1}$ polymyxin B sulphate, 0.001 $g.L^{-1}$ nalidixic acid, 0.02 $g.L^{-1}$ cefotaxine and 1 $g.L^{-1}$ streptomycin sulphate. The explants produced from this treatment remained healthy and retained their dark-brownish colour and were axenic. Other treatments involving the use of various combinations of 1.5% w/v KI (potassium iodide), 70% v/v ethanol and 1% v/v hypochlorite (Chlorox™) produced either bleached (dead) but axenic explants or healthy but non-axenic explants.

A battery of different types and forms of media formulated from Murashige and Skoog (MS) basic medium and Provasoli's Enriched Seawater (PES) medium had been investigated for tissue culture of *G. changii*. The different forms of MS and PES

media were used in their basal forms, incorporated with vitamins and incorporated with vitamins and growth substances. The growth substances were incorporated either singly or in combinations of an auxin and a cytokinin. Solid and semi-solid MS, MS + vitamins, MS + vitamins + growth substances, PES + vitamins and PES + vitamins + growth substances did not succeed in generating either callus or shoot formation and neither did liquid PES + vitamins. Branching or shoot formation was only observed after 35 days in culture for explants cultured in liquid PES + vitamins incorporated with a single growth substance. PES + vitamins media containing 0.01 $mg.L^{-1}$ of NAA and 0.001 $mg.L^{-1}$-IAA induced the highest incidence of branching followed by 0.001 $mg.L^{-1}$ kinetin. Incorporation of 2,4-D into the media failed to induce branching. Although it was reported in literature that branching from chopped explants of some species of red algae were able to regenerate in to shoots without the need for growth substances, it was shown not to be the case for *G. changii* as no incidence of shoot formation was recorded in liquid PES + vitamins media containing no addition of growth substances. It was suggested that shoot formation, a form of organogenesis, in *G. changii*, bypassed the callus stage in the presence of a single growth substance.

Friable callus-like structures (CLS) and branching or shoot formation were initiated from explants cultured PES + vitamins medium containing different combinations of 2,4-D (auxin) and kinetin (cytokinin). Highest incidence of CLS and shoot formation were recorded for explants cultured in PES + vitamins medium containing 10 $mg.L^{-1}$ 2,4-D and 0.001 $mg.L^{-1}$ kinetin. The initiation of CLS and shoot formation increased proportionately in medium containing higher concentrations of kinetin and lower concentrations of 2,4-D. Other auxins tested (IAA and NAA) in

combination with kinetin failed to induce either CLS or shoot formation. It may be concluded from this investigation that while 2,4-D when used singly in liquid PES + vitamin medium failed to induce shoot formation, was required for the both the formation of CLS and shoots.

Work on protoplast isolation required the determination of the optimal osmoticum concentration to be used together with enzymes contained in the protoplast isolating solution. Concentration of mannitol over the range of 0.5 to 1.3 M was investigated and 1 M was found to be the optimal concentration required for the induction of the highest number of sufficiently-plasmolysed cells of *G. changii*. Mannitol concentrations below 0.8 M failed to induce plasmolysis and concentration above 1.0 M produced over-plasmolysed cells.

Protoplast isolation from finely chopped axenic explants was investigated using abalone acetone powder, cellulase, pectinase, pectolyase and agarase. These enzymes were tested individually and in combination in different concentrations and time of incubation. All the enzymes tested alone except agarase liberated the highest number of protoplast after 2 hours of incubation. Abalone acetone powder tested, when tested alone in concentration of 1% w/v, liberated the highest number of viable protoplasts (5.0×10^4 protoplast g^{-1} fresh weight) followed by 2% w/v cellulase (3.2×10^4 protoplast g^{-1} fresh weight), 1% w/v pectinase (2.34×10^4 protoplast g^{-1} fresh weight) and 1% w/v pectolyase (1.67×10^4 protoplast g^{-1} fresh weight). Agarase (3% w/v) liberated 1.75×10^4 protoplast g^{-1} fresh weight after 1.5 hour of incubation.

A combination of 1% w/v each of the enzymes mentioned above produced a maximum of 3.0×10^4 protoplast g^{-1} fresh weight after 2 hours of incubation. This is not comparable to that achieved using abalone acetome powder alone (5.0×10^4 protoplast g^{-1} fresh weight). This suggests that a combination of all the five enzymes may have resulted in cell death due to the plasma membrane being digested.