ABSTRACT

A system for tissue culture and protoplast isolation was investigated for *Gracilaria changii* (Rhodophyta) Abbott, Zhang and Xia. A plant sterilisation protocol for the generation of healthy and axenic explants required for both tissue culture and protoplast isolation was established. This method involved treating the seaweed thalli in ultra high quality (UHQ) water for an hour and sonicated for three to four minutes. This is then followed by treatment in 1% w/v povidone-iodine and incubated overnight in sterile seawater containing a series of antibiotics (0.1 $g.L^{-1}$ kanamycin, 0.3 $g.L^{-1}$ penicillin G, 0.22 $g.L^{-1}$ polymyxin B sulphate, 0.001 $g.L^{-1}$ nalidixic acid, 0.02 $g.L^{-1}$ cefotaxine and 1 $g.L^{-1}$ streptomycin sulphate).

Different forms and constituents of Murashige and Skoog (MS) and Provasoli's Enriched Seawater (PES) media were tested for callus induction and plant regeneration. Branching or shoot formation was only recorded in liquid PES + vitamins medium supplemented with different concentrations of growth substances. All the growth substances tested, except 2,4-D, induced branching. IAA (0.001 $mg.L^{-1}$) induced the highest incidence of branching (20%) followed by 0.001 $mg.L^{-1}$ kinetin, 0.1 $mg.L^{-1}$ NAA and 0.01 $mg.L^{-1}$ IAA in which 13.33% of the explants branched.

Friable callus-like structure (CLS) and shoots were initiated from cross-sections of explants in liquid PES + vitamins medium supplemented with different combinations and varying concentrations of 2,4-D and kinetin. A combination of 10 $mg.L^{-1}$ 2,4-D and 0.001 $mg.L^{-1}$ kinetin generated 100% incidence of CLS and shoot

formation. Incidence of CLS and shot formation was positively related to higher 2,4-D concentrations and lower kinetin concentrations.

Mannitol concentration of 1 M was found to be an excellent osmotic stabiliser for *G . changii* required for enzymatic cell wall digestion during protoplast isolation. Cells were sufficiently plasmolysed at this osmoticum concentration.

Enzymes such as abalone acetone powder, cellulase, pectinase, pectolyase and agarase were tested individually and in combinations in various concentrations and time of incubation for their effectiveness in protoplast isolation. Incubation of tissues in 1% w/v abalone acetone powder generated the highest protoplast yield (5.0×10^4 protoplast g^{-1} fresh weight). A combination of 1% w/v each of all the enzymes mentioned failed to achieve the maximum achieved using 1% w/v abalone acetone powder alone.

ABSTRAK

Sistem untuk pengkulturan tisu dan pengasingan protoplast *Gracilaria changii* (Rhodophyta) Abbott, Zhang dan Xia telah disiasat. Satu kaedah untuk menghasilkan bahagian tumbuhan yang sihat dan asenik telah berjaya ditetapkan. Kaedah ini melibatkan rendaman bahagian tumbuhan di dalam air berkualiti tinggi, UHQ *(ultra high quality)* selama satu jam dan sonikasi selama tiga hingga empat minit. Kemudian, bahagian tumbuhan tersebut dibersihkan dengan 1% w/v *povidone-iodine* dan direndamkan selama satu hari di dalam air laut yang mengandungi pelbagai jenis antibiotik (0.1 $g.L^{-1}$ kanamisin, 0.3 $g.L^{-1}$ penisilin G, 0.22 $g.L^{-1}$ polimisin B sulfat, 0.001 $g.L^{-1}$ asid nalidisik, 0.02 $g.L^{-1}$ sifotaksin dan 1 $g.L^{-1}$ streptomisin sulfat).

Medium Murashige dan Skoog (MS) dan *Provasoli's Enriched Seawater* (PES) dalam bentuk dan komposisi yang berlainan telahpun diuji untuk perkembangan kalus dan anak pokok. Hanya medium PES + vitamin yang mengandungi hormon-hormon pertumbuhan mengizinkan pertumbuhan anak pucuk. Semua hormon pertumbuhan yang telah diuji kecuali 2,4-D menyebabkan perkembangan anak pucuk. IAA (0.001 $mg.L^{-1}$) menyebabkan peratusan perkembangan anak pucuk yang paling tinggi (20%) diikuti dengan 0.001 $mg.L^{-1}$ kinetin, 0.1 $mg.L^{-1}$ NAA dan 0.01 $mg.L^{-1}$ IAA di mana 13.33% perkembangan anak pucuk telah direkodkan.

Struktur serupa kalus, CLS (*callus-like structure*) yang berbentuk longgar didapati berkembang daripada keratan rentas tumbuhan yang dikultur di dalam medium PES + vitamin yang mengandungi kombinasi kepekatan 2,4-D dan kinetin

yang berlainan. Kombinasi 10 $mg.L^{-1}$ 2,4-D dan 0.001 $mg.L^{-1}$ kinetin menyebabkan 100% perkembangan CLS dan anak pucuk. Peratusan perkembangan CLS dan anak pucuk bertambah dalam kepekatan 2,4-D yang tinggi dan kepekatan kinetin yang rendah.

Kepekatan manitol 1 M didapati bertindak sebagai penstabil osmotik yang baik untuk *G. changii* yang diperlukan semasa pencernaan dinding sel dalam proses pengasingan protoplast. Sel-sel telah diplasmolisiskan dengan sempurna dengan kepekatan manitol ini.

Enzim-enzim seperti serbuk *abalone acetone*, selulase, pektinase, pektoliase dan agarase telah diuji secara berasingan dan berkombinasi dalam kepekatan dan masa inkubasi yang berlainan semasa pengasingan protoplast. Tisu yang diinkubasikan dalam 1% w/v serbuk *abalone acetone* menghasilkan bilangan protoplast yang paling tinggi (5.0×10^4 protoplast g^{-1} berat basah). Kombinasi 1% w/v setiap enzim yang tersebut di atas tidak berjaya mencecah bilangan protoplast yang terhasil apabila hanya 1% w/v serbuk *abalone acetone* digunakan.