Isolation and culture of protoplasts from the medicinal plants Centella asiatica

ABSTRACT

In the present investigation, protoplasts were isolated from cell suspensions initiated from leaf laminae and petioles using an enzyme mixture consisting of 1.5% (w/v) Cellulase R10, 1.0% (w/v) Macerozyme R10 and 0.5% (w/v) Driselase in CPW salts solution with 13% (w/v) mannitol as osmotic stabilizer. Yields and viabilities of isolated protoplasts were $1.2 \times 10^5 \pm 0.1\ g^{-1}$ fresh weight and 20.8 ± 4.4% for protoplasts from lamina-derived cell suspensions and $7.9 \times 10^5 \pm 1.5\ g^{-1}$ fresh weight and 79.3 ± 13.4% for protoplasts from petiole-derived cell suspensions. Protoplasts from lamina explant-derived cell suspensions were cultured at plating densities of 0.25×10^5 – 2.0×10^5 protoplasts ml-1 in half-strength B5 based medium containing 0.1 mgl^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.3 mgl1 zeatin, dispensed as semi-solid agarose droplets (each approx. 70 µl in volume) in 5.5 cm diameter Petri dishes (10 droplets per dish). First mitotic divisions of protoplast-derived cells were observed after 4 d of culture at an optimum plating density of 0.5×10^5 protoplasts ml^{-1}, giving an initial plating efficiency at this time of 12.7 ± 0.6%. After 42 d of culture, protoplast-derived cell colonies were creamy-white in colour and each approx. 1 mm in diameter, with a final plating efficiency of 0.6 ± 0.2%. Cell colonies transferred to semi-solid proliferation medium containing 2,4-D (4.0 mgl^{-1}) and zeatin (0.2 mgl^{-1}) were creamy-yellow in appearance, whereas colonies cultured on medium devoid of these growth regulators became light green and compact. In the case of protoplasts from petiole-derived cell suspensions, culture in Murashige and Skoog (1962)-based medium supplemented with 2.0 mgl^{-1} alpha-naphthaleneacetic acid and 0.5 mgl-1 6-benzylaminopurine resulted in an initial plating efficiency of 19.3 ± 4.2% at an optimum plating density of 1.0×10^5 protoplasts ml^{-1}. However, mitotic division was not sustained after this stage. Plant regeneration studies are on-going from protoplasts isolated from lamina-derived cell suspensions.