

FORMATION AND REGENERATION OF PROTOPLASTS OF ORCHID  
MYCORRHIZAL FUNGI

Coelho, I.S., Queiroz, M.V., Costa, M.D. Kasuya, M.C.M., Araújo, E.F.  
Departamento de Microbiologia/BIOAGRO, Universidade Federal de Viçosa,  
Viçosa/MG, Brasil

Although mycorrhizal fungi present a biotechnological potential, little is known about their physiology and genetic. This study was conducted in order to standardize the formation conditions and regeneration of *Epulorhiza repens* and *Ceratorhiza* sp. protoplasts. For *E. repens*, the highest production of protoplasts,  $8,0 \times 10^6$  protoplasts/mL, was obtained in KCl 0,6 M, with 15 mg/mL of "Lysing Enzymes" and 0,5 g of mycelium at 2 days. The best regeneration frequency, 8,5 %, was achieved when sucrose 0,5 M was used as an osmotic stabilizer. From total protoplasts obtained, 58,6 % were uninucleate, 21,4 % binucleate and 20 % anucleate. For *Ceratorhiza* sp. the highest production of protoplasts,  $4,0 \times 10^7$  protoplasts/mL, was obtained in NaCl 0,6 M, with 15 mg/mL of "Lysing Enzymes" and 15 mg/mL of Glucanex, and 0,5 g of mycelium at 2 days. The best regeneration frequency, 6,7 %, was achieved when sucrose 0,5 M was used as an osmotic stabilizer, and from total protoplasts obtained, 61 % were uninucleate, 24,6 % binucleate, 3,2 % trinucleate and 11,2 % anucleated. It is important to establish optimized protocols for obtaining and regenerating protoplasts of *E. repens* and *Ceratorhiza* sp. to allow the establishment of techniques of genetic transformation, mutant isolation, electrophoretic karyotype determination and crossings between strains.