Highly efficient somatic embryogenesis and plant regeneration via suspension cultures of banana (Musa spp.)

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ABSTRACT

A protocol has been developed for the high efficient regeneration of the banana cultivar Dwarf Brazilian (Musa spp. AAB group) via cell suspension. Primary somatic embryos were produced when explants of immature male flower buds were cultured on Murashige and Skoog (MS) medium plus 1 mg/l biotin, 100 mg/l malt extract, 100 mg/l glutamine, 4 mg/l 2,4dichlorophenoxyacetic acid, 1 mg/l indole-3-acetic acid (IAA), 1 mg/l . -naphthaleneacetic acid, 30 g/l sucrose and 2.6 g/l Phytagel, pH 5.8 (M1 medium) and then transferred to M1 medium plus 200 mg/l casein hydrolysate and 2 mg/l proline. Suspension cultures were initiated from embryogenic tissues placed in liquid medium supplemented with 2,4-D (1mg/l), biotin (1 mg/l), L-glutamate (100 mg/l), malt extract (100 mg/l), and sucrose (45 g/l), the pH of the medium was adjusted to 5.3. The packed cell volume (PCV) of the suspension increased 2-5 fold with each monthly cycle. The somatic embryos were developed when suspension culture aspirated on MS medium supplemented with biotin (1 mg/l), malt extract (100 mg/l), Glutamine (100mg/l), NAA (1mg/l), Kinetin (0.5 mg/l) Zeatin (0.2 mg/l), sucrose (45 g/l), and phytagel (2.6 g/l). Differentiated embryos were transferred to MS medium supplemented with 5 mg/l 6-benzylaminopurine (BA) for development of the mature somatic embryos, which were isolated and cultured on hormone-free MS medium for germination and development into plantlets. Approximately 90% of the somatic embryogenesis germinated and developed into plantlets, and these were subcultured onto MS medium plus 0.1% activated charcoal and 1 mg/l IAA. Approximately 900-1050 plants were obtained from initial starting material (regeneration 90%) of 0.5 ml PCV suspension culture in 4-5 months. Morphologically normal banana plants were developed from all regenerated plants. Somatic embryogenesis via cell suspension might be an excellent technique for mass production, developing a breeding strategy and genetic transformation of banana.

Key words: In vitro, plant tissue culture, regeneration, somatic embryogenesis, cell suspension, banana.

Abbreviations MS: Murashige and Skoog, medium (1962), BA: 6-benzylaminopurine, 2,4-D; dihlorophenoxyacetic acid, IAA: indole-3-acetic acid, NAA: -naphtaleneacetic acid, SE:somatic embryos, PCV: packed cell volume.