

Rapid Sugarcane Shoot Regeneration for Plants Transformation

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Sugarcane has played an important role in Brazilian agribusiness contributing with more than 34% of GDP. In order to reach the growing demand induced by the improvement of technological applied systems on ethanol and sugar production it is necessary the frequent release of improved varieties, which can be obtained through classical breeding and genetic transformation. The success to obtain transgenic plants is highly dependent on the efficiency of in vitro plant regeneration. In this research we shown the establishment of direct sugarcane shoots regeneration protocols from immature leaves as a very promising methodology to be used to obtain transgenic plants in less than two months. Immature leaves in a disc roll were pre-induced in the dark for 0, 3, 6, 9 and 12 days in three different culture media MS3C, MS3K and MS8. Their basic compositions have the salts and vitamins of MS medium (Murashige and Skoog, 1962) supplemented with sucrose 30 g.L⁻¹ and pH 5.8. The MS3C includes 2,4-D (3 mg.L⁻¹) and coconut water (50 mL.L⁻¹); the MS3K contain 2,4-D (3 mg.L⁻¹) and kinetin (0.1 mg.L⁻¹); the MS8 contains 2,4-D (8 mg.L⁻¹). After incubation in the dark, the immature leaf discs were transferred to MRP medium (basic MS medium supplemented with NAA (3.72 mg.L⁻¹) and 6-BAP (0.45 mg.L⁻¹) in the light. The number of shoots formed was counted after 30 days. The best results in terms of final regeneration of plants were observed in the treatment MS3K with incubation in the dark for 3 days which produced an average of 70,4 plants per leaf disc. This methodology has the advantage of avoiding the stage of callus formation which may increase somaclonal variation and thus it is expected high number of stable transgenic plants using this regeneration protocol.

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