Plant Embryo Culture: Methods and Protocols

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Biolistic-Mediated Transformation Protocols for Maize and Pearl Millet Using Pre-Cultured Immature Zygotic Embryos and Embryogenic Tissue

Martha M. O'Kennedy, Hester C. Stark, and Nosisa Dube

CSIR, Biosciences Plant Biotechnology, Pretoria, South Africa

Corresponding author: MOKennedy@csir.co.za

Abstract

Maize (Zea mays L.) is the most important cereal food crop in sub-Saharan Africa and Latin America, and a key feed crop in Asia, whereas pearl millet [Pennisetum glaucum (L.) R. Br.] is a staple food that supplies a major proportion of calories and protein to large segments of the populations living in the semi-arid tropical regions of Africa and Asia. The limitations of biological gene transfer with Agrobacterium tumefaciens specifically related to recalcitrant cereal crops, led to the development of alternative methods of which high-velocity microprojectiles, biolistic genetic transfer is the most successful and also the most widely employed. Agrobacterium facilitated transformation is the method of choice especially for deregulation of commercial transgenic food crop products, but biolistic-mediated transformation are still valid for proof of concept and functional genomics applications. Biolistic-mediated transformation and the production of transgenic plantlets via somatic embryogenesis of two maize strains viz. Hi-II (a laboratory strain) and M37W (a South African elite white maize genotype) as well as a pearl millet strain (842B) are described in this chapter. The stages described include: 1) proliferation of immature zygotic embryos for biolistic-mediated transformation, 2) induction and maintenance of transgenic embryogenic tissue on selection medium; 3) maturation (both morphological and physiological) of transgenic somatic embryoids; and 4) germination of the somatic embryoids to transgenic putative primary events. Maize and pearl millet cultures were regenerated via somatic embryogenesis as they are bipolar structures that shoot and root simultaneously. The culture media described in this chapter rarely induced or regenerated plantlets via organogenesis.