Somatic Embryogenesis and Plant Regeneration of Mango (*Mangifera indica* L.)

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INTRODUCTION

In the last 15 years there has been a tremendous increase in areas dedicated to Mango production in the tropics and subtropics. Mango production is appealing because the fruit is nutritionally important and constitutes an attractive option to increase income and reduce poverty in the rural sector of developing countries.

Despite its importance and worldwide distribution, mango suffers from a long juvenile period, erratic flowering and alternate bearing habits. In fruit crops, control of flowering is a critical aspect in the production system, since it determines the seasonality of fruit supply to the market. One of the great advantages of the tropics is the possibility of producing during the whole year, nevertheless fruit producers and markets face major challenges to supply fruits of high quality throughout the year. Biotechnology can potentially be used to manipulate existing cultivars by targeting specific genetic traits, such as flowering behavior. Somatic embryogenesis has been reported in some mango cultivars[1,2], most of which are poly-embryonic.

This research aims to manipulate the expression of a target set of developmental genes known to modulate flowering. However, to apply these tools, a consistent embryogenesis and regeneration protocol is required.

MATERIALS AND METHODS

Plant Material

Immature fruits (45-60 days after anthesis) were collected from commercial orchards located in Tolima, Cundinamarca, and Valle del Cauca Departments in Colombia. The cultivars included mono-embryonic Florida certified clones for export market, also important in Africa: Keitt, Tommy Atkins, Kent, Haden and Irwin.; and the Colombian local selection poly-embryonic clones for local market: Magdalena River, Jobo, 505-4, Sufaida, Arauca, Manzano, Vallenato, Azúcar' and Yulima.

Explant Excision and Culture

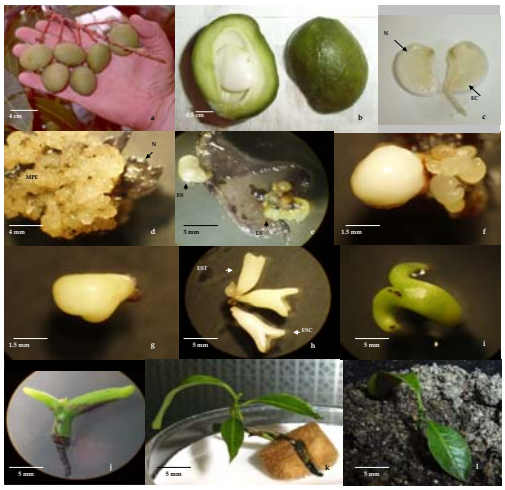

Figure 1. Somatic embryogenesis from nucellar tissues of Keitt and Magdalena River cultivars of mango. *(a-j)* Somatic embryogenesis of Magdalena River Mango. *(a)* Immature Fruits, source of nucellar explants; *(b)* immature fruits cut longitudinally; *(c)* ovules cut open lengthwise showing nucellus and zygotic embryo; *(d)* proembryogenic masses from nucellar tissue of Keitt; (*e-f*) globular somatic embryos; *(g)* somatic embryo at heart stage; *(h)* torpedo somatic embryos; *(i)* cotyledonary somatic embryo; *(j)* germinated somatic embryo *(k)*; plantlet in rooting medium (l) Plantlet acclimated in the greenhouse. N: nucellus; PEM: proembryogenic masses; SE: somatic embryos generated *in vitro*; TSE: somatic embryo at torpedo stage; CSE: somatic embryo at cotyledonary stage.

Genetic Transformation

Agrobacterium mediated transformation was conducted using the C58C1RifR strain harboring the binary vector pATARC3-B1b containing the nptII (neomycin phosphotransferase II), the *uidA* (GUS) and the arc5-I genes (Goossens et al., 1999). Cultures were subjected to standard genetic transformation protocol including a stepwise selection first on kanamycin 200 mg l/1, and after two months on kanamycin 400 mg l/1. Kanamycin resistant healthy growing tissues were assayed for histochemical GUS expression.

RESULTS

❖ The highest somatic embryogenesis was observed in Keitt (39%), 505-4 (33%), Jobo (31%) and Magdalena River (15%) cultivars.

❖Poly-embryonic cultivars generate somatic embryos directly from the nucellar tissue without callus formation.

❖Reproducible response depends on homogeneity of physiological stage of the mango trees selected; standardization of fruits age/size chosen; and commercial genotypes (cultivars) used.

❖A combination of low concentrations of 2,4D (auxin) with TDZ (cytokinin) significantly improved the embryogenic response from nucellar tissue.

❖Mango somatic embryos with the sequential developmental stages (globular, heart, torpedo and cotyledonary stages) were produced.

❖A significant reduction in apical shoot necrosis was obtained by changing the macronutrient composition in the germination medium of somatic embryos.

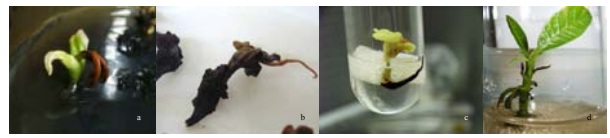

Figure 2. Shoot apical necrosis in mango. *(a)* First symptom: terminal portion of shoot becomes brown and dies; *(b)* dead germinated-somatic embryo affected by apical necrosis; *(c)* somatic embryo with cotyledons germinated using the new medium macronutrient composition (macro salts modified from reported mango medium); *(d)* healthy growing plant with expanded true leaves.

❖Somatic embryos continue a normal development into plants without shoot tip necrosis symptoms when transfer to this new salt formulation after root initiation, and transplanted to the greenhouse.

❖Histochemical assay to detect β-glucuronidase activity demonstrated *gus* expression in tissues after stepwise selection on kanamycin at lethal concentrations. Plant regeneration from putative transgenic proembryogenic masses (PEM) is in progress.

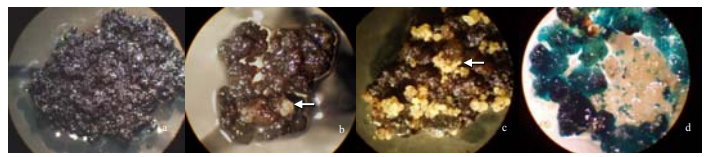

Figure 3. Putatively transformed proembryogenic masses (PEM) of 'Keitt' mango cultivar. *(a)* Non-transformed PEM (dead) control cultured on kanamycin selection medium-; *(b-d)* PEM growing on selection medium containing 400 mg l^{-1} kanamycin and *(d) Gus* expression from PEM after four-months of selection on medium containing 400 mg l^{-1} kanamycin.

Acknowledgements

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