

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

Flórez-Ramos, María E. Buitrago, James Cock and Zaida Lentini*

* Corresponding author: z.lentini@cgiar.org

International Center for Tropical Agriculture (CIAT), A.A. 6713
Cali, COLOMBIA



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 accounts for an estimated 38 percent of total tropical fruit output in 2002 with a total production of 25 million tons. In Africa mango production is currently at the level of 2.6 million tons annually (FAO 2004) with a worth estimated in US\$ 42 million (ICIPE 2004). Smallholders supply over 90% of mangoes produced (ICIPE 2004). 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 polyembryonic. This research aims to manipulate the expression of a target set of developmental genes known to modulate flowering in other fruit tree species such as citrus. To apply these tools, a reproducible embryogenesis and regeneration protocol is required.

Results

- ❖ The highest somatic embryogenesis was observed in Keitt (39%), 505-4 (33%), Jobo (31%) and Magdalena River (15%) cultivars.
- ❖ Polyembryonic 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 2,4-D (auxin) with TDZ (cytokinin) significantly improved the embryogenic response from nucellar tissue.
- ❖ A large number of secondary somatic embryos were generated when using the immersion temporary system.
- ❖ 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
- ❖ Somatic embryos continue a normal development into plantlet without shoot tip necrosis symptoms when transfer to this new salt formulation after root initiation, and transplanted to the greenhouse.
- ❖ Histochemical assay to detect B-glucuronidase activity demonstrated *gus* expression in tissues after stepwise selection with kanamycin at lethal concentrations for control tissues. Plant regeneration from putatively transgenic PEM is in progress.

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 monoembryonic Florida certified clones for export market, also important in Africa: Keitt, Tommy Atkins, Kent, Haden and Irwin.; and the Colombian local selection polyembryonic clones for local market: Magdalena River, Jobo, 505-4, Sufaida, Arauca, Manzano, Vallenato, Azúcar' and Yulima.

Explant Excision and Culture

Immature fruits were washed and surface sterilized. Fruits were excised and the ovules dissected symmetrically. The nucellus from each ovule was transferred onto culture medium in the dark at 28±2°C. Embryogenic response was evaluated as number of nucellus producing proembryogenic masses or somatic embryos generated. Pro-embryogenic masses (PEM) were isolated and sub-cultured. Masses of globular embryos were transferred to recovery medium. Somatic embryos at torpedo stage were transferred to culture vessels with aeration and incubated at 16h photoperiod to reach the cotyledonary stage. Germination was induced on embryos at advanced cotyledonary stage. Induction of Secondary Embryogenesis was optimized using Temporary Immersion (RITA). Somatic embryos with roots and green cotyledons (1-2 cm long) showed shoot tip necrosis, a typical bottleneck reported for mango at this stage of *in vitro* development. Serial experiments were conducted to overcome this problem. Healthy looking plants were transplanted to the greenhouse.

Genetic Transformation

Agrobacterium mediated transformation was conducted using the C58C1Rif^r strain harboring the binary vector pATARC3-B1b containing the *nptII* (neomycin phosphotransferase II), the *uidA* (intron-GUS) and the *arc5-1* genes (Goossens *et al.*, 1999). Cultures subjected to standard genetic transformation protocol including a stepwise selection 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.

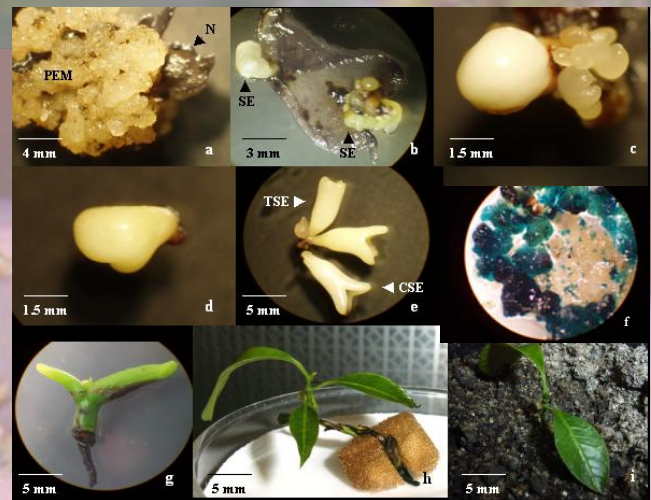


Figure 1. Mango somatic embryogenesis from nucellar tissues of 'Keitt' and 'Magdalena River' cultivars. (a) indirect somatic embryogenesis in 'Keitt' cultivar, PEM formation; (b) direct somatic embryogenesis from nucellar tissue of 'Magdalena River' cultivar; (c) somatic embryo at globular stage; (d) somatic embryo at heart stage; (e) torpedo and early cotyledonary somatic embryos; (f) advanced cotyledonary stage somatic embryo; (g) *gus* expression in PEM after stepwise selection on kanamycin; (h) plantlet in rooting medium; (i) 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.

Acknowledgements

This work is supported by the grant Project No. 2004 FS 034 from The Rockefeller Foundation, NY, USA.

References

1. Litz, R. 2004. Biotechnology and mango improvement. *Acta Hort* 645: 85-92.
2. Litz, R. & Lavi, U. 1997. Biotechnology. In *The Mango* (ed. Litz, R.E.), CAB International, Wallingford, UK, pp 401-423.
3. Pateña, L, Carlos-Refuerzo, L & Barba, R. 2002. *In vitro* Cell Dev. Biol.-Plant. 38: 173-177
4. Rivera-Domínguez, M; Manzanilla-Ramírez, M.A.; Robles-González, M. & Gómez-Lim, M.A. 2004. *Plant Cell Tiss. Org Cult.* 79: 101-104
5. Flórez-Ramos, C.P.; Buitrago, M.E.; Cock, J. and Lentini, Z. 2006. Somatic embryogenesis and plantlet regeneration of mango (*Mangifera indica*). *Acta Horticulturae in press.*