

## ABSTRACT

### **Agrobacterium mediated transformation of *Theobroma cacao* L**

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A number of *in vitro* experiments were conducted with the objective of developing an agrobacterium mediated transformation protocol for producing transgenic plants of *Theobroma cacao* L.

During the first phase, experiments were conducted to optimize somatic embryogenesis in cacao using floral and zygotic explants. Genotype, explant type, size of flower bud from which the explants were derived, duration of exposure to the initiation medium Lopez-Baez *et al.* (1993) (MI) and modification of the components of the initiation medium and the expression medium of Lopez-Baez *et al.* (1993) (ME), all significantly influenced callusing in floral explants, however due to the sporadic nature of embryo production (0-12.8 %), the significance of embryogenesis could not be determined. The results indicated that petal explants derived from buds 3-4 mm, exposed to three weeks in MI medium, containing 1/2 MS, 40 g/l sucrose and coconut water from ripe fruit and ME medium containing coconut water from ripe fruit were optimal for embryogenesis.

The stage of development of zygotic explants and the modification of the protocol of Pence *et al.* (1979) in terms of basal salt and age of coconut water significantly influenced callusing and embryogenesis. The concentration of NAA in the medium was not found to be significant. Embryogenesis frequencies up to 33.6 % were produced with zygotic explants.

In the second phase, experiments were conducted to optimize putative transformation. Kanamycin at 75 mg/l was found to inhibit callusing and embryogenesis in non-transformed petal and staminode while a concentration of 50 mg/l was adequate to inhibit growth of non-transformed sectors in zygotic embryo explants. Up to 70 % of the zygotic explants exhibited transient transformation after 1 day, however, transient transformation was not detected in petal and staminode explants. After 21 days, 62.5 % of the zygotic explants demonstrated putative integrative expression.

In zygotic embryo explants, the frequency of putative transformation and embryogenesis were found to decrease with increasing co-cultivation time. The agrobacterium concentration, length of inoculation time, presence of acetosyringone and length of preculture period significantly influenced putative transformation and embryogenesis, while tobacco

feeder plates and the length of the recovery period did not. Putatively transformed embryos were produced at frequencies up to 21.3 %.

In the final phase, the successful production of viable plantlets was not achieved in this work. Somatic embryos increased in size and germinated however only two or three small leaves were produced. Hardening of these plantlets was unsuccessful. The use of liquid maturation medium was found to increase the frequency of hypocotyl elongation, root formation and shoot formation.

**Keywords:** Agrobacterium, transformation, Theobroma, cacao, co-cultivation