

Physical and biological parameters affecting transient GUS and GFP expression in banana via particle bombardment

ABSTRACT

Physical and biological parameters for DNA delivery into banana cultivar, Rastali (AAB) explants were optimised by monitoring transient *gusA* and *gfp* gene expression. Optimisation of the physical factors was carried out under the following conditions; helium pressure (450, 900, 1100, 1350 and 1550psi); distance from stopping plate to target tissue (3, 6, 9 and 12 cm); vacuum pressure (26, 27, 28 and 29 mmHg), number of bombardments (1, 2 and 3 times) per Petri dish and gold microcarrier size (0.6 and 1.0 μm). Distance from rupture disk to macrocarrier and macrocarrier to stopping screen was fixed at 4mm and 11mm, respectively. Two controls were also incorporated i.e. tissues without bombardment and bombardment of microcarrier without DNA. The biological parameters included the explant type (single bud and corm slice), influence of explant sizes (3, 5 and 10 mm), preculture treatment prior bombardment (0, 1, 2, 3 and 4 days), DNA concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 μg) and effect of post-bombardment incubation time (3, 6, 9, 12, 15 and 18 days). Optimised bombardment conditions for single bud was bombarding twice at 1100 psi, 9cm target distance, 28 mmHg, 1 μM gold particle size, 3mm in size range, 1.5 μg DNA per bombardment, three days preculture prior to bombardment and six days post bombardment. For corm slice, the optimised bombardment conditions was bombarding once at a helium pressure of 1100 psi helium pressure, 9cm (*gusA* gene) or 6cm (*gfp* gene) target distance, 28 mmHg vacuum pressure and 1 mM gold particle size, 5mm in size range, 1.5 mg DNA per bombardment, one day preculture prior to bombardment and nine days post bombardment. Combinations of optimised physical and biological parameters and an effective selection system were developed which allowed high-efficiency of DNA delivery combined with minimum damage to target banana tissues.

Keyword: Particle bombardment; Banana; Transient gene expression