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Abstract

Transient transformation approaches show promise for rapidly assessing specific genes and gene constructs in live tissue. This is especially true in the case of plant leaf tissues, which are hard to culture and provide easy targets for transformation techniques. In the case of maize leaves, the use of biolistic approaches circumvent the problems of *Agrobacterium* host range.

To achieve reliable expression, transient biolistic transformation of maize leaf tissues (taken from within the non-emergent leaf whorl of 8-week-old maize plants) was optimised using a Taguchi design-of-experiments approach and Generalised Linear Modelling. Bombardments were carried out on plants from the B73 inbred line using the PDS-1000/He biolistic device. Input conditions (burst disc pressure, microparticle loading, DNA loading and sample selection within the non-emergent leaf whorl) were varied across three levels over the course of nine trials.

Bombardments were carried out using a BioRad PDS-1000/He gene gun, M17 tungsten microparticles and a pAHC25 reporter gene plasmid; the reporter gene construct consisting of a ubiquitin promoter, intron, beta glucuronidase coding region and NOS terminator. Samples were assessed for beta glucuronidase expression by histological staining in 5-Bromo-4-chloro-1*H*-indol-3-yl β-D-glucopyranosiduronic acid (X-Gluc) solution. This resulted in visible blue 'spots' (each corresponding to a transformed cell) which were then counted under a dissecting microscope.

Additional, qualitative results were generated by sectioning bombarded leaf samples and wet-mounting the sections for observation under a stereo-microscope. An improved sectioning techniques, hand cryo-sectioning, was developed to surmount some of the challenges posed by the thin, friable samples. For the final experiment an additional factor; sample distance along the leaf axis from the ligule (the leaf sheath extension which in non-emergent leaves is coincident with the leaf base) was included due to publication of similar research by another group.

The results established new optimal conditions for maize leaf bombardments and confirmed that ligule distance is an important input factor for achieving high transient transgene expression. Optimal conditions for maize leaf bombardment were determined to be: high burst disc pressures (1350PSI), particle loadings above 2mg of tungsten microparticles per bombardment, DNA loadings of 5ug per bombardment ($2.38x10^{-6}$ umol of pAHC25, or $4.8x10^{11}$ copies), leaf sample selection from the central leaf whorl out to the 3rd or 4th non-emergent leaves and a distance from the leaf ligule of 0-3cm. In addition, a simple empirical model for microparticle penetration (the newtonian penetration approximation, giving an estimated penetration of 20-40um for the conditions tested) was assessed and found to be well-correlated with the results seen in maize leaf bombardments when attempting to estimate impact depth.

Additional modelling concerning the area ratio of the nucleus versus the rest of the cell was performed, with the result that even when accounting for morphological differences in cell/nuclear ratio between cells closer to the leaf base and those further away, there was still a significant difference in transformation efficiency.

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Declaration of Originality

Full names of student: Thomas Gerard Schmidt

Student number: 04399846

Topic of work: Transient Biolistic Transformation in Maize Leaves

Declaration

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List of Abbreviations

2,4-D – 2,4-dichlorophenoxyacetic acid

ANOVA – Analysis of Variance

EDTA – Ethylenediaminetetraacetic acid

GFP – Green Fluorescent Protein

GLM – Generalised Linear Model

GUS – Beta glucuronidase

PIG – Particle Inflow Gun

SASRI – South African Sugarcane Research Institute

S/N ratio – Signal-to-Noise ratio

X-Gluc – 5-Bromo-4-chloro-1*H*-indol-3-yl β-D-glucopyranosiduronic acid

ZFN – Zinc-Finger Nuclease