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Miscanthus giganteus Biolistic Transformation Using the Visible RUBY Red Marker Gene to Monitor Transformation Efficiency

Background/Objective

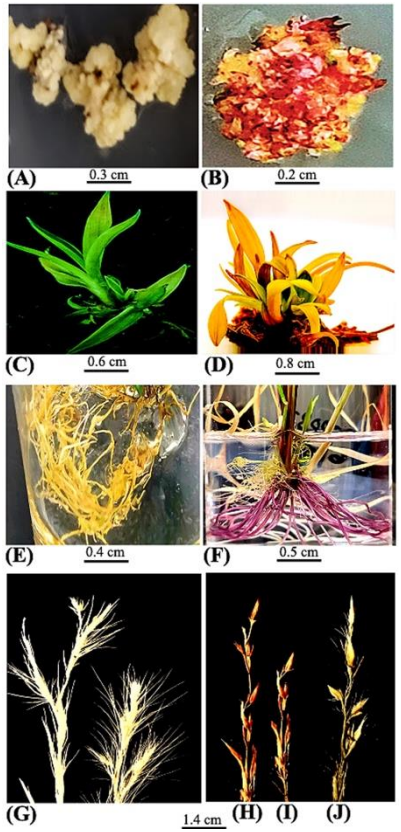
Miscanthus × *giganteus* (*M*×*g*) is a high-yielding perennial C4 bioenergy crop, but genetic improvement by breeding is constrained by triploid sterility and clonal propagation. Improving genetic transformation methods for *M*×*g* would provide opportunities for advantageous trait introgression. Use of an easy to phenotype reporter gene is a promising strategy to improve transformation processes and efficiency. This study presents an efficient novel method for biolistic transformation of inflorescence-derived callus in *M*×*g* and demonstrates its efficacy using RUBY, a betalain-based noninvasive reporter that is visible throughout the transformation process.

Approach

Here we provide the first biolistic transformation protocol for *M*×*g*. From a total of 360 inflorescences, 170 produced friable callus for a 47.2% callus induction rate, similar to previously published work. Of these, 120 calli were randomly selected and subjected to biolistic transformation, yielding 21 independent event transgenic plants using two RUBY constructs, resulting in a transformation efficiency (TE) of 17.8%. Transformation was confirmed via selection on hygromycin media and confirmation of the RUBY gene by PCR.

Results

RUBY expression (*Zea mays* codon optimized) was visible from callus stage through plantlet development into maturity. The *Zea mays* codon optimized hygromycin selection marker was driven by previously established promoters for Miscanthus, ZmUBI and 2×35S, while the RUBY gene expression was controlled by a known *Zea mays* C4 promoter, *Brachypodium* UBI10, newly employed in Miscanthus. The construct containing the 2×35S promoter for hygromycin had a 15.1% TE while the ZmUBI promoter had a 20.5% TE.



Comparison of RUBY independent transformants from RC00336 construct (B, D, F, H, I, J) and wild type (A, C, E, G) callus (A, B), regenerated plantlet leaves (C, D) and roots (E, F), and inflorescence (H-RC00336-3, I-RC00336-5, J-RC00336-13 (null)).

Significance/Impacts

This study provides a novel, highly efficient protocol for successful biolistic transformation of *M*×*g* for stable expression. Our work also demonstrates that RUBY expression can be used as a convenient and powerful monitor of transformation in ongoing and future work to engineer *M*×*g* into an improved feedstock and offers future opportunities for improving transformation methods and realizing even greater improvements in efficiency.

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