Genetic transformation of Fusarium avenaceum by Agrobacterium tumefaciens mediated transformation and the development of a USER-Brick vector construction system - DTU Orbit (29/01/2019)

Genetic transformation of Fusarium avenaceum by Agrobacterium tumefaciens mediated transformation and the development of a USER-Brick vector construction system

The plant pathogenic and saprophytic fungus Fusarium avenaceum causes considerable in-field and post-field losses worldwide due to its infections of a wide range of different crops. Despite its significant impact on the profitability of agriculture production and a desire to characterize the infection process at the molecular biological level, no genetic transformation protocol has yet been established for F. avenaceum. In the current study, it is shown that F. avenaceum can be efficiently transformed by Agrobacterium tumefaciens mediated transformation. In addition, an efficient and versatile single step vector construction strategy relying on Uracil Specific Excision Reagent (USER) Fusion cloning, is developed. Results: The new vector construction system, termed USER-Brick, is based on a limited number of PCR amplified vector fragments (core USER-Bricks) which are combined with PCR generated fragments from the gene of interest. The system was found to have an assembly efficiency of 97% with up to six DNA fragments, based on the construction of 55 vectors targeting different polyketide synthase (PKS) and PKS associated transcription factor encoding genes in F. avenaceum. Subsequently, the Delta FaPKS3 vector was used for optimizing A. tumefaciens mediated transformation (ATMT) of F. avenaceum with respect to six variables. Acetosyringone concentration, co-culturing time, co-culturing temperature and fungal inoculum were found to significantly impact the transformation frequency. Following optimization, an average of 140 transformants per 10^6 macroconidia was obtained in experiments aimed at introducing targeted genome modifications. Targeted deletion of FaPKS6 (FA08709.2) in F. avenaceum showed that this gene is essential for biosynthesis of the polyketide/nonribosomal compound fusaristatin A. Conclusion: The new USER-Brick system is highly versatile by allowing for the reuse of a common set of building blocks to accommodate seven different types of genome modifications. New USER-Bricks with additional functionality can easily be added to the system by future users. The optimized protocol for ATMT of F. avenaceum represents the first reported targeted genome modification by double homologous recombination of this plant pathogen and will allow for future characterization of this fungus. Functional linkage of FaPKS6 to the production of the mycotoxin fusaristatin A serves as a first testimony to this.

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