A rapid *Brachypodium distachyon* transformation method using leaf whorl explants

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Project Goals: Understanding the interactions, localization, and dynamics of grass rhizosphere communities at the molecular level (genes, proteins, metabolites) to predict responses to perturbations and understand the persistence and fate of engineered genes and microbes for secure biosystems design. To do this, advanced fabricated ecosystems are used in combination with gene editing technologies such as CRISPR-Cas and bacterial virus (phage)-based approaches for interrogating gene and microbial functions *in situ*—addressing key challenges highlighted in recent DOE reports. This work is integrated with the development of predictive computational models that are iteratively refined through simulations and experimentation to gain critical insights into the functions of engineered genes and interactions of microbes within soil microbiomes as well as the biology and ecology of uncultivated microbes. Together, these efforts lay a critical foundation for developing secure biosystems design strategies, harnessing beneficial microbiomes to support sustainable bioenergy, and improving our understanding of nutrient cycling in the rhizosphere.

Abstract

To fully understand grass rhizosphere communities, it is important to consider the role of the plant root in shaping the rhizosphere microbiome. However, this is poorly understood, in part due to the difficulties in generating plant mutants, since plant transformation, particularly of grasses, is time-consuming and laborious. The m-CAFEs project uses *Brachypodium distachyon*, as it is an excellent model for bioenergy feedstocks. The current transformation method for *B. distachyon* ecotype Bd21-3 uses immature embryos as the explant material, and has a cycle of 22-31 weeks from non-transgenic seed to the first generation of transgenic seed. Immature embryos are both technically challenging to isolate and only available from plants during a short developmental window. Recently, a novel method using leaf whorls as the explant material has been developed in sorghum (Silva et al., 2020). Here, we demonstrate that the leaf whorl-based transformation can be adapted to *B. distachyon*, thereby shortening the cycle to 14-20 weeks. Advantages include that since the explant material is derived from 3-week-old seedlings, rather than mature plants, total experiment length is reduced. Second, callus can be generated from the plants continuously until the plant starts to set seed (~8-12 weeks depending on ecotype). Third, isolating leaf whorls is much less technically challenging and laborious compared to immature

embryos. Finally, we have been successful in generating calli from late-flowering ecotypes, such as Bd1-1, which can be difficult to isolate immature embryos from under lab conditions (Schwartz et al., 2010). This method can be integrated with other advances in plant transformation, such as the expression of morphogenic regulations (e.g. BBM/WUS or GRF-GIF4) to further accelerate plant transformation.

References

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