

Development of Transgenic Sorghum Lines to Enhance Water Use and Photosynthetic Efficiencies

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Project Goals: This project aims to leverage *Setaria viridis* as a model system to develop novel technologies and methodologies to redesign the bioenergy feedstock Sorghum bicolor to enhance water use and photosynthetic efficiencies.

Our objectives are towards meeting the needs for stable sorghum transgenics for this project and we are extending these capabilities into new transformation technologies for sorghum. We have established a reliable pipeline for the introduction of stable genetic constructs into sorghum for the projects outlined in the proposal. Our first goal has been accomplished by developing reliable protocols using standard *Agrobacterium*-mediated transformation of BTx430 for routine analyses and the transformation the sorghum reference genome, sorghum (BTx623). The Kausch Lab has generated a pipeline for this project and has many transgenic lines and events in various stages of regeneration for our initial constructs. This pipeline in collaboration with the Voytas and Cousins labs includes the following constructs driven by the maize Ubiquitin promoter and using the BAR gene as a selectable marker: (1) pNG026, BE3 nuclease; (2) pNG028, 1bCPF1 nuclease; (3) pMZ64, Ta Cas 9; (4) pMZ69, dTa Cas9; and , (5) pMZ263, dCas9 transcriptional activator under control of the Zm PEPC promoter targeting all four isoforms of carbonic anhydrase in the sorghum genome. One of our goals is to engineer photosynthesis to improve performance under water stress via (1) a transgenic approach using stably integrated constructs; (2) a transcriptional activation route employing dCas9; and (3) modification of PEPC kinetics to enhance the efficiency of C4 photosynthesis. We are currently in the process of generating these transgenic lines. Numerous events for each are currently in T₀ plants in regeneration for each. Another goal of our project is to develop methods to edit genes and to manipulate gene expression *in vivo* in Sorghum. To accomplish this goal we will use: (1) Targeted mutagenesis with nucleases (2) Targeted mutagenesis with base editors (3) Targeted gene replacement; and, (4) Targeted gene insertion. We are now exploring the use of the transient expression of morphogenic regulators and gene editing functions for rapid target validation and regeneration of edited events. There are currently four publications published on our results, one review paper, and three presentations from our work. Co-PI Kausch and former Co-PI Quemada were active in the discussion and publication regarding the Regulatory and Stewardship implications for using genome editing in sorghum and other crops. Both presented invited presentations at the SIVB meeting on this topic in June 2018.

Publications

1. Kausch A.P., Nelson-Vasilchik K., Hague J., Mookkan M., Quemada H., Dellaporta S., Fragoso C., Zhang Z.J. (2019) Edit at Will: Genotype Independent Plant Transformation in the Era of Advanced Genomics and Genome Editing, Plant Science, in press, available online January 14 2019, <https://doi.org/10.1016/j.plantsci.2019.01.006>
2. Do, P. T., Lee, H., Nelson-Vasilchik, K., Kausch, A., & Zhang, Z. J. (2018). Rapid and efficient genetic transformation of sorghum via *Agrobacterium*-mediated method. Current Protocols in Plant Biology, 3, e20077. doi: 10.1002/cppb.20077
3. Mookkan, M., Nelson-Vasilchik, K., Hague, J., Zhang, Z.J., and Kausch, A.P. (2018) Morphogenic regulator-mediated transformation of maize inbred B73. Current Protocols in Plant Biology 3 (4), e20075. doi: 10.1002/cppb.20075
4. Nelson-Vasilchik, K., Hague, J., Mookkan, M., Zhang, Z.J., and Kausch, A.P. (2018) Transformation of recalcitrant sorghum varieties facilitated by baby boom and wuschel2. Current Protocols in Plant Biology 3(4), e200076. doi: 10.1002/cppb.20076
5. Mookkan, M., Nelson-Vasilchik, K., Hague, J., Zhang, Z.J., and Kausch, A.P. (2017) Selectable marker independent transformation of recalcitrant maize inbred B73 and sorghum P898012 mediated by morphogenic regulators *BABY BOOM* and *WUSCHEL2*. Plant Cell Reports 36 (9), doi:10.1007/s00299-017-2169-1

Presentations

1. Albert Kausch* Invited Speaker* (2018) The Era of New Agricultural Product Development Via Genomics, Plant Transformation, and Genome Editing. Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI 02892 Society for In Vitro Biology Meetings, June 2-5 2018 St. Louis MO.
2. Albert Kausch* Invited Speaker* (2018). Public Policy, Societal Impacts, and Considerations for Advanced Biology, Genomics and Gene Editing in Pharmaceutical, Medical and Agricultural Applications. Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI 02892 Society for In Vitro Biology Meetings, June 2-5 2018 St. Louis MO.
3. Hector Quemada* and Albert Kausch Invited Speaker* (2018) Regulation of Genome Edited Events. Donald Danforth Plant Science Center, Saint Louis, MO 63132 Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI 02892 Society for In Vitro Biology Meetings, June 2-5 2018 St. Louis MO

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