Transformation of American chestnut founder lines with OxO

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Scope of Work

The overall goal of the proposed work is to generate at least one American chestnut culture line (founder line) representing each of the broad sub-regions of the range of the tree (New England, North Central, Mid-Atlantic and Southern) capable of making trees carrying and expressing the OxO gene. These transgenic OxO lines can be used to ensure that there are regionally-adapted OxO American chestnut "founders" for each region for breeding and other purposes. To accomplish this goal, we propose to do the following work over the next two years:

Our lab already has over 90 new American chestnut embryogenic lines representing each of the four sub-regions, which were initiated during summer 2020 with the help of TACF cooperators. We will screen these "founder" lines by running them through our production pipeline to characterize each for its potential to produce somatic embryos and somatic seedlings. This work has already begun, with 34 lines now in the pipeline. We will use the results of the screen to choose 3-4 lines from each region that appear to have the highest potential for making somatic embryos and plantlets to be used as target material for *Agrobacterium*-mediated transformation with the OxO gene. Currently, the plan is to share copies of these lines with the Powell Lab at SUNY-ESF and each lab will conduct transformations using its own system. Each lab will probably start out working on different lines and if one lab or the other has difficulties applying its system to get transgenic cultures for a given line, the other lab can take over to see if its approach does better. This approach would also apply if one lab has problems regenerating plantlets from a given line or event. Although not settled yet, we will probably do the transformations with a wound-inducible promoter-OxO fusion (Win3-OxO) created by the Powell Lab, rather than using the 35S-OxO fusion used to generate the Darling 58 transgenic line. In our lab at UGA, the only molecular assays we will do will be to test putative transgenic events for presence of the OxO gene using PCR. Copies of PCR-positive events will be sent to the Powell lab for further molecular assays for copy number and OxO expression level. Once this information is available, we will generate somatic embryos and somatic seedlings from single-copy events showing acceptable expression levels from multiple events in each background using our standard protocols.

Deliverables to TACF will be somatic seedlings representing 3-5 Win3-OxO events in at least one founder line background from each of the four regions. We will work with the Powell Lab to get to these numbers for the four regions.

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Budget Justification

We are requesting salaries and benefits for one 0.5-time Postdoc (Heather Gladfelter), one 0.5-time Research Professional II (Ryan Tull) and one undergrad student assistant, and operating expense (tissue culture, transformation, molecular genetics supplies) for two years.

It is the policy of The American Chestnut Foundation not to pay indirect costs on grants.

Total: $163,000

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BUDGET

UNIVERSITY OF GEORGIA RESEARCH FOUNDATION, INC.
IRS Entity Number 58-1353149

Budget period: 07/01/21 - 06/30/23

Principal Investigator: Scott Merkle

		Year 1	Year 2	Total
A	**Salaries and Wages**			
1)	Research Professional II (0.5 time)	$ 21,000	$ 22,000	$ 43,000
2)	Postdoctoral Associate (0.5 time)	$25,000	$ 26,000	$ 51,000
3)	Student assistant	$6,000	$6,000	$ 12,000
	Total Salaries and Wages	$ 52,000	$ 54,000	$ 106,000
B	**Fringe Benefits**			
1)	48% of A1	$ 10,080	$ 10,560	$ 20,640
	38% of A2	$ 9,500	$ 9,880	$ 19,380
	Total Fringe Benefits	$ 19,580	$ 20,440	$ 40,020
C	**Permanent equipment**	$ -		$ -
D	**Travel**	$ -		$ -
E	**Other Direct Costs**			
1)	Materials and Supplies	$ 8,000	$ 8,980	$ 16,980
	Total Other Direct Costs	$ 8,000	$ 8,980	$ 16,980
F	**TOTAL DIRECT COSTS**	$ 79,580	$ 83,420	$ 163,000
G	**F&A (TACF no overhead)**	$ -		$ -
	TOTAL PROJECT COSTS	$ 79,580	$ 83,420	$ 163,000