

Final Project Report

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Project Title: “Utilization of the *Arabidopsis FT* Gene to Facilitate Rapid Tobacco Variety Development”

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The objective of this project was to produce early-flowering versions of six KTTII inbred lines of tobacco. Previous work at NCSU and in this laboratory has shown conclusively that overexpression of the Arabidopsis floral regulator gene *FLOWERING LOCUS T* (*FT*) in tobacco gives plants that will flower in as little as 40 days from germination. We chose the KTTII burley parental inbred lines TKF1112D, TKF1116A, TKF1176A, and TKF4028E2-A and the two dark inbred lines D1012C1 and D1084F1 for transformation. All were transformed independently with a genetic construct carrying the *FT* cDNA gene clone from *Arabidopsis thaliana*. Dr. Gao also transformed two of the burley lines (TKF1112D and TKF1176A) with a similar construct carrying the cDNA for a related Arabidopsis gene known as *TWIN SISTER OF FT* (*TSF*) that has an analogous function in regulating flowering time.

The project commenced in late 2013, with the first transgenic plants flowering in January 2014. The work was quite routine, although there were several minor issues: seeds of both dark lines (D1012C1 and D1084F1) were heavily contaminated with fungal spores, and the initial attempts to grow seedlings in sterile culture were unsuccessful. This was overcome by increasing seed sterilization times and sowing single seeds per container. The first transformed shoots of burley line TKF4028E2-A did not survive, and the transformation with the *35S:AtFT* gene construct was repeated. Also, we initially had only a single early-flowering version of TKF1176A, so more were produced in the following months.

As shown in the table below, we were successful in producing early-flowering versions of all six tobacco inbred lines (2 to 4 independent transformants per line). All of the plants expressing *FT* were crossed back to their parental lines, with two exceptions, and also self-pollinated. Of the five lines expressing *TSF*, two were backcrossed and selfed, and three were only self-pollinated.

We thank the Council for Burley Tobacco for their generous funding of this project. The early flowering lines will allow us to produce new tobacco varieties in less time than with conventional methods, which will benefit tobacco producers in Kentucky and Tennessee.

Tobacco Inbred	Early-flowering gene	T0#	Backcrossed	Selfed	Date pollinated	Date seed harvested
TKF1112D	FT	1	√		3/14/14	4/16/14
TKF1112D	FT	1		√		4/2/14
TKF1112D	FT	2	√		3/14/14	4/16/14
TKF1112D	FT	2		√		4/2/14
TKF1112D	FT	3		√		4/2/14
TKF1112D	TSF	1	√		3/14/14	4/14/14
TKF1112D	TSF	1		√		4/15/14
TKF1112D	TSF	2		√		5/22/14
TKF1112D	TSF	3		√		5/22/14
TKF1116A	FT	1	√		7/14/14	9/8/14
TKF1116A	FT	1		√		9/3/14
TKF1116A	FT	2	√		7/22/14	9/3/14
TKF1116A	FT	2		√		9/3/14
TKF1116A	FT	3	√		7/14/14	9/3/14
TKF1116A	FT	3		√		9/3/14
TKF1116A	FT	4	√		7/21/14	9/3/14
TKF1116A	FT	4		√		9/3/14
TKF1176A	FT	1	√		1/10/14, 1/22/14	2/16/14, 2/25/14
TKF1176A	FT	1		√		2/16/14
TKF1176A	FT	2	√		3/11/14	4/21/14
TKF1176A	FT	3	√		6/13/14	7/17/14
TKF1176A	FT	3		√		7/17/14
TKF1176A	FT	4	√		6/13/14	7/17/14
TKF1176A	FT	4		√		7/17/14
TKF1176A	FT	5	√		7/21/14	9/11/14
TKF1176A	FT	5		√		9/11/14
TKF1176A	TSF	1	√		7/17/14	9/11/14
TKF1176A	TSF	1		√		9/11/14
TKF1176A	TSF	2		√		9/11/14
TKF4028E2-A	FT	1	√		5/28/14	7/11/14
TKF4028E2-A	FT	1		√		7/11/14
TKF4028E2-A	FT	2		√		7/11/14
D1012C1	FT	1	√		1/10/14	2/26/14
D1012C1	FT	1		√		2/26/14
D1012C1	FT	2	√		1/31/14	3/11/14
D1012C1	FT	2		√		2/26/14
D1084F1	FT	1	√		5/28/14	7/2/14
D1084F1	FT	1		√		9/3/14
D1084F1	FT	2	√		5/28/14	7/2/14
D1084F1	FT	2		√		9/3/14