Manipulation of Stomatal Density in Fine Hardwoods

Shaneka Lawson*, Charles Michler and Paula Pijut

Department of Forestry and Natural Resources and Hardwood Tree Improvement and Regeneration Center (HTIRC)
Purdue University, West Lafayette, Indiana 47907

PURDUE UNIVERSITY

ABSTRACT

In the wake of Dutch elm disease, green ash (*Fraxinus pennsylvanica*) has been planted as a replacement for the American elm (*Ulmus americana*). Naturally a drought tolerant tree, this woody ornamental has been used for agroforestry, conservation, and urban street plantings. We are using genetic engineering to generate trees with increased water conservation abilities to alleviate pressures placed on current water availability. Overexpression of the *AtEPF1* gene in *Arabidopsis* results in reduced stomatal density, a characteristic directly related to water-use efficiency and indirectly to drought tolerance. Successful use of *Arabidopsis* and poplar model systems indicates this knowledge may be transferable to green ash. Here, we demonstrate a new technique for improving green ash hardiness without adverse effects, thus providing a foundation for the development of environmentally attuned fine hardwoods.

INTRODUCTION

Decreasing water loss by altering stomatal density has been achieved in *Arabidopsis* (Fig. 2A-B). Here we attempt to mirror those results using the model tree species poplar and green ash, a fine hardwood. In *Arabidopsis*, EPF1 (epidermal patterning factor 1) is involved in stomatal complex development and controls number and spatial distribution of stomata. We will overexpress *At*EPF1 in *Arabidopsis*, poplar, and green ash along with a "putative" poplar ortholog *Pta*EPF1 (Fig. 1). We aim to decrease leaf water loss while improving water-use efficiency (WUE), a measure of biomass obtained to units of water used. In green ash we hope to improve this ratio without adversely affecting growth. Often found in riparian areas such as stream banks, green ash has proven itself capable of tolerating a wider range of environments, thus we feel our proposed improvements will allow green ash populations to tolerate the highly variable future climate events predicted to occur.

BACKGROUND

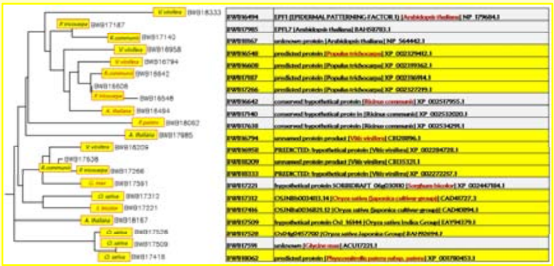
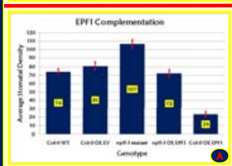


Fig. 1. Phylogenetic analysis. Homology between *Arabidopsis* EPF1 gene and top 20 results.



Microscopy Results		Results: Lawson
Col-0	Normal	Normal
Empty Vector	Normal	Normal
epf1-1	2-3x more stomata	1.4-1.5x more stomata
epf1-1 OE EPF1	normal	normal
Col-0 OE EPF1	2-3X less stomata	4X less stomata

Fig. 2. *Arabidopsis* complementation. (A) *At*EPF1 experiment. (B) Comparison between published and observed results.

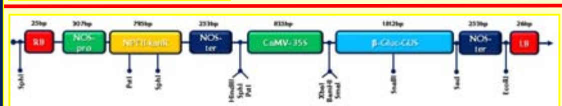
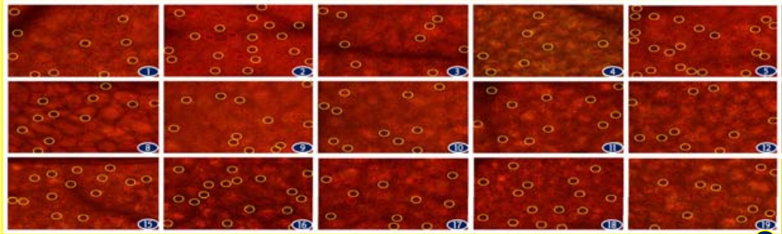


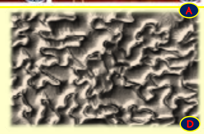
Fig. 3. Empty pBI121 vector.

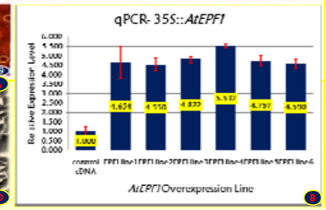
MATERIALS AND METHODS

- Transform poplar, *Arabidopsis* , and green ash with pBI121-based binary vectors (Fig. 3)
- Screen and verify transformants using transgene-specific primers
- Measure transgene expression levels with qRT-PCR (Fig. 4C)
- Propagate transformants and determine transpiration rate by analysis of water loss (measured gravimetrically) and stomatal density (Figs. 4A&B, 5A-D)
- Verify transgene integration with Southern blots using transgene-specific probes

RESULTS: *Arabidopsis*



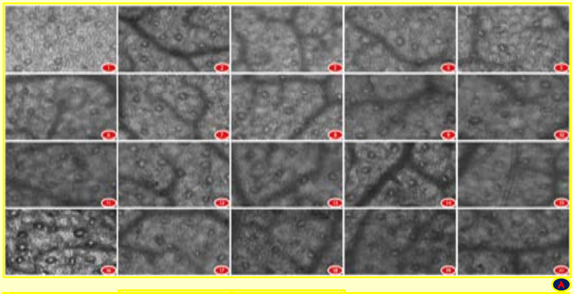


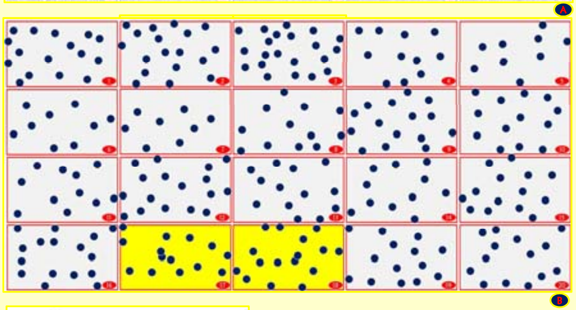


#	Mutant Lines	Average Density (A=1mm²)	Average Water Loss (36hrs)
1	CDC6	10	2.33
2	CDKB1;1	22	2.36
3	CDT1	9	2.65
4	Col-0 (control)	8	3.86
5	EDT1	24	1.13
6	EPF1	16	9.43
7	ER	12	2.12
8	ERL1	11	1.26
9	FAMA	13	8.74
10	FLP	11	5.6
11	MKK4	12	1.07
12	MKK5	10	5.61
13	MPK3	10	6.27
14	MPK6	12	6.79
15	MUTE	16	2.77
16	SDD1	17	2.99
17	SPCH	13	2.03
18	TMM	15	0.75
19	YODA*	12	1.11
	*Leaf size significantly smaller		

Fig. 4. *Arabidopsis* staining. (A) Panels 1-19 represent available stomatal density mutants with stomata highlighted. (B) A magnified unstained leaf. (C) Chart identifying mutant lines, their average stomatal density, and gravimetric water loss over 36 hours. (D) qPCR analysis of six *At*EPF1 transgenic overexpression lines.

RESULTS: *Poplar*





#	Line Name	Average Density (A=1cm²)
1	E1	16
2	E6	18
3	E9	21
4	E10	11
5	E12	9
6	E25	9
7	E26	8
8	E42	12
9	E43	15
10	E46	13
11	E50	11
12	E55	17
13	E58	13
14	E83	11
15	E89	14
16	E91	16
17	E100	14
18	E100	16
19	E101	14
20	717 (wt)	14

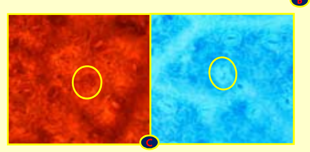


Fig. 5. Poplar staining. (A) Panels 1-20 indicate abaxial staining of 19 transgenic poplar lines overexpressing *At*EPF1. (B) Representative stomatal densities of the transgenic lines. (C) Observed alterations in stomatal patterning. (D) Mutant line stomatal densities.

PRELIMINARY RESULTS: *Green Ash*

Fig. 6. Green ash preliminary results. (A) Transformation and regeneration illustration (B) Control vector transformed green ash callus (According to Du and Pijut 2009). (C) Regenerated "putative" transgenic plantlets.

DISCUSSION

- Stomatal density variations in *Arabidopsis* and poplar were obtained with *AtEPF1* overexpression constructs (Figs. 4,5)
- Observation of *Arabidopsis* stomatal density mutant transpiration rates indicate a connection to water-use efficiency (Fig. 4B)
- qRT-PCR results indicate stomatal densities vary with *AtEPF1* overexpression construct levels (Fig. 4C)

REFERENCES

Du, N.; Pijut, P.M. Regeneration of plants from *Fraxinus pennsylvanica* hypocotyls and cotyledons. Sci. Hortic. 118:74-79; 2009

ACKNOWLEDGEMENTS

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