THE EFFECT OF DELTA-12 FATTY ACID DESATURASE (FAD) GENE EXPRESSION ON SEEDLING VIGOR UNDER COOL TEMPERATURES

John L. Snider
Department of Crop and Soil Sciences, University of Georgia, Tifton, GA
Kent D. Chapman and Shanmukh Salimath
Department of Biological Sciences, University of North Texas, Denton, TX
Viktor Tishchenko
University of Georgia, Griffin, GA

Introduction

Seedling vigor is an important characteristic for ensuring uniform and healthy stand establishment, and in some instances, poor seedling vigor and stand establishment can negatively impact yield or force growers to make the costly decision to replant (Collins and Whitaker, 2012; Snider et al., 2014; Wanjura et al., 1969). Vigorous early season plant growth 1) maximizes light interception, 2) improves competitiveness with weedy species, and 3) lessens the long-term damage that can be caused by early-season insect herbivory. Given the well-established relationship between temperature and cotton development, it is not surprising that below optimum temperature conditions during the early growing season will slow growth and development, and if temperatures are cool enough, result in chilling injury in cotton (Kratsch and Wise, 2000; Wise et al., 1983).

One plant characteristic that influences tolerance to either high or low temperatures is the level of membrane fluidity. That is, chilling-sensitive species, like cotton, tend to have poor membrane fluidity at low temperatures, resulting in a number of negative physiological consequences. The level of fatty acid saturation in plant cell membranes will influence membrane fluidity in much the same way that fatty acid saturation determines whether fats used for cooking purposes are a liquid (canola oil, olive oil, etc.) or a solid (lard, butter, etc.) at room temperature. Membrane fluidity and tolerance to cool, early season temperatures could potentially be improved in cotton if fatty acids were less saturated during the seedling stage.

The delta-12 fatty acid desaturase (FAD2) is an enzyme in plants that accomplishes fatty acid desaturation, specifically to create omega-6 polyunsaturated fatty acids. Consequently, the current study sought to assess whether transgenic cotton lines overexpressing a cotton isoform of the delta-12 fatty acid desaturase (FAD2-4; Zhang et al., 2009) would exhibit greater seedling vigor than their parent genotype under cool temperatures imposed under controlled environment conditions. Thus the main objective of the current study was to assess the response of leaf area, plant fresh weight, and plant height three weeks after planting under cool (20/15C) and optimal (30/20C) day/night temperature conditions for one parental line (Coker 312) and six different third-generation transgenic lines of Upland cotton.

Materials and Methods

Seeds of Coker 312 (the parental line; L1) and six FAD2-4 transgenic lines (L2 through L7) were grown for three weeks in two large Conviron walk-in controlled environment chambers (model CG72) at the Georgia Envirotron at the University of Georgia Griffin Campus. From planting until the end of the three-week growth period, chamber temperatures were maintained at a 30/20 ± 0.5C day/night temperature regime (optimal growth temperature regime for cotton) or a 20/15 ± 0.5C day/night temperature regime (sub-optimal temperature regime). Light intensity for both temperature regimes in the chambers was maintained equal throughout the growth period (~700 µmol m^{-2} s^{-1} photosynthetically active radiation). Seeds were planted at a 2.5 cm

depth in 1 liter pots filled with Pro-Mix growth medium and watered to capacity every two days. Pots were spaced 15 cm apart and the experimental design was a completely randomized design with seven lines and five replications of each.

Following three weeks of growth at each temperature regime, a number of different measures of seedling vigor were obtained. For brevity, only three parameters are discussed in the current report: leaf area per plant, plant fresh weight, and plant height. Plant height was measured in cm; whole plants were then cut at the base, and fresh weight was measured immediately following excision; all leaves were then cut from the plant, and a LI-3100 leaf area meter (Li-Cor; Lincoln, NE) was used to quantify total leaf area per plant.

To assess the importance of FAD2-4 overexpression on seedling vigor under cool conditions, relative to the vigor of the parent line (Coker 312), the effect of genotype (L1 through L7) on leaf area, fresh weight, and plant height at three weeks past planting was assessed using a one-way analysis of variance (ANOVA) at each temperature regime. Post-hoc analysis was conducted using Fisher's LSD ($\alpha = 0.05$).

Results and Discussion

When leaf area is assessed for all lines under optimal temperature conditions, no significant genotype effect is observed ($P < 0.05$; Figure 1). Under cool conditions (20/15C), two notable trends are observed: 1) leaf area development is substantially reduced under cool temperature conditions relative to optimal temperature conditions and 2) genotype strongly influences leaf area development under cool temperatures. Specifically, L4 produced significantly greater leaf area than the parent line (L1) under the cool temperature regime.

Under optimal conditions, plant fresh weight is significantly affected by genotype, where L3 and L6 produced the lowest plant fresh weight. L5 produced the greatest fresh weight and was not statistically different than L1, L2, L4, or L7. Under cool temperatures, L1-L3 produced the lowest plant fresh weight, whereas L4-L7 produced the greatest plant fresh weight. Similar to leaf area per plant, plant height demonstrated no significant cultivar effects under optimal conditions, but a significant cultivar effect under cool temperatures was observed. Specifically, L4 plants were the tallest plants measured under cool temperatures and were not statistically different than L5 through L7. L4, L5, L6, and L7 were all taller than the parental line under the 20/15C temperature regime.

Our findings indicate that multiple FAD2-4 overexpressing lines assessed demonstrated promise for improving seedling vigor under cool conditions, relative to Coker 312. Specifically, L4 demonstrated greater seedling vigor than the parental line in all parameters measured under the 20/15C temperature regime. Importantly, with the exception of L3 for plant fresh weight, all FAD2-4 transgenic lines perform similarly to Coker 312 under optimal temperature conditions, indicating no negative impacts of fatty acid desaturase over expression under optimal conditions. Thus, fatty acid desaturation appears to be a promising approach for improving seedling vigor under cool temperature conditions while not negatively impacting performance under optimal growth temperatures. The genotypes assessed in the current study are currently being evaluated in the field using planting date to expose all lines to cool early-season temperatures.

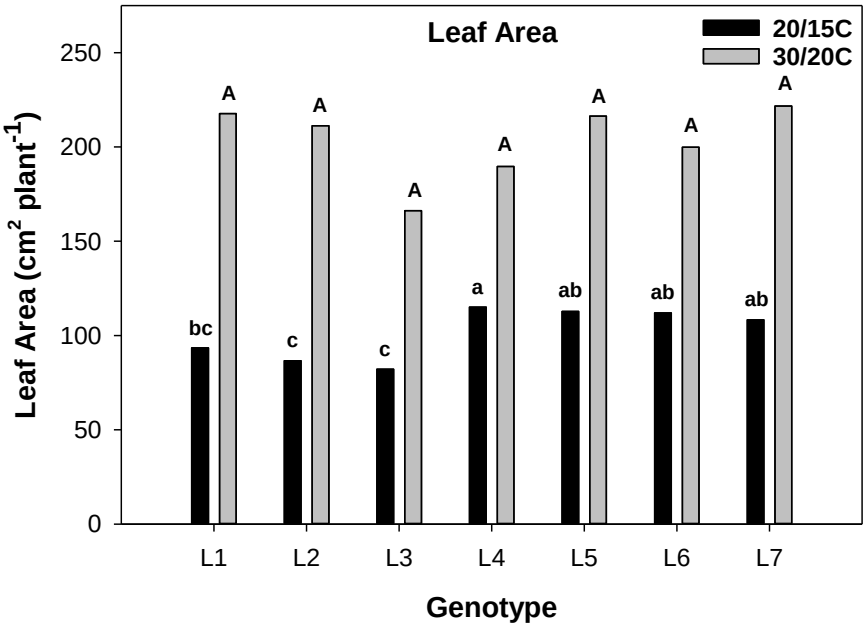

Figure 1. Average leaf area per plant for seven cotton genotypes (lines). L1 is the parent line (Coker 312), and L2 through L7 are third-generation transgenic lines engineered to overexpress fatty acid desaturase (FAD), which should improve seedling vigor under cool temperatures. Each column represents the mean leaf area of five plants. Columns not sharing a common letter within a given temperature regime are statistically different.

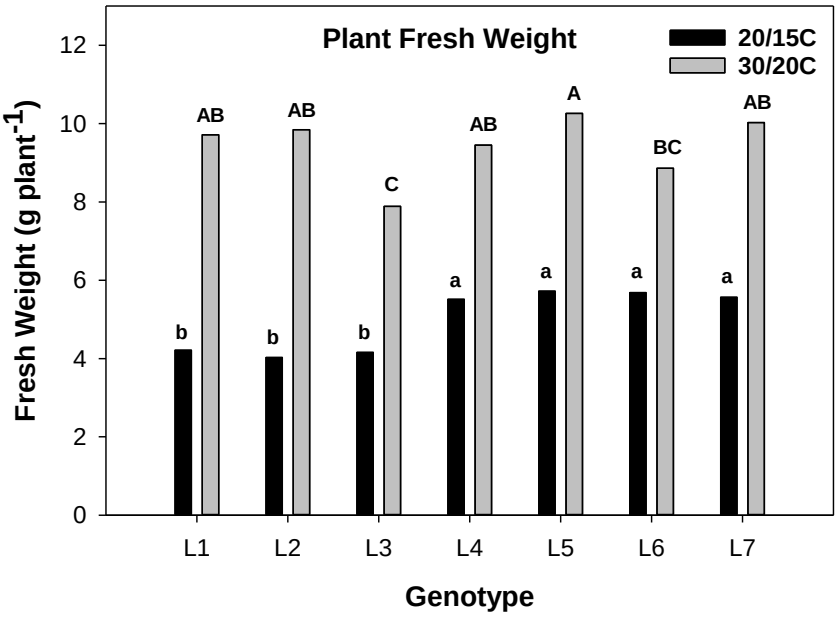

Figure 2. Average fresh weight per plant for seven cotton genotypes (lines). L1 is the parent line (Coker 312), and L2 through L7 are third-generation transgenic lines engineered to overexpress fatty acid desaturase (FAD), which should improve seedling vigor under cool temperatures. Each column represents the mean fresh weight of five plants. Columns sharing a common letter within a given temperature regime are not statistically different.

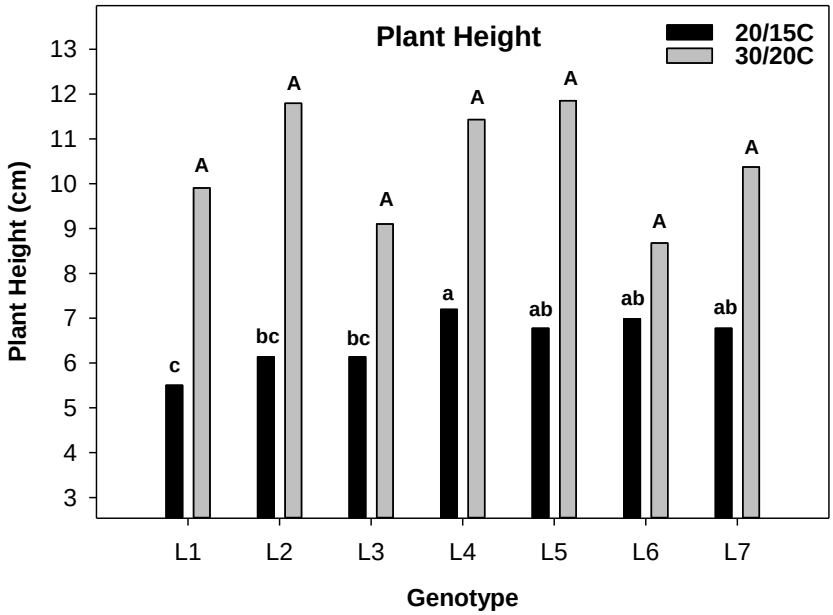

Figure 3. Average height per plant for seven cotton genotypes (lines). L1 is the parent line (Coker 312), and L2 through L7 are third-generation transgenic lines engineered to overexpress fatty acid desaturase (FAD), which should improve seedling vigor under cool temperatures. Each column represents the mean height of five plants, and columns not sharing a common letter within a given temperature regime are statistically different.

Acknowledgement

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