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At*RAV1* and At*RAV2* overexpression in cotton increases fiber length differentially under drought stress and delays flowering

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ABSTRACT

There is a longstanding problem of an inverse relationship between cotton fiber qualities versus high yields. To better understand drought stress signaling and adaptation in cotton (*Gossypium hirsutum*) fiber development, we expressed the Arabidopsis transcription factors *RELATED_TO_ABA-INSENSITIVE3/VIVIPAROUS1/(RAV1)* and *AtRAV2*, which encode APETALA2-Basic3 domain proteins shown to repress transcription of *FLOWERING_LOCUS_T* (*FT*) and to promote stomatal opening cell-autonomously. In three years of field trials, we show that AtRAV1 and AtRAV2-overexpressing cotton had ~5% significantly longer fibers with only marginal decreases in yields under well-watered or drought stress conditions that resulted in 40–60% yield penalties and 3–7% fiber length penalties in control plants. The longer transgenic fibers from drought-stressed transgenics could be spun into yarn which was measurably stronger and more uniform than that from well-watered control fibers. The transgenic AtRAV1 and AtRAV2 lines flowered later and retained bolls at higher nodes, which correlated with repression of endogenous *GhFT-Like* (*FTL*) transcript accumulation. Elevated expression early in development of ovules was observed for *GhRAV2L*, *GhMYB25-Like* (*MYB25L*) involved in fiber initiation, and *GhMYB2* and *GhMYB25* involved in fiber elongation. Altered expression of RAVs controlling critical nodes in developmental and environmental signaling hierarchies has the potential for phenotypic modification of crops.

1. Introduction

Seed epidermal cells of cotton are the most important source of spinnable fiber [1]. Cotton fibers share many similarities with leaf trichomes and citrus juice sacs for cell fate determination [2] and are a good model system for understanding plant cellular processes such as differentiation and elongation, carbon partitioning to cellulose sinks, and signaling between maternal and embryonic tissues. Fiber cells initiate three days before anthesis and undergo rapid elongation at approximately 3–18 days post anthesis (DPA) [3]. Negative correlations exist between cotton fiber fineness and strength, and for in-boll yields versus fiber length and maturity [4], representing longstanding bottlenecks for breeders which, in addition to a narrow germplasm base and allopolyploidy, pose challenges potentially addressed by genetic engineering. Fiber quality is a key trait because immature or coarse fibers caused by stress results in poor yarn spinning performance and marketability. Sustainable cotton production is the ultimate challenge facing farmers drawing on the southern Ogallala Aquifer, a non-renewable resource where 90% of groundwater used in the southern High Plains produces one-third of all cotton in the U.S. [5].

Basic3 (B3) domain transcription factors (TFs) are unique to plants. The cognates of the family, maize Viviparous1 [6] and Arabidopsis orthologue ABSCISIC ACID-INSENSITIVE3 (ABI3) [7], physically and functionally interact with basic-leucine-zipper TFs of the ABI5 clade [8,9] in hierarchical control of TFs controlling seed and seedling growth [10]. In Arabidopsis, there are

Abbreviations: ABA, abscisic acid; ABI, abscisic acid-insensitive; B3, basic3 domain; DAS, days after sowing; DPA, days post-anthesis; DI, deficit irrigation; eIF, elongation initiation factor; *FLC*, *FLOWERING LOCUS C*; *FT*, *FLOWERING_LOCUS_T*; *FTL*, *FT-Like*; KSNT, kanamycin-sensitive non-transgene control; *RAV*, *RELATED_TO_ABA-INSENSITIVE3/VIVIPAROUS1*; *TEM*, *TEMPRANILLO*; TPR, topless; TFs, transcription factors; *TSF*, *TWIN SISTER OF FT*; WW, well watered.

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Table 1
Effect of drought stress on Coker312 seed cotton yields and fiber length in the field from 2011 to 2013.

Year, water and treatment	Average fiber length, AFIS (by weight; inches) ±s.e.m		Seed cotton yield (lb./acre) ±s.e.m	% Yield penalty	% Fiber length penalty	Ratio of yield/length penalties
	Low nodes 6–9	High nodes 10–12				
2011[a] ($n=9$)						
Full	1.04 ± 0.012	0.97 ± 0.011	2550 ± 150	–	–	–
Deficit	0.98 ± 0.011	0.96 ± 0.016	1420 ± 160	44.1	3.5	12.6
2012 ($n=3$)						
Full	1.04 ± 0.009	1.02 ± 0.006	4470[b] ± 210	–	–	–
Deficit	1.03 ± 0.010	1.01 ± 0.003	3650[b] ± 210	20.4	1.0	20.4
Dryland	0.95 ± 0.015	0.95 ± 0.009	1720[b] ± 100	61.6	7.8	7.9
2013[c] ($n=3$)						
Full	0.99 ± 0.015		3780 ± 190	––	–	–
Deficit	0.97 ± 0.006		3200 ± 240	15.5	2.0	7.7
Dryland	0.95 ± 0.016		2470 ± 120	34.8	4.0	8.6
						Avg. 11.4

[a] All hand harvested; yields extrapolated from 1 meter subplot samples.
[b] $n=6$, machine harvested.
[c] Fiber measurements the average of three hand- and six machine-harvested samples.

over 100 B3-class TFs, with two homologues of the RELATED TO ABA-INSENSITIVE3/VIVIPAROUS1 (RAV) clade defining the eudicot-specific Group I APETALA2-B3 family [11]. RAVs bind as monomers to bipartite sequence motifs that contain consensus elements for both the AP2 and B3 domains [12]. RAVs contain a R/KLFGV conserved motif that functions as a repression domain [13]. AtRAV2-Like (RAV2L) and RAV1 physically interact with TOPLESS (TPR) corepressors that facilitate recruitment of histone deacetylases and methyltransferases [14]. AtRAV2L has been shown to be an integrator of internal (brassinosteroid, auxin) and external (blue light) signals in hypocotyl physiology [15]. RAV2 is required for induction of many genes involved in stress and defense pathways in different species [16,17]. RAVs have also been described as ethylene response DNA-binding factors and are induced by numerous stimuli [18,19]. Previous characterization of RAV functions in brassinosteroid response [20,21], reactive oxygen species scavenging [22], suppression of RNA silencing by viruses [16], control of flowering time [23–27], sylleptic tree branching [28], cytokinin signaling [29], senescence [30,31], and salt tolerance [32,33] support the function of RAVs as nodes in crosstalk networks integrating external and internal signals [34].

Drought is the most important environmental stress affecting agriculture. Plants growing in environments subjected to abiotic stresses do not meet their genetic potential and suffer yield penalties [35]. Stresses tend to induce early flowering through an elaborate network of floral signaling pathways [36]. In Arabidopsis, the *RAV2* and *RAV2L* genes (also named *TEMPRANILLO2 [TEM2]* and TE*M1*, respectively) are important modulators of flowering time via direct repression of the "florigen" component *FLOWERING LOCUS T (FT; AT1G65480)* [24] a small protein that moves from leaves to apical meristems. Ectopic expression of AtFT in photoperiodic cotton increases determinate plant growth, affects sympodial growth to promote compact architecture, and overcomes photoperiodism [37,38]. Conversely, ectopic expression of a cotton FT homologue, GhFT1/GhFTL, can accelerate flowering in Arabidopsis and partially rescue the late flowering phenotype of an *ft* mutant, demonstrating similar conserved functions of FT homologues between species [39]. AtRAV2/TEM2 and AtRAV2L/TEM1 bind GIGANTEA to effect AtFT repression [40]. AtFT is regulated positively by CONSTANS (the output of the photoperiod pathway), negatively by FLOWERING LOCUS C (FLC) (which integrates the vernalization pathway), and by myriad developmental programs [41] including an ABA-dependent drought escape pathway [36] and other autonomous and light-quality pathways [42]. AtFT is also regulated epigenetically by histone methylation and deacetylation of chromatin [43–45].

Altering the expression of regulatory genes has the potential for phenotypic modification to address global challenges such as climate change and sustainability. Although many genes are expressed during fiber development and associate with quantitative traits [46–54], little is known of the molecular mechanisms underlying cotton fiber initiation and elongation, and a role for the plant stress hormone abscisic acid (ABA) in the process has not been established other than by association [55,56]. Other than altered expression under greenhouse growth conditions of assimilate enzymes *Sucrose Synthase* [57,58] and vacuolar *Invertase* [59], or alteration of tissue-specific brassinosteroid, auxin, ethylene, gibberellin, and jasmonic acid biosynthesis, transport, or responses [60–68], there have been few reports on improving cotton fiber traits by altering expression of candidate signaling genes: *GhWRINKLED1* (*WRI*), an AP2/EREB class TF [69]; a Fasciclin-Like Arabinogalactan protein gene *GhFLA1* [70]; *WIDELY EXPRESSED LIN-11-Isl1-MEC3-Like* (*GhWLIM1a),* an actin remodeler/zinc finger TF influencing polyphenolic deposition during fiber maturation [71,72]; calcium sensor *GhCaM7* [73]; a peptide hormone gene *GhPSK* [74]; red light photoreceptor *GhPHYTOCHROME A1* [75]; and *Response to Drought 22-Like-1* (*GhRDL1*), which is transactivated by GhMYB2 [76] and interacts with expansin to control fiber wall loosening [77]. Here, we addressed by three years of field trials whether *AtRAV1* or *AtRAV2* overexpression in transgenic cotton could impact fiber initiation and elongation without compromising yields, and characterized *GhFT-Like* (*GhFTL*) expression as a potential molecular mechanism underlying delayed flowering and improved drought–stress response traits of these lines.

2. Materials and methods

2.1. Plant materials

The transgenic lines in Coker312 genetic background (USDA Germplasm Resources Information Network #529278; PVP #007200100) were those generated previously [78] from hypocotyl explants via *Agrobacterium tumefaciens* GV3101-mediated transformation and selection on kanamycin [79]. Briefly, pUNI51 full-length cDNA donor clones were recombined with pKYLX-pro*35S*:myc9::loxP binary acceptor vector (ABRC stock CD3-677) [80] using Cre recombinase [81]. Four independent RAV1, eight RAV2, three RAV2L, and two ABI5 transgenic events were generated