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Overexpression of a Potato Sucrose Synthase Gene in Cotton Accelerates Leaf Expansion, Reduces Seed Abortion, and Enhances Fiber Production

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ABSTRACT Sucrose synthase (Sus) is a key enzyme in the breakdown of sucrose and is considered a biochemical marker for sink strength, especially in crop species, based on mutational and gene suppression studies. It remains elusive, however, whether, or to what extent, increase in Sus activity may enhance sink development. We aimed to address this question by expressing a potato *Sus* gene in cotton where *Sus* expression has been previously shown to be critical for normal seed and fiber development. Segregation analyses at T1 generation followed by studies in homozygous progeny lines revealed that increased Sus activity in cotton (1) enhanced leaf expansion with the effect evident from young leaves emerging from shoot apex; (2) improved early seed development, which reduced seed abortion, hence enhanced seed set, and (3) promoted fiber elongation. In young leaves of Sus overexpressing lines, fructose concentrations were significantly increased whereas, in elongating fibers, both fructose and glucose levels were increased. Since hexoses contribute little to osmolality in leaves, in contrast to developing fibers, it is concluded that high Sus activity promotes leaf development independently of osmotic regulation, probably through sugar signaling. The analyses also showed that doubling the Sus activity in 0-d cotton seeds increased their fresh weight by about 30%. However, further increase in Sus activity did not lead to any further increase in seed weight, indicating an upper limit for the Sus overexpression effect. Finally, based on the observed additive effect on fiber yield from increased fiber length and seed number, a new strategy is proposed to increase cotton fiber yield by improving seed development as a whole, rather than solely focusing on manipulating fiber growth.

Key words: cotton fiber; invertase; leaf expansion; seed abortion; seed development; sucrose synthase; sugar signaling.

INTRODUCTION

Developing cotton seeds are unique in that phloem-unloaded assimilates are partitioned in opposite directions to two highly active sinks each for distinctive developmental endpoints: outwards to fiber cells on seed coat epidermis for cell elongation and cellulose biosynthesis and inwards to filial tissues for embryonic development and oil biosynthesis (Ruan, 2005). This feature of resource partitioning renders cotton the most important source of natural fiber for textiles and a valuable oil crop worldwide.

Each cotton fiber is a single cell that initiates from ovule epidermis at, or prior to, anthesis. Thereafter, the fibers undergo a rapid and synchronized elongation for about 16–20 d before they switch to intensive secondary cell wall cellulose biosynthesis that lasts for about 15 d. By maturity, fibers are about 3 cm long and contain more than 95% of cellulose on a dry weight basis (Basra and Malik, 1984). Owing to these developmental characteristics and its accessibility for sampling and phenotyping, cotton fiber has proven to be an ideal single-celled model for studying cell expansion and cellulose synthesis (Ruan, 2007; Qin and Zhu, 2011). In addition, as a maternal seed tissue, fiber needs to coordinate its development with

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that of endosperm and embryo. Thus, progress in fiber research also contributes to our understanding the metabolic interaction between seed maternal and filial tissues (Ruan et al., 2008; Pugh et al., 2010).

One major advance in cotton fiber biology over the last two decades or so has been a better understanding of how sucrose import and metabolism regulate fiber and seed development. Sucrose is the major form of photoassimilates, which is translocated through phloem from source leaves to sink tissues such as developing seed and fruit. Once unloaded into recipient sink cells, sucrose is cleaved into hexoses by either sucrose synthase (Sus, EC 2.4.1.13) or invertase (INV, EC 3.2.1.26) for use in metabolism, biosynthesis, storage, and signaling (Weber et al., 2005; Fallahi et al., 2008; Ruan et al., 2010; Brill et al., 2011). While molecular regulation of cotton fiber growth by INV is only beginning to be understood (Wang et al., 2010), substantial progress has been made in elucidating the role of Sus in cotton fiber and seed development. In this context, early studies established the spatial pattern of Sus expression in developing cotton ovule and seed (Nolte et al., 1995; Ruan et al., 1997) and revealed the presence of abundant Sus in fiber initials and its absence in either adjacent normal seed coat epidermal cells or the epidermal cells of a fibreless seed mutant (Ruan and Chourey, 1998; Ruan et al., 2005). These early findings indicated a role of Sus for fiber growth. Apart from its expression in fibers, Sus is also abundantly detected in transfer cells located at the innermost layer of the seed coat and in developing filial tissues, where Sus is involved in transfer cell wall ingrowth (Pugh et al., 2010) and endosperm cellularization (Ruan et al., 2008). Indeed, suppressing the expression of Sus in cotton seed coat leads to a fiberless phenotype (Ruan et al., 2003), whereas silencing its expression in the filial tissue results in stunted and unviable seeds and loss of transfer cells (Ruan et al., 2003, 2008). Together, these studies demonstrated unequivocally that high expression of Sus is required for normal development of both cotton fiber and seed.

The above findings, together with the report that a Sus gene is genetically located within a quantitative trait loci (QTL) associated with cotton fiber yield (Rong et al., 2005), identify Sus as an ideal candidate for overexpression in transgenic cotton to improve fiber yield and quality. Such a study is not only logical from an applied perspective, but also essential for determining the plasticity of Sus in regulating fiber growth, bearing in mind that inhibition of growth and development by silencing the expression of a candidate gene is not necessarily indicative of achieving a positive phenotypic effect through its overexpression (e.g. Walford et al., 2011). This issue is particularly relevant to Sus, since its overexpression has yielded conflicting results on plant growth and biomass accumulation in several species (Coleman et al., 2006; Bieniawska et al., 2007; Barratt et al., 2009; Coleman et al., 2009).

This study aimed to investigate the effect of Sus overexpression on cotton fiber and seed development. A potato Sus gene was expressed in cotton under the control of constitutive segment seven promoter (S7) isolated from subterranean clover stunt virus. Segregation studies at T1 generation coupled with further analyses in homozygous transgenic progeny lines revealed that elevation of Sus activity in young leaves accelerated leaf expansion resulting in larger leaf area as compared to segregating null controls or wild-type plants. Significantly, higher Sus activity in the seed enhanced cotton fiber elongation and early seed growth and reduced seed abortion, which, together, led to higher seed set and fiber production per fruit. This positive impact on fiber yield is probably attributed to a combination of locally enhanced sink strength and increased supply of photoassimilate.

RESULTS

Transformation and Confirmation of Transgenic Events

To increase Sus activity in cotton, we made a Sus overexpression construct, expressing a full-length *Sus* cDNA isolated from potato (GenBank accession number M18745) under the control of constitutive segment seven promoter (S7) from subterranean clover stunt virus. The choice of the potato *Sus* was based on the consideration that its transcript level is more than 10-fold higher in developing tuber than that in source leaves (Salanoubat and Belliard, 1987). Thus, it is likely the potato *Sus*, once expressed, may play particular roles in sink tissues such as seed and fiber. The S7 promoter has been previously shown to be mainly active in developing leaves, fiber, and seed filial tissues of transgenic cotton (Schünmann et al., 2003) with a pattern of expression similar to that of the more commonly used 35S promoter of Cauliflower Mosaic Virus. The construct was introduced into cotton by using Agrobacterium-mediated method (Murray et al., 1999).

We obtained 26 primary (T0) transgenic lines. Among them, 21 lines were fertile, whereas five were sterile (Figure 1A). The presence of the transgene in all of these T0 plants was initially verified by PCR detection of the S7 promoter–*Sus* transgene fragment. Southern analysis was conducted on 10 fertile lines to confirm their transgenic nature and to determine the transgene copy numbers. Figure 1B shows an example of the Southern blot analysis, probed with a 3′ UTR fragment of the potato Sus cDNA, where lines 168, 100–1, and 152 showed a single insertion of transgene, whereas line 267 had two copies of the transgene. We selected six single-copy lines (100–1, 65, 292, 152, 168, and 168–1) and one two-copy line, 267 for segregation analyses at T1 generation (Figure 1C).

Segregation Analyses Showed Enhanced Early Seed Growth from Overexpression of Sus

We focused the analyses at T1 generation on (1) identifying any Sus overexpressers in the segregants from multiple lines and (2) characterizing any phenotype detected from these plants. We sowed 20 T1 seeds from each of the seven selected lines. The T1 seedlings were screened by PCR for the presence or absence of the transgene (see Figure 1C). To gain valid assessments of the impact of transgene expression on plant