Sucrose synthase, a major biomarker for sink strength in cotton

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Abstract

Cotton seeds are unique in that photosynthetic assimilates are translocated in opposite directions to two strong active sinks, outwards to epidermal cells for fibre elongation and cellulose biosynthesis and inwards to filial tissues for embryonic development and oil biosynthesis. The regulation of resource partitioning between the developing embryo and the fibre cells is crucial in determining the fibre yield and seed quality and hence the survival of the species. Sucrose synthase plays a key role in managing the tug of war for nutrients between the active sinks by maintaining the osmotic gradient through generation of hexoses and effectively supplying UDP glucose as substrate for cellulose synthase. This paper highlights the significance of SUS activity in cotton seed and fibre development.

Keywords: Sucrose synthase, biomarker, cotton


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Introduction

Life on planet earth primarily depends on the photosynthetic carbon fixation and light energy in energy-rich sugar molecules, leading to concomitant production of oxygen. The processes of sugar synthesis, translocation, consumption and storage are dynamic and tightly linked to cellular physiology, organ identity, environmental inputs and developmental stages (Sheen, 1999). Photosynthesis and sink demand are rigorously coordinated, and this is arbitrated by both metabolic regulation and specific sugar-signaling mechanisms. Although, sucrose is the major mobile sugar in plants, import of sucrose depends on the source or sinks status of the leaf cells. Photosynthates produced in excess are stored transiently as starch in the chloroplast during the day and subsequently gets converted in to sucrose (Smith et al., 2005).

Import of sucrose in sink tissues such as young leaves and developing seeds is either through plasmodesmata (symplastic transport) or cell wall (apoplastic transport). In sink cells, sucrose is cleaved either by invertase (INV) or sucrose synthase (SUS). Invertase (EC 3.2.1.26) is a hydrolyase which cleaves sucrose into glucose and fructose. In contrast, sucrose synthase (EC 2.4.1.13) is a glycosyl transferase (UDP glucose: D fructose 2 glycosyl transferase) which in the presence of UDP converts sucrose into UDP glucose and fructose. In the vacuole, vacuolar invertase (VIN) is a major intracellular source for hexose generation in expanding tissues. In the apoplast, extracellular sucrose is hydrolysed by Cellwall (CW)-INV, a major driving force in phloem unloading and therefore sink strength (Roitsch and Gonzalez, 2004) (Fig. 1). Cleavage of sucrose by invertase is generally linked to growth and expansion (Ricardo, 1970) whereas SUS cleavage activity has been reported to be highly correlated with sink strength of various starch storing organs including potato tubers, carrot roots, maize kernels and pea embryos (Tang and Sturm, 1999) and cellulose synthesis (Albrecht and Mustroph, 2003; Fuji et al., 2010) by supplying UDP-glucose as substrates.

In cotton, SUS plays a substantial role in seed and fibre development. The tug of war for nutrients between the fibre initials and the embryo is regulated by SUS in transfer cells. Advances in cotton biotechnology research resulted in identification of four distinct isoforms of SUS genes (SusA, SusB, SusC and SusD) in Gossypium hirsutum involved in fiber development (Brill et al 2011) and seven SUS genes from Gossypium arboreum (Chen et al 2012). Recently, availability of whole genome sequence data of Gossypium raimondii resulted in further identification and isolation of novel SUS isoforms. Till date, the genomes of G. arboreum, G. raimondii and G. hirsutum contain 8, 8, and 15 SUS genes, respectively (Zou et al., 2013) and they exhibit distinct and partially overlapping expression patterns during fiber development. This review elucidates the importance of SUS and INV in cotton seed and fibre development.

Post fertilization, the transition of ovule to seed and ovary to fruit is characterized by the metabolic interaction of maternal and the two filial tissues, the embryo and the endosperm (Barnabas, et al., 2008 and Ruan et al., 2010). In most plant species, carbon and energy requirement by the sink organs are supplied in the form of sucrose. In the developing cotton seed coat, the sucrose transported in the phloem from the photosynthetic leaves is transported symplastically to the transfer cells located at the innermost layer of the coat for efflux to the apoplastic space between the maternal and filial tissues (Ruan et al., 1997; Ruan et al., 2001). Before entering into the filial tissues, the sucrose gets cleaved by several isoforms of INV and SUS, which are localized in different subcellular compartments. Maintaining the sucrose supply and its degradation into hexoses is key to male fertility, seed and fruit set (Ruan et al., 2012). SUS exists both free in the cytosol and in association with the plasmalemma (Amor et al., 1995; Carlson and Chourcy, 1996) and a tonoplast-associated form of SUS has also been reported in red beet (Etxeberria and Gonzalez, 2003). The phosphorylation status of the enzyme determines the extent to which SUS is soluble or membrane-bound (Winter et al., 1997).

Fig.1. Sucrose cleavage in sink cells (Based on Rolland et al., 2006)

Glu - Glucose; Fru - Fructose; Suc - Sucrose
V-INV – Vacuolar Invertase ;
C-INV – Cytoplasmic Invertase
CW-INV – CellWall Invertase
   – Hexose transporter
   – Sucrose transporter

Developing young cotton bolls are very strong carbohydrate sinks. The bolls may increase in weight by 15% per day (Schubert et al., 1986). In cotton, during the early stage of seed development, comparison of relative activities of SUS and INV in the seed coat suggested that SUS predominates over INV (Hendrix, 1990, Ruan et al., 2008). SUS activity and hexose levels are significantly higher in the endosperm than in the embryo during early seed development. In 10 Days after Anthesis (DAA) seeds, endosperm showed higher SUS activity whereas the activity was dropped to trace levels in the embryo (Ruan et al., 2008). By 25 DAA, endosperm gets crushed by the expanding embryo in cotton seeds (Stewart, 1986). At this stage, the activity of SUS in the residual endosperm was declined to 20% of that in the 10 day endosperm. Coincidently, the SUS activity was approximately 35 fold higher in the embryo, compared with that in early stage. As that of SUS, soluble invertase activity was higher in endosperm at 10 DAA and dropped to undetectable level at 25 DAA. In the embryo, the activity of acid invertase and alkaline invertase was barely detectable at 10 DAA. The alkaline invertase activity was only 20% of SUS and acid invertase in the endosperm at 10 DAA. UDP glucose thus produced by SUS could be transported to fibre cells and directly used in cellulose biosynthesis (Haigler et al., 2001; Doblin et al., 2002; Ruan, 2007). Hence SUS plays a predominant role in fibre initiation and development. Since cotton fibre is derived from epidermal cells of seeds, seed size and seed number are the key determinants of fibre yield. Increase in seed size has been highly correlated positively with fibre yield through evolution and domestication (Applequist et al., 2001; Pugh et al., 2010). This correlation could be attributed to the translocation efficiency of seeds to the filial tissues (Pugh et al., 2010). Silencing SUS in the embryo or endosperm blocked the seed and hence the fibre development entirely, whereas suppressing SUS in the seed coat inhibited fibre growth without affecting seed development (Ruan et al., 2003 & 2008). In the tetraploid species of cotton, seed development is not affected among all fibreless mutants. Hence fibre development is dependent on seeds but not vice-versa (Ruan, 2013). Over-expression of SUS, driven by a promoter active in cotton embryo, fibre and leaves elevated seed number by 22% contributing 18% increase in mature fibre weight (Xu et al., 2012). However, SUS over-expression by a fibre-specific promoter induced no positive effect on fibre yield. Similarly, over-expression of Indole-3- Acetic Acid (IAA) biosynthetic genes driven by a fibre-specific promoter did not enhance fibre growth, in spite of significantly higher accumulation of free IAA content in transgenic fibres. However, over-expression of the IAA biosynthetic gene iaaM, driven by petunia MADS box gene Floral Binding Protein 7 (FBP 7) known to be active in the entire ovule and seed coat (Colombo et al., 2011) significantly increased fibre cell number, final yield and quality (Zhang et al., 2011). Though the data were interpreted as fibre-cell effect by Zhang et al. (2011), the positive impact of over-expressed iaaM may be correlated to Increased IAA biosynthesis within the ovules (Ruan, 2013). In addition, exogenous application of IAA transport inhibitor, 1-N-Napthylphthalamic acid (NPA) to wild-type cotton ovary pedicels reduced IAA content and number of fiber cells (Zhang et al., 2011). This result indicates that IAA in fibre initials is imported from ovule tissues rather than synthesized within fibres. As fibre initiation and hence the yield potential are controlled by ovules rather than by fibre themselves, enhancing seed development (seed number and size) will pave way to improve fibre yield and quality (Ruan , 2013). Cotton yield is determined by the number of bolls per unit area, number of seeds per boll, number of
fibers per seed, and average weight per fiber (Bowman, 2001). Hassan et al., (2005) studied the performance of Egyptian cotton cultivars and found significant positive correlation between seed index and seed cotton yield. Fiber number and lint mass per unit of seed surface area are linked to seed size, which should be considered when selecting for increased lint mass or fiber number per unit of seed surface area (Bednartz et al., 2007).

**SUS in transfer cells: Regulator of resource partitioning between fibre cells and embryo**

Transfer cells (TCs) are plant cells with secondary wall ingrowths (Gunning and Bate, 1969) located at bottlenecks within nutrient transport pathways (Offler et al., 2003) with the wall ingrowth/plasma membrane complex frequently polarized to the direction of solute flow. The wall ingrowths amplify their Plasma Membrane Surface Area (PMSA) and hence, the capacity to transport nutrients. In developing seeds of many species, TCs differentiate from cells located at one or both sides of the maternal/filial interface. The morphology of individual wall ingrowths is either reticulate or rib-like/flange (McCurdy et al., 2008). In tetraploid (AD genome) and diploid cotton (A or D) seeds, TCs are found at the inner surface of the seed coat juxtaposed to the embryo (Kyser et al., 1988; Ruan et al., 1997). They are symplastically connected with inner seed coat and vascular system located in the outer seed coat (Ruan et al., 1997). Cotton seed coat TCs are characterized by sequential development of flange and reticulate wall ingrowths (Pugh et al., 2010). The tetraploid cotton species and their diploid A and D genome progenitors displayed high, medium and low seed and fibre biomass yield, respectively. TCs from the tetraploid species developed substantially more flange and reticulate wall ingrowths and exhibited a higher degree of reticulate wall ingrowths formation than their progenitors. Hence the estimated PMSA of TCs of tetraploid species was about 4 and 70 times higher than of the A and D genome progenitors respectively (Pugh et al., 2010).

Developmentally, TC wall ingrowths are either not formed (AD genome) or had just initiated (A and D genomes) at 10 DAA when seed biomass was less than 10% of that at maturity. However, during the rapid phase of biomass accumulation in the three species, wall ingrowths formation in the trans-differentiating seed coat TCs was substantial. This TC wall ingrowths formation increased PMSA of these cells and their potential sucrose transport capacity. SUS is abundantly expressed in seed coat transfer cells from the tetraploid species but not its diploid progenitors. SUS is a key enzyme required for construction of TC wall ingrowths and function (Offler et al., 2003; Ruan et al., 2003). Antisense suppression of SUS expression in cotton seed blocked TC formation entirely (Ruan et al., 2003). SUS signal increased dramatically from 10 DAA to 20 DAA corresponding to a period of significant increase in flange and reticulate wall ingrowths and seed and fibre biomass yield in the AD genome species. The low to undetectable level of SUS in the A and D genome species correlates with lower levels of of wall ingrowths formation, seed yield and fibre biomass.

**SUS regulates development of TCs in many ways. Fructose released from sucrose by SUS reaction may be converted to glucose by hexose isomerase. Glucose and fructose play important signaling roles in regulating gene expression through hexose kinase-dependent or independent pathways. Glucose and to a lesser extent, fructose have been recently shown to activate the promoter of ZmMARP-1 encoding a MYB-like transcription factor specifically expressed in maize basal endosperm TCs (Barrero et al., 2009). Thus SUS may possibly be involved in upstream regulation of TC initiation by providing hexoses as signaling molecules. Consistent with this claim, loss of a TC-specific cell wall invertase, hydrolyzing sucrose into glucose and fructose, in maize endosperm inhibits TC development (Kang et al., 2009). Recent studies by Wang et al. (2010) revealed a link between invertase-mediated sugar sensing and cell wall signaling. SUS contributes to TC function by providing energy (ATP) via glycolysis (Ruan et al., 1997; Ruan et al., 2001). ATP provides energy for active transport of nutrients through the activity of plasma membrane-bound H⁺-ATPase that hydrolyzes ATP to extrude H⁺ to the apoplasm for establishing a transmembrane electrical chemical gradient (Lalonde et al., 2003). Seed TCs are enriched with energy-dependent H⁺/antiporter for influx from seed coats and H⁺/symporter for influx into filial tissues (Patrick, 1997; Offler et al., 2003). H⁺-ATPase has been co-localized with an H⁺/sucrose symporter in TCs of Vicia faba cotyledons (Harrington et al., 1997). Sucrose transporters are abundantly expressed in TCs of cotton seed (Ruan et al., 2001). The potential energy coupled transport of sucrose from seed coat TCs to the filial tissues is mediated by SUS. Thus, the absence of SUS in TCs of 20 DAA seed from A or D genome progenitors indicates these TCs may be no longer functional for nutrient transport at this stage, contributing to their small seed size as compared to that of the AD genome seed where SUS was abundantly expressed in seed TCs at 20 DAA.

About 25% of cotton seed coat epidermal cells develop into long cellulosic fibers (Ruan, 2007). The fiber accounts for about 45% and 40% of total seed biomass at maturity in AD genome species and its A genome progenitor, respectively. Thus, cotton seeds from these genotypes have two strong sinks: fibers developing from epidermal cells of the outer seed coat and the embryo enclosed within the inner seed coat. The assimilates unloaded from the phloem located in the outer seed coat must flow in two opposite directions to support fiber and embryo development (Ruan et al., 1997), resulting in a potential competition for resources between the two sinks (Ruan, 2005). In this ‘tag of war’, the development of TCs at the innermost cell layer of the seed coat may serve as a strategy to enhance nutrient flow to the embryo to ensure the survival of seeds, hence the species during evolution. In this context, the fiberless seed from the D genome species had the least TC wall ingrowths formation whereas the seed from the AD genome with the highest amount of fiber development had the most extensive wall ingrowths (Pugh et al., 2010).

**Roles of sucrose synthase and sucrose phosphate synthase for fiber development**

Cotton fibre originates from epidermal cells of ovules, and its growth and development is a gene-regulated process involving four distinct, but overlapping stages: cell initiation (~3 to 0 DAA, elongation (~0 to ~20 DAA), secondary wall synthesis (~16 to ~30 DAA, and maturation (~30 to ~60 DAA). At the onset of secondary cell wall formation stage during cotton fibre development, the rate of cellulose synthesis abruptly rises over 100-fold compared to the elongation and primary cell wall synthesis stage (Delmer, 1999). Amor et al. (1995) made a breakthrough discovery that a substantial amount of SUS, a protein traditionally thought to be exclusively located in the cytosol is tightly associated with the plasma membranes of cotton fibre. SUS acts degradatively to channel UDP glucose to the cellulose synthase, releasing a fructose molecule with each cycle (Fig. 2). According to this model, the initial carbon source for cellulose synthesis is sucrose and that the UDP glucose used directly by cellulose synthase is not from the general intracellular UDP glucose pool (Fig.2) Immunolocalization in
cryogenically preserved tracheary elements and cotton fibers revealed that SUS is proximal to the sites of cellulose synthesis in the plasma membrane (Kimura et al., 1999). In tracheary elements, SUS is found at the sites of secondary wall deposition and microtubules. Actin is localized between SUS and the microtubules across the cell surface of tracheary elements (Salnikov et al., 2001) thus, correlating that sucrose synthase is an actin-binding protein (Winter et al., 1998). In cotton fibers at secondary wall stage, just as cellulose synthesis occurs throughout the fibre cells, SUS and microtubules were co-distributed over the whole fiber. It is estimated that sucrose entering the fibre cells is partitioned in the ratio of 3:1 between membrane (PM-) and soluble (S-) SUS at the secondary cell wall stage (Delmer, 1999). Immunolocalisation analyses at the electron microscopic level showed that the occurrence of PM-SUS is developmentally regulated. The immunogold labelled PM-SUS is sporadically present in fibres at fibre elongation stage (10 DAA) but becomes more evident at 20 DAA when the fibres are at secondary cell wall cellulose stages. PM-SUS may form a complex with the cellulose synthase on the plasma membrane either directly or indirectly, to channel carbon directly from sucrose to cellulose (Haigler et al., 2001; Salnikov et al., 2003) as proposed by Delmer (1999). Coupling between the PM-SUS and cellulose synthase has the many advantages viz., synthesis of cellulose from sucrose with no additional energy input, avoiding competition for use of UDP-glucose by other metabolic pathways, and immediate recycling of UDP, an inhibitor of the reaction catalysed by cellulose synthase (Haigler et al., 2001). In this model, fructose released from PM-SUS gets phosphorylated by fructokinase or hexokinase (Anderson-Gunnaras et al., 2006). After fructose phosphorylation, sucrose phosphate synthase can synthesise sucrose phosphate, which is then dephosphorylated by sucrose-6-phosphate phosphatase (Fig. 2). The energy and hexoses required for the maintenance of cell growth is provided by the soluble SUS in the cytosol.

The membrane association of SUS might involve de-phosphorylation of the serine residue(s) at the N-terminal region of the protein and interaction with actin. Phosphorylation of serine (Ser) 15 of the SUS1 protein in maize resulted in soluble phase localisation of SUS, and de-phosphorylation tends to activate membrane association (Winter and Huber, 2000). The phosphorylation of Ser 15 is developmentally regulated in maize leaves and N-terminal conformation is affected in a way that may stimulate the catalytic activity of SUS and influence membrane association (Hardin et al., 2004). However, the level of ^32P-labelling of SUS is similar between membrane and soluble fractions in cotton fibres and the role of de-phosphorylation in plasma membrane association is yet to be elucidated (Haigler et al., 2001; Salnikov et al., 2003).

SUS and VIN in maintenance of turgor during fibre elongation

Cotton fibre elongation is a result of a complex interplay between cell turgor and cellwall extensibility (Smart et al., 1998; Ruan et al., 2001). During the most active elongation period (5–25 DAA), vigorous cell expansion with peak growth rate of ~2 mm/day is observed in upland cotton, coupled with cell expansion and a specific set of metabolite syntheses (Qin and Zhu, 2011). Cell turgor is generated and maintained through influx of water down the water potential difference driven by the relatively high concentration of osmotically active solutes (Cosgrove, 1997). In elongating fibres, soluble sugars, potassium and malate are the three major osmotic active solutes, together accounting for ~80% of fibre sap osmolality (Ruan, 2005; Dhindsa et al., 1975). Potassium and sucrose are imported from the underlying seed coat cells, either symplastically through the plasmodesmata or apoplasmically across cell wall space and plasma membrane by their respective transporters, ATP being provided by the plasma membrane and tonoplast H^+ ATPases, respectively (Smart et al., 1998; Ruan et al., 2001). GhSUT1 encodes a cotton K^+ transporter, weakly expressed in fibres at 6 DAA when the plasmodesmata are open. The mRNA abundance of GhSUT1 increases significantly at 10 DAA when plasmodesmata are closed and then rapidly decline to undetectable level at 16 DAA when plasmodesmata are re-opened (Ruan et al., 2001). Similar temporal expression was exhibited by GhSUT1, a plasma membrane H^+ sucrose symporter which is localized at the base regions of the fibres. The closing and re-opening of plasmodesmata are mediated by deposition and degradation of callose catalyzed by β,1,3-glucan synthase and β,1,3-glucanase, respectively. The duration of plasmodesmata closure correlates positively with the fibre length at maturity, indicating the critical role of the same in regulating fibre elongation (Ruan et al., 2004). The closure of plasmodesmata and the simultaneous upregulation of GhSUT1 and GhSUT2 at the mid-phase of elongation (fibres are ~1 cm long at this stage) may provide a cellular basis for the fibres to sustain turgor pressure to drive fibre elongation (Ruan et al., 2001, Ruan et al., 2004, Ruan, 2005).

Once taken up into the fiber cells, sucrose could be degraded into UDP glucose and fructose by sucrose synthase (SUS) in the cytoplasm (Ruan et al., 2003) or hydrolysed by acid invertase into glucose and fructose in the vacuole thus doubling the osmotic contribution of sucrose (Li et al., 2010). Though the expression level of GhSUS gene members are high throughout the fiber elongation period, GhVIN, a major vacuolar invertase (VIN) in cotton fibre, shows evidently high expression level early in fiber elongation (0-10 DAA) matching high VIN activity observed in this stage (Li et al., 2010). It is evident from transgenic analysis by

**Fig. 2.** Cellulose biosynthesis in cotton

![Cellulose Biosynthesis Diagram]

- **HK** – Hexokinase
- **PGI** – Phosphoglucoisomerase
- **PGM** – Phosphoglucomutase
- **SPS** – Sucrose Phosphate Synthase
- **SPP** – Sucrose Phosphate Phosphatase
- **CesA** – Cellulose Synthase
- **SUS** – Sucrose Synthase

suppression of GhSUS and GhVIN1 that GhSUS play roles in both fibre elongation and cellulose biosynthesis through osmotic regulation and supplying UDP-glucose as substrate for cellulose production and hexoses for generation of ATP as an energy source for a variety of transport and metabolic processes. High SUS activity may produce more hexoses, hence a higher osmotic potential, leading to high turgor to drive cell elongation (Ruan, 2005; Wang et al., 2010). The hexose concentration increased to 30% in 10 DAA fibres in SUS overexpressed transgenic lines which would increase the osmotic potential to drive influx of water generating a higher cell turgor to ‘push’ fibre elongation (Ruan et al., 2001). GhVIN1 is specifically required for the formation and enlargement of the central vacuole, crucial for early fibre elongation. In-situ hybridization analysis showed that GhVIN1 transcript exhibited highest level in fibres at 0-2 DAA and were maintained at high level at 5 DAA and decreased at 10 DAA onwards. Since cotton fibres start their elongation at 0-2 DAA through formation of a large central vacuole, high VIN activity at this stage would rapidly hydrolyse sucrose into two molecules of hexoses in the vacuole, providing a major osmotic force to drive the influx of water to expand the vacuole (Li et al., 2010). Around this time at 5 DAA, a tonoplast aquaporin, GhTIP1 & 2 exhibited higher expression in cotton fibre as compared to later stages of elongation, which would facilitate the influx of water across the tonoplast and hence the fibres protrude above the seed epidermal surface. With the rapid enlargement of fibre cell, it becomes increasingly difficult to maintain cell turgor. The expression of multiple tugor- generating genes such as GhSUT1, GHKTI, GhPEPSc along with GhVIN1 and GhSUS become necessary to maintain lower water potential in the fibres to drive continuous influx of water for the rapid phase of elongation (Wang and Ruan, 2010). At 10 DAA, transcription levels of GhSUT1 and GHKTI are high and the fibre plasmodesmata are closed which may provide a mechanism to generate and maintain a high turgor driving the maximum rate of elongation occurring at 12-13 DAA. By ~16 DAA onwards, the expression of tugor-generating genes is dramatically reduced and fibre plasmodesmata are re-opened which releases turgor. This low expression of turgor related genes accompanied by diminished expression of expansins curtailed the elongation process. While potassium and sucrose are imported into fibres from phloem in the seed coat, malate is synthesized within the fibre cells by refixing CO2 through the activity of Phosphoenolpyruvate carboxylase (GhPEPCs). The transcription levels of GhPEPSc1 and GhPEPc2 and malate concentrations are relatively high early in elongation, increase to maximal level around 10-12 DAA, and decline rapidly at 15 DAA onwards when elongation is slowed down (Li et al., 2010).

Interaction of sucrose synthase (SUS) and ethylene in fibre development

Ethylene is synthesized by the conversion of S Adenosyl-L-Methionine (SAM) to 1-aminoacyclopropane-1-carboxylic acid (ACC) and the oxidative cleavage of ACC, which are catalyzed by the rate limiting enzymes, ACC synthase (ACS) and ACC Oxidase (ACO), respectively. Transcripts of three GhACOs are accumulated in 10 DAA fibers as compared with 10 DAA ovules. Exogenously applied ethylene stimulates fiber growth, whereas L-[2-aminoethoxyvinyl]- glycine (AVG), an ethylene biosynthesis inhibitor, inhibits fiber growth. The biosynthesis of saturated or monounsaturated very long-chain fatty acids (VLCFAs, fatty acids > C18) has important roles in plant growth and development (Zheng et al., 2005; Chen et al., 2006 and Qin et al., 2007). Rapidly elongating fibre cells contain three to five times the amount of VLCFAs (from C20 to C26) and a higher amount of unsaturated fatty acid (ω-linolenic acid C18:3) than do ovules (Qin et al., 2007). VLCFAs may promote fiber cell elongation by activating ethylene synthesis (Qin et al., 2007).

SUS is preferentially expressed in elongating fiber cells but not in adjacent normal epidermal cells, and it is induced significantly upon exogenous ethylene treatment (Shi et al., 2006). On the basis of comparative proteomic and bioinformatic analyses, nucleotide sugar metabolism is reported to be the most significantly up-regulated biochemical process during fiber elongation (Pang et al., 2010). UDP glucose initially produced by SUS throughout the primary cell wall synthesis and fiber elongation stages may be utilized for biosynthesis of pectin precursors. Cellulose biosynthesis may start to function at the end of the primary cell wall extension period to utilize the UDP glucose that is continuously produced by SUS and UGP for secondary cell wall biosynthesis and deposition (Qin and Zhu, 2011)

Transgenic cotton with sucrose synthase gene for fiber quality and yield

Importance of SUS activity has been validated through transgenic studies by suppression or overexpression of SUS genes (Table. 1). Recently novel sucrose synthase (SUSA1) gene was isolated from a superior quality fiber germplasm line 7235 in Gossypium hirsutum and validated for its association with fiber quality traits in recombinant inbred lines. The suppression of the gene expression through RNA interference resulted in reduction in boll size and seed weight along with negative effect on the fiber quality traits. Over expression of the SUSA1 gene driven under 35S promoter resulted in increased biomass during the seedling and boll stages and significant increase in the fiber length and moderate improvement of fiber strength was noticed in SusA1 over expressed transgenic lines. Increase in fiber length may be attributed to increased turgor pressure, by degrading sucrose into UDP-glucose and fructose, SUS could generate osmotic potential, hence turgor pressure, for fiber elongation. The enhancement in fiber strength could be attributable to the increased availability of substrate UDP glucose for cellulose and non cellulose biosynthesis required for higher rate of secondary wall thickening (Jiang, et al., 2012). Similarly, Over expression of potato sucrose synthase gene driven under S7 promoter which is known to express actively at filial tissues of cotton seed (subterranean clover stunt virus (SCSV) resulted in 30 % increase in fiber yield of cotton. The yield increase was attributed to increased seed number resulted from reduced abortion of seed contributed by enhanced seed sink strength due to higher SUS activity in the filial tissues (Xu, et al., 2012).

Conclusion

Sustaining and increasing the crop yield under changing environmental conditions is a major challenge in modern agriculture. Seed constitutes an integral yield component in most of the crops with seed set determined at the flowering. Inadequate nutrient supply and abiotic stresses affects the seed set and result in seed abortion resulting in irreversible yield loss (Boyer and McLaughlin, 2007). It is evident that preventing or reducing abortion would benefit seed crops immensely. Increasing SUS activity in cotton by overexpressing SUS gene enhanced leaf expansion, improved early seed development, which reduced seed abortion and promoted fiber elongation. Elevation of SUS activity by overexpressing SUS increases seed number at maturity in cotton due to alleviation of seed abortion. The higher SUS activity in the filial tissues may significantly enhance seed sink strength (Pugh et
al., 2010) and consequently reduce seed abortion. Higher concentration of sucrose and hexoses represses programmed cell death genes in the ovaries, thereby preventing abortion (Boyer and McLaughlin, 2007; Li et al., 2011). Hence elevating SUS activity may provide a better strategy to increase cotton fiber yield through improved seed development.

References


Table 1: Transgenic validation of role of sucrose cleaving enzymes in Cotton

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Gene manipulated</th>
<th>Promoter used</th>
<th>Approach</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
</table>
| 1     | Sucrose synthase (SusA1) gene from *Gossypium hirsutum* | 35S promoter derived from cauliflower mosaic virus (CaMV) | Silencing through RNA interference Over expression | • Reduction in boll size and seed weight  
• Negative effect on the fiber quality trait  
• Increased biomass during the seedling and boll stages  
• Significant increase in the fiber length and moderate improvement of fiber strength | Jiang et al., 2012 |
| 2     | Sucrose synthase gene from potato | S7 promoter of the subterranean clover stunt virus (SCSV) | Over expression | • 30% Increase in yield attributed to increase seed number  
• Increase in fiber length and strength | Xu et al., 2012 |
| 3     | GhVIN (Vacuolar invertase from *Gossypium hirsutum*) | RD22-like1 (RDL1) fiber specific promoter from *Gossypium arboreum* for RNAi 35S promoter from CaMV | RNA interference Over expression | • Reduced fiber elongation  
• Enhanced fiber elongation | Wang et al., 2010 |
| 4     | Sucrose synthaseA from *Gossypium hirsutum* | Filial tissue-specific D12 Des promoter | RNA interference | • Arrest of early seed development | Ruan et al., 2008 |
| 5     | Sucrose phosphate synthase from spinach | 35S promoter from CaMV | Over expression | • Enhanced leaf sucrose synthesis  
• Higher fiber micronaire and maturity ratio | Haigler et al., 2007 |
| 6     | Sucrose Synthase Gene from *Gossypium hirsutum* | S7 promoter of the Subterranean clover stunt virus | RNA interference | • Reduction in cotton Fiber Cell Initiation, Elongation, and Seed Development | Ruan et al., 2003 |