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Brief Communication

Sucrose enhanced reactive oxygen species generation promotes cotton fibre initiation and secondary cell wall deposition

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Cotton fibre is a single cell that derived from the ovule epidermis. Fibre cell initiation and elongation determine cotton fibre yield and quality. Sucrose is the most important substrate for cellulose synthesis and energy production, as well as provides turgor pressure to promote fibre elongation. However, varieties with high-sugar content in fibres usually have short fibres, and the mechanism behind this is unclear.

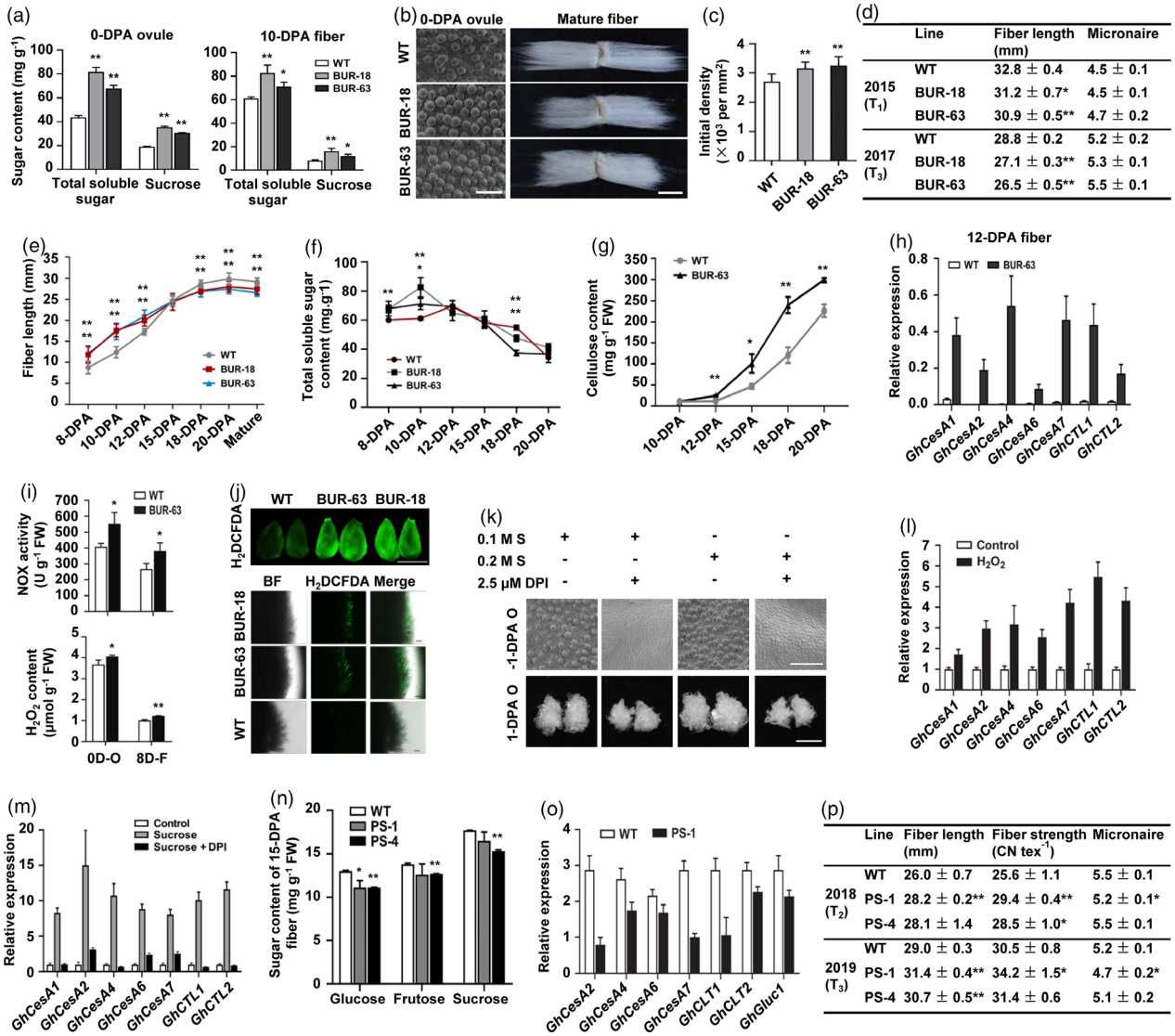
Sugar transporter SUTs (sucrose transport proteins) and SWEETs (Sugars Will Eventually be Exported Transporters), which are responsible for phloem loading or sucrose import into sink organs, are the important regulators for carbon allocation in plants (Chen *et al.,* 2012; Ruan *et al.,* 2001; Sauer, 2007; Sun *et al.,* 2019; Zhang *et al.,* 2017). To reveal roles of sucrose in cotton fibre development and assess effects of sugar regulation on fibre quality, we use a fungal SUT gene (UmSrt1) that displays a significantly higher affinity (20 to 200 folds) compared with plants (Wahl *et al.,* 2010) and a cotton SWEET gene (GhSWEEET15) to increase or decrease the level in fibres.

A seed coat-specific promoter *pBAN* was used to direct expression of *UmSrt1* in the ovule epidermis and fibres to promote sucrose influx of cotton fibre cells. Sugar contents in 0-DPA ovules and 10-DPA fibres of *pBAN::UmSrt1* (BUR) cotton were significantly enhanced (Figure 1a). Interestingly, as sugar content increased, the fibre initial density of transgenic BUR-18 and BUR-63 cotton was enhanced (Figure 1b,c), while the length of mature fibres of the transgenic lines was significantly decreased, compared with that of the wild-type control (Figure 1b,d). During the rapid elongation stage of fibre (8-15 DPA), transgenic fibres grew more rapidly than that of the wild type (Figure 1e). However, since then, the elongation rate of transgenic fibres was slower than that of wild type (Figure 1e). Nevertheless, the soluble sugar content was decreased and cellulose amount in transgenic fibres was higher compared to wild type after 15 DPA (Figure 1f,g). Notably, the expression of secondary cell wall (SCW) synthetic relative genes in transgenic cotton was largely promoted (Figure 1h). These data demonstrate that the increased sugar level promotes fibre initiation and SCW synthesis. It has been known that the initiation of SCW synthesis is usually accompanied by the slow-down of fibre elongation (Singh *et al.,* 2009). This can be seen in our results: the transgenic fibres were shorter than the wild type (Figure 1b,d).

The reactive oxygen species (ROS) is an important signalling molecule in the regulation of fibre elongation (Potikha *et al.,* 1999). Sugar metabolism can produce NADH, the oxidation of which provides the main source of ROS production (Ravera *et al.,* 2015; Sledow and Umbach, 1995). Detecting NADH oxidase (NOX) activity of 0-DPA ovules and 8-DPA fibres, we found that the enzyme activity in transgenic lines was significantly higher than that of wild-type cotton (Figure 1i). Consisting with the increase of NOX activity, ROS content in transgenic ovules and fibre was significantly increased (Figure 1i,j). Adding DPI, a ROS inhibitor, to the medium, the fibre initiation and fibre elongation of wild-type cotton was suppressed even the ovules were cultured under high-sugar concentration (Figure 1k). With the presence of ROS in the medium for ovule culture, the expression of SCW synthesis related genes was enhanced (Figure 1l). Conversely, with ROS scavenger, the expression of these genes was suppressed (Figure 1m). These results indicated that sucrose-increased ROS production not only stimulated fibre initiation, but also promoted the secondary cell wall biosynthesis.

The increased sugar content in fibres resulted in the advanced initiation of SCW synthesis and decreased fibre length. This promotes us to propose a strategy to increase fibre length by decreasing of sucrose level during SCW synthesis stage. We found *GhSWEET15 was a* sucrose efflux gene which was preferentially expressed in 16- and 20-DPA fibres. We then generated transgenic cotton lines, in which G*hSWEET15* was up-regulated during SCW synthesis stage. In *GhSWEET15* up-regulated fibres, the sugar content (Figure 1n) was decreased and the expression of SCW synthesis associated genes was down-regulated (Figure 1o). Two consecutive field experiments (2018–2019) showed that, as designed, the *GhSWEET15* up-regulated fibres became thinner, longer and stronger than the wild-type control (Figure 1p).

Collectively, our results indicate the relationship between sucrose and ROS generation and their impact on cotton fibre initiation, elongation and SCW deposition. We show that the increase of sugar content can promote ROS generation, which stimulates the fibre initiation and results in more fibres; the enhanced ROS level also promotes secondary cell wall biosynthesis, which in turn arrests the fibre elongation and results in thinner and shorter fibres. Our study provides an answer to the puzzle in cotton breeding: why 'high sugar' varieties usually have poor fibre



	Line	Fiber length (mm)	Micronaire
2015 (T_1)	WT	32.8 ± 0.4	4.5 ± 0.1
	BUR-18	31.2 ± 0.7*	4.5 ± 0.1
	BUR-63	30.9 ± 0.5**	4.7 ± 0.2
2017 (T_3)	WT	28.8 ± 0.2	5.2 ± 0.2
	BUR-18	27.1 ± 0.3**	5.3 ± 0.1
	BUR-63	26.5 ± 0.5**	5.5 ± 0.1

	Line	Fiber length (mm)	Fiber strength (CN tex^{-1})	Micronaire
2018 (T_2)	WT	26.0 ± 0.7	25.6 ± 1.1	5.5 ± 0.1
	PS-1	28.2 ± 0.2**	29.4 ± 0.4**	5.2 ± 0.1*
	PS-4	28.1 ± 1.4	28.5 ± 1.0*	5.5 ± 0.1
2019 (T_3)	WT	29.0 ± 0.3	30.5 ± 0.8	5.2 ± 0.1
	PS-1	31.4 ± 0.4**	34.2 ± 1.5*	4.7 ± 0.2*
	PS-4	30.7 ± 0.5**	31.4 ± 0.6	5.1 ± 0.2

Figure 1 Sucrose enhances reactive oxygen species generation and thus promotes cotton fibre initiation and secondary cell wall deposition. Total soluble sugar content and sucrose content in 0-DPA ovules and 10-DPA fibres of *pBAN::UmSrt1* transgenic lines and wild type. (b) Scanning electron microscopy of fibre initials of 0-DPA ovules (left column, bar = 50 μm) and the length of mature fibre (right column, bar = 1 cm) of transgenic plants and wild type. (c) Fibre initial densities of 0-DPA ovules of transgenic and wild-type cotton. (d) Fibre quality traits of *pBAN*::*UmSrt1* transgenic lines and wild type. (e and f) Fibre length (e) and sugar content (f) of *pBAN*::*UmSrt1* transgenic lines and wild type at various stages of cotton fibre development. (g) Cellulose content of BUR-63 and wild type. (h) qRT-PCR analysis of genes relative to secondary wall (SCW) synthesis in 12-DPA fibres of transgenic lines and wild type. (i) H_2O_2 content and NOX (NADH oxidase) activity of 0-DPA ovules and 8-DPA fibres of *pBAN::UmSrt1* transgenic lines and wild-type cotton. 0D-O, 0-DPA ovule; 8D-F, 8-DPA fibre. (j) Reactive oxygen species (ROS) staining of 0-DPA ovules (bar = 2 mm) and 1-DPA fibres (bar = 60 μm) of transgenic lines and wild-type cotton. BF, bright field; H_2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; Merge, the merge of bright field and ROS signal. (k) Fibre initiation and elongation of −1-DPA ovules and 1-DPA ovules cultured for 48 h and 8 days with or without DPI (diphenyleneiodonium). Wild-type ovules cultured for 48 h (−1-DPA ovules), or 8 d (1-DPA ovules) on BT media with 0.1 M, or 0.2 M sucrose (S), and with or without 2.5 μM DPI, respectively. +, added; −, not added. Bar = 100 μm (−1 DPA ovules) and 0.5 cm (1-DPA ovules). (l) qRT-PCR analysis of genes related to the synthesis of SCW in the wild-type fibres treated with H_2O_2, sucrose and DPI. Control, BT medium; H_2O_2, BT medium + 10 μM H_2O_2. sucrose, BT medium + 0.1 M sucrose; sucrose + DPI, BT medium + 0.1 M sucrose and 0.1 μM DPI. (m) Sugar content in 15-DPA fibre cells of *pGhSWEET15::GhSWEET15* cotton (PS-1 and −4 lines) and wild type. (o) qRT-PCR analysis of genes related to the SCW synthesis in 15-DPA fibres of PS-1 line and wild type. (p) Fibre quality of *pGhSWEET15*::*GhSWEET15* transgenic lines and WT. Data are means ± SD of three repeats in (a), (d), (e), (f), (g), (h), (i), (l), (m), (n), (o) and (p), and eight repeats in (c). Asterisks indicate significant differences between transgenic lines and WT (Student's *t*-test, *, $P < 0.05$; **, $P < 0.01$). BUR-18 and BUR-63 in (a–j): *pBAN::UmSrt1* transgenic homozygous lines. PS-1 and PS-4 in (n) and (p): two transformants of *pGhSWEET15::GhSWEET15* transgenic cotton (T_2). WT: wild-type cotton. DPA: days post-anther.

qualities. We also suggest a strategy to improve cotton quality and yield through sugar manipulation: up-regulating the sugar content in fibre initiation and early elongation stage to increase fibre number (thus enhancing fibre yield), while down-regulating the sugar content in fibre SCW synthesis period to increase fibre length and fibre strength.

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Conflicts of interest

The authors declare no conflict of interest.

Author Contributions

YP designed the research; XD, XL, LW, LH, XL and JZ performed the experiments of gene cloning and manipulation, cotton transformation and plant management. XD and XL performed data analyses. SS participated in tissue culture of cotton. LH and FW conducted the stereo fluorescence microscopy and confocal spectral microscope. YP and XD wrote the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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