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 cotton wild type after 15 DPA (Figure 1f,g). Notably, the expression of carbohydrate amount in transgenic fibres was higher compared to wild type (Figure 1e). However, since then, the elongation rate of transgenic fibres grew more rapidly than that of wild type (Figure 1f,d). During the rapid elongation stage of fibre (8-15 DPA), the synthetic relative genes in transgenic 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 shorter 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 1f,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-enhanced 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 GhSWEET15 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 quality.

Keywords: fibre initiation, fibre elongation, secondary cell wall deposition, sucrose, sugar transporter, reactive oxygen species.

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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.

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) Cellulose content of BUR-63 and wild type. (d) qRT-PCR analysis of genes relative to secondary wall (SCW) synthesis in 12-DPA fibres of transgenic lines and wild type. (e) H2O2 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. (f) 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; H2DCFDA, 2',7'-dichlorodihydrofluorescein diacetate; Merge, the merge of bright field and ROS signal. (g) 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 μM or 0.2 μM 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). (h) qRT-PCR analysis of genes related to the synthesis of SCW in the wild-type fibres treated with H2O2, sucrose and DPI. Control, BT medium; H2O2, BT medium + 10 μM H2O2, sucrose, BT medium + 0.1 μM sucrose + DPI, BT medium + 0.1 μM sucrose and 0.1 μM DPI. (m) Sugar content in 15-DPA fibre cells of pGhSWEET15::GhSWEET15 transgenic 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 (T2). WT: wild-type cotton. DPA: days post-anther.
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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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