

SuSy, A MASTER GENE CONTROLLING COTTON FIBRE DEVELOPMENT

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Background

Cotton is the most important textile crop due to its cellulose-enriched mature fibres, single-celled hairs derived from ovule epidermis at anthesis. Despite the great potential for increasing cotton productivity through genetic engineering of fibre development, little progress has so far been made in this area. This is in sharp contrast to the success of pest and herbicide resistant transgenic cotton that have already made a large impact on agriculture in both the U.S. and Australia (1). The major impendence to fibre engineering is due to our poor understanding of the biology of the cotton fibre, particularly, the identities and functions of genes controlling various fibre development processes.

Cotton fibres are metabolically active cells in utilising hexose or its derivatives from phloem-imported sucrose for its initiation, elongation and cellulose synthesis (2,3,4,5). Sucrose synthase (SuSy) is the key enzyme in cotton fibre to break down incoming sucrose into fructose and UDP-glucose (2,3). The latter is the immediate substrate for cellulose synthesis (6). However, previous evidence on the role of SuSy in cotton fibre development is largely correlative in nature (2,3,4). Here, by using reverse genetic approach, we have now demonstrated that SuSy indeed plays a crucial role in cotton fibre initiation, elongation and cellulose synthesis.

Approach

We made sense and antisense SuSy suppression constructs, consisting of the 3' end of the SuSy cDNA, SS3, isolated from cotton fibres, driven by the promoter from subterranean clover stunt seven virus (S7). The two suppression constructs were transformed into cotton by *Agrobacterium*-mediated method. We analysed several lines of primary transformants and segregating individuals of the T1 generation by using molecular, cellular and biochemical approaches. We focused on 0 to 3 days after anthesis (DAA) for fibre initiation and early elongation and 25 DAA when fibre undergoes intensive cellulose synthesis.

Results

We obtained several lines of direct evidence to demonstrate the crucial and rate-limiting role of SuSy in cotton fibre initiation and elongation. First, the transformation of cotton

with SuSy suppression constructs leads to a fibreless phenotype (Fig 1) in multiple independent lines, which was inherited and co-segregated with the transgene. On the other hand, all the T1 progeny where the transgene segregated out showed wild type phenotype in fibre and seed growth. This demonstrates that the presence of the SuSy transgene is the causal basis of the fibre-repressed phenotype observed. Second, immunolocalization analysis and enzyme assay revealed that the expression of SuSy protein was suppressed by 70% or more in the ovule epidermis of the fibreless lines. Electron microscopy showed shrunken or collapsed fibre initials in the transgenic ovules. Importantly, the degree of SuSy suppression corresponds well with the level of inhibition on fibre cell initiation, without affecting the activity of invertase, a functionally closely related enzyme hydrolysing sucrose into glucose and fructose. Finally, analysis among a range of transgenic lines established a close linear correlation between the reduction of SuSy at 0 DAA and the repression of fibre elongation at 3 DAA. These results demonstrate that cotton fibre cell initiation and elongation are highly sensitive to changes of SuSy expression.

At 25 DAA, a small portion of seeds in the fruit had SuSy suppressed only in the seed coat fibres and transfer cells but not in the embryos. These seeds showed wild type seed phenotype except for much reduced fibre length and cellulose level. The latter is due to suppression of SuSy in the fibres. The remaining seeds in the fruit, however, had SuSy suppression both in the maternal seed coat and in the embryos. These seeds were shrunken with loss of seed coat transfer cells and only less than 5% of wild type seed size and fibre mass.

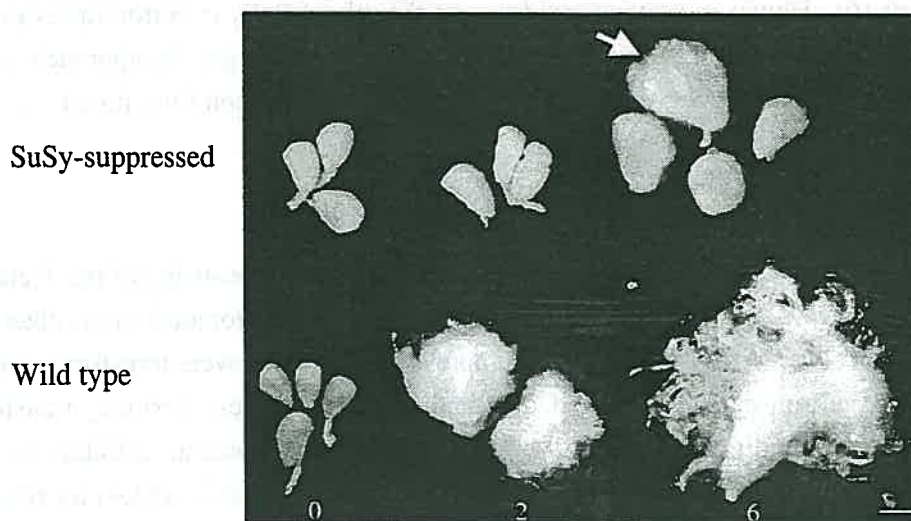


Figure Fibreless phenotype due to suppression of SuSy-gene expression in cotton seed. The number indicates days after anthesis (DAA). Arrow indicates seed with SuSy suppression occurred only in maternal tissue but not in embryos (see Results).

Conclusions

Our results provide direct evidence that the expression of sucrose synthase (SuSy) in the ovule epidermis is crucial for fibre initiation and elongation. We also found that the reduction of SuSy in fibres inhibits secondary cell wall cellulose synthesis. Finally, we discovered that while fibre initiation and early elongation is largely maternal controlled, additional expression of SuSy in the embryo is required for late fibre development and is essential for seed coat development and achieving normal seed size. The results provide important novel insights on the role of SuSy in controlling plant cell and seed development and offer opportunities to modify fibre and seed development by genetically engineering SuSy gene expression.

References:

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