

**MECHANISMS OF COOL TEMPERATURE
INHIBITION OF COTTON FIBER
CELLULOSE SYNTHESIS**

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Abstract

The long-term goal of this research is to improve the cool temperature tolerance of cotton fiber secondary wall deposition through targeted genetic engineering. It is well known that the nightly cool period (less than 28 - 22°C or 82 - 72°F) of the diurnal temperature cycle in temperate growing regions such as the Texas Southern High Plains adversely affects fiber yield and quality, particularly maturity, during a limited growing season. In previous research, we have established that the cotton ovule/fiber culture system established by C.A. Beasley is a valid model for the field cool temperature response of fiber development (Haigler et al., 1991). By feeding ovules with ¹⁴C-glucose and monitoring apparent rates of crystalline cellulose synthesis and respiration (CO₂ release) by previously published methods (Roberts et al., 1992), we established that some existing cultivars of *Gossypium hirsutum* L. have improved tolerance to cool temperatures through at least two mechanisms: (1) ability to conserve the maximum rate of cellulose synthesis at 15°C (59°F); and (2) ability to recover after rewarming from 15°C to 34°C (93°F). These studies established Paymaster HS-200 and Paymaster HS-26 as partially cool-tolerant cultivars for mechanisms (1) and (2), whereas Acala SJ-1 performed poorly for both, and transformable Coker 312 was equally improved for mechanism (2) and intermediate for mechanism (1). However, analysis of fiber weight gain after first exposure of cultured ovules/fibers to 15°C on 18 days post anthesis (DPA; 18 DPA = time of secondary wall deposition) indicates that even Acala SJ-1 has potential to adapt to cool temperatures, but only after a lag period of more than 3 days. The cultivar-specific results have been discussed more fully previously (Haigler et al., 1994).

Recently we have analyzed metabolites, glucose-6-P and UDP-glucose, that are putatively associated with cellulose synthesis in cultured ovules, which were fed with exogenous glucose as substrate. Metabolites were analyzed enzymically (Lowry and Passonneau, 1972; Kanabas et al., 1986) after extraction from fibers of Paymaster HS-200, Acala SJ-1, and Coker 312 on the 34°C and 15°C sides of a temperature cycle on successive days after first exposure to 15°C on 18 DPA. The results suggest that a major cool temperature hindrance of fiber cellulose synthesis occurs

after the uptake of glucose and its conversion by glucokinase to glucose-6-P. Furthermore, the ability to elevate glucose-6-P on the 15°C side of the cycle, which is more pronounced at an earlier time in Paymaster HS-200 than in Acala SJ-1 or Coker 312, may aid in acclimation to cool temperatures. A similar suggestion has been made for cool temperature tolerance of photosynthesis in barley (Labate and Leegood, 1989) and spinach (Holaday et al., 1992), perhaps because elevated glucose-6-P compensates for cool temperature induced changes in enzyme velocity and kinetics (Labate and Leegood, 1989). The cotton cultivars analyzed did not show such pronounced changes in the UDP-glucose pool at 15°C compared to 34°C, and all three behaved similarly.

Elevated glucose-6-P could support higher activity of phosphoglucomutase and UDP-glucose pyrophosphorylase, leading to higher flux through the pool of cytosolic UDP-glucose, which is the likely precursor of cellulose synthesis. However, recent evidence suggests that cellulose synthase is closely coupled to sucrose synthase, which degrades sucrose to yield UDP-glucose that is immediately passed to the cellulose synthase (Amor et al., 1995). In this model, the cytosolic pool of UDP-glucose is not related directly to cellulose synthesis. However, if sucrose is an obligatory substrate for fiber cellulose synthesis due to the metabolic channeling described above, cytosolic UDPG and fructose-6-P would be required substrates for sucrose synthesis from exogenous glucose in cultured ovules. Fibers on cultured ovules do contain sucrose that has been synthesized from exogenous glucose (Carpita and Delmer, 1981; confirmed by us), and it could be that elevated glucose-6-P at 15°C enhances production of sucrose that is required for cellulose synthesis. Since we do not yet know the form in which photosynthate enters the fiber cell, it remains possible that a similar mechanism operates in plant-grown fibers.

These results demonstrate that specific metabolic differences can be identified in existing cultivars that correlate with the cool temperature hindrance of fiber cellulose synthesis and acclimation to cool temperature. Consequently, we are optimistic that further beneficial changes can be made through targeted genetic engineering.

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