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# BIOCHEMICAL FACTORS INFLUENCING FIBER QUALITY AND COLOR FORMATION IN NATURALLY COLORED COTTON VARIETIES.

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**Annotation:** This article analyzes recent research on fiber quality and pigmentation in colored cotton (G. hirsutum L.) samples. The studies provide information about the biochemical characteristics of colored fiber, particularly pigment biosynthesis and its importance in producing high-quality fibers. Special attention is given to the role of bioactive metabolites (such as flavonoids and anthocyanins) affecting color inheritance in colored fibers, strategies used to improve fiber quality, and the outcomes of genetic selection.

**Keywords:** natural colored cotton, flavonoid, cellulose, GhF3H, hormones, quantitative trait loci (QTL) analysis

Naturally colored cotton is considered an important resource for the future of the textile industry. Products made from naturally colored fiber are gaining significant attention as environmentally friendly goods manufactured without harming the environment. The cultivation of colored cotton is somewhat more complex compared to conventional white cotton, and various biochemical and genetic studies are being conducted to improve its pigmentation and fiber quality. In this article, we examine the main pigmentation processes of naturally colored cotton and the genes involved in them, the factors influencing color formation, as well as an analysis of studies conducted on genetic analysis and gene modifications.

Naturally colored cotton is an environmentally friendly resource for human society, as it does not require chemical dyeing during textile processing. As a result, it significantly reduces pollution and conserves both water and energy (Weng et al., 2018; Wang et al., 2020). Colored cotton varieties produce thicker fibers compared to conventional white cotton, making these characteristics valuable in the fashion industry as well as in other sectors (Rani et al., 2020). Despite these advantages, naturally colored cotton varieties generally have lower yields than white cotton, making them less economically profitable. Naturally colored cotton fibers are usually weaker and create challenges during industrial processing compared to white cotton, and molecular biotechnological research is needed to improve them to the level of white fiber quality (Abbasi, 2019). However, improving essential traits such as color spectrum and low fiber yield through conventional breeding alone can be difficult (Wen et al., 2024). Therefore, numerous biochemical studies have been carried out to enhance fiber quality, focusing on the regulatory role of flavonoids in fiber quality traits and the mechanisms influencing fiber coloration [3,4,5,6,7,13,18]. Since the inheritance of fiber color is one of the most important traits in colored cotton, studying the mechanisms regulating color formation is a current and significant research challenge. In this regard, studies conducted by Hua et al. (2022) demonstrated the role of proanthocyanidins (*PAs*), particularly gossypol-like structures, and their oxidation in producing brown pigments in brown cotton fibers, and identified the involvement of the *PPO* enzyme, the *ANR* gene, and *GhMATE* transport genes in pigment transport and polymerization. Furthermore, research conducted by Wang et al. (2019) and Xiao, Y.H. et al. (2014) showed through *KEGG* and *WGCNA* analyses that the activity of flavonoid and phenylpropanoid pathways directly influences pigmentation. Similar studies have also been carried out on green cotton fibers, revealing that the phenylpropanoid pathway, particularly metabolites associated with pigmentation (cinnamic acid, flavonoids) and their regulatory genes (*PAL, CHS, FLS, LAR*), are also present in green-colored cotton. The period between 18–24 *DPA* has been identified as a key stage for pigment formation in colored fibers (Zhang, X. et al., 2019). Thus, the flavonoid biosynthesis pathway regulates pigment formation in both brown and green cotton fibers. The biosynthetic pathways of metabolites responsible for color formation in naturally colored fiber (anthocyanin, flavonoid, and phenylpropanoid pathways) have been extensively studied and analyzed. The environmental advantages of the selected varieties have also been highlighted (Ali, M. et al., 2022).

At present, brown and green colored cotton are among the main types of naturally colored cotton cultivated by farmers worldwide. Physiological studies of plants by Yuan et al. (2012) suggested that the low fiber quality observed in naturally colored cotton may be due to insufficient cellulose and flavonoid biosynthesis. Li et al. (2019) identified cinnamic acid and its derivatives, including ferulic and caffeic acids, as pigments in green cotton. Flavonoids such as flavone, flavonol, anthocyanin, and flavanols also participate in the pigmentation of green-colored cotton fibers. In particular, the high expression of the *PAL, CHS, F3H, FLS,* and *LAR* genes plays a key role in the biosynthesis of pigments, and the period between 18–24 *DPA* has been identified as the main stage of pigmentation. Several experiments have shown that the amount of cellulose affects the development of the fiber cell wall and influences fiber length, strength, and fineness. Therefore, cellulose content may be an important indicator in determining fiber quality (Dutt et al., 2004; Fan et al., 2013). The maximum rate of cellulose accumulation and the overall level of cellulose deposition in colored cotton are lower than those in white cotton; the chlorophyll content (evaluated by *SCMR*) is lower than that of white cotton, and the rate of chlorophyll degradation is relatively higher, as identified by Zhang Xiang et al. (2019) during their studies. Differences between colored and white fibers have been observed based on cellulose accumulation, sugar content, and chlorophyll levels. Fiber growth in colored cotton was delayed (Liu, C. et al., 2017).

It has been determined that in naturally colored cotton, the biosynthesis of anthocyanins and *PAs* is regulated through the *MYB113* transcription factor. Fan et al. (2023) described the mechanism by which the *MYB113* transcription factor regulates anthocyanin biosynthesis in cotton fibers. This indicates important genetic control for pigmentation in colored fibers (Wang, S. et al., 2023). So far, only a few genes related to anthocyanin homeostasis in cotton (Gossypium hirsutum) have been cloned and functionally studied (Wen et al., 2018; Yan et al., 2018). In this issue of *Plant Physiology*, Wang and colleagues reported that the transcription factor *MYB113* regulates anthocyanin biosynthesis in cotton. Ectopic expression of *MYB113* activated anthocyanin and *PA* synthesis, resulting in red-leafed cotton and brown fibers (Wang et al., 2022a). Through *MYB* transcription factors, pigment delivery to fibers (Qiu et al., *Plant Journal*, 2020) is controlled by *GhTT19*, *GhMYB25*, and *GhMYB113*, which regulate pigment biosynthesis and vacuolar transport. This can be useful as a genetic marker for selection and may serve as an important indicator for achieving genetic compatibility and phenotypic stability in colored cotton breeding.

Some studies have shown that endogenous hormones play a very important role in enhancing fiber development. Certain types of hormones can improve the final fiber quality (Zhang et al., 2012). In naturally colored cotton, there are some studies aimed at improving fiber quality by regulating physiological processes and the application of exogenous hormones. The use of exogenous hormones can be beneficial in enhancing the fiber quality traits of naturally colored cotton (Zhang et al., 2009).

Increasing yield and improving fiber quality are considered among the important objectives in breeding. Enhancing the development of these traits through traditional methods is a rather complex process; therefore, constructing molecular linkage maps using neutral genetic markers has been recognized as important in genetic selection (Wu et al., 2009). Identifying quantitative trait loci (*QTLs*) closely associated with fiber quality traits using markers can lead to the cultivation of improved cotton varieties in the future. In the *QTL* analysis conducted by Tang et al. (2021), markers affecting the L, a, b color parameters were identified, which may be important for future marker-assisted selection (*MAS*). Key *QTLs* controlling fiber color and quality in colored cotton have been identified. On seven linkage groups (*LGs*), there are 19 color-related *QTLs* and 8 fiber quality *QTLs*, which are useful for breeding purposes (Xu, H. et al., 2021). In brown and green cotton, the slower rate of fiber development results in lower fiber length, strength, maturity, and micronaire values compared to white cotton. One of the important factors contributing to the lower fiber quality of brown and green cotton is the reduced content of *IAA* and *ABA*. Increasing *IAA* and *ABA* levels in these fibers through $GA_3$ and *ABA* spraying improved fiber quality (Zhang Xiang et al., 2016). According to the research of Hongli Zheng et al. (2022), in *Gossypium hirsutum* L., the *GhF3H, GhF3’H,* and *GhF3’5’H* genes exhibit different expression patterns in colored and white cotton fibers, with strong expression mainly during the development stages of brown fibers. Differences in *ATPase,* sucrose invertase, *SS*, and *UDPG-PPase* enzymes between colored and white cotton fibers and their effects on fiber development have been demonstrated (Sun, Y. et al., 2018). A decrease in the transcription levels of hydroxylase genes led to varying degrees of lightening in brown fiber color. The expression of *GhF3H* has the greatest influence on fiber color, reflecting a characteristic specific to naturally colored cotton varieties. Silencing of *GhF3’H* and *GhF3’5’H* disrupts the balance between the two hydroxylases, resulting in varying degrees of fiber color lightening (Hongli Zheng et al., 2022).

In summary, multiple studies indicate that pigmentation in naturally colored cotton fibers is biologically complex, with flavonoids, proanthocyanidins, cinnamic acid derivatives, and their genetic regulation playing a crucial role. Enzymes and transcription factors involved at each stage of the biosynthetic pathways directly influence pigment formation. At the same time, genetic mapping, *QTL* identification, and molecular selection are being recognized as important approaches for improving fiber quality and color stability in naturally colored cotton.

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