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Towards improving BT and NC cotton fibre quality

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Abstract
Cotton is a plant popular for its abundant cellulose fiber. All parts of the cotton plant are useful, especially the fiber. Development of genetically modified cotton against insect attack has regained cotton glory with impending limitations such as inferior fiber quality observed in *Bacillus thuringiensis* Cotton (Bt cotton), and Naturally Colored Cotton (NC cotton) fiber thus, paving the way to continuous dyeing of white cotton fiber which has a significant adverse effect in the environment, health, and climate. However, the formation of colors in NC cotton fiber involves certain biochemical and physiological processes usually through the flavonoid pathways. Bt and NC cotton are mainly from the *Gossypium hirsutum* species which is the majorly cultivated cotton species worldwide. So, with the demand for a sustainable future, it becomes imperative to improve *G. hirsutum* fiber quality. "Consequently, it has been found that *G. barbadense*, Espansin, and Myb genes family, etc. have the potential to improve cotton fiber quality. Therefore, in this work, we are proposing the integration of the genes associated with cotton development, which have been predicated on having importance in Cotton fiber quality and are essential in the molecular improvement and genetic engineering of cotton plants towards the development of Bt and NC cotton with superior fiber quality for a sustainable healthy environment. We also highlighted several other realizable recommendations.

Keywords: Cotton, *Bacillus thuringiensis* cotton, naturally colored cotton, fiber quality, molecular improvement, and genetic engineering

Introduction

Cotton is a plant popular for its abundant cellulose fiber. Cotton fiber is a soft and fluffy staple fiber that grows in the boll or a protective capsule around the seeds (Nnaemeka and Sun, 2021) [38, 42]. Cotton (Gossypium spp.) is an important economic crop and the largest source of textile fiber in the world. All parts of the cotton plant are useful, especially the fiber or lint. Therefore, cotton researchers and marketers approach cotton issues with seriousness.

However, cotton farming has declined globally due to many factors mostly insect attacks in the past years, and fiber quality in recent times, etc. These have deprived huge economic benefits from cotton. Remedial measures for the effect of these losses have been approached with insecticide application the only combating alternative. This measure has significant damage to the environment and human health. The revitalization of cotton for its fiber, human safety, and healthy environment, was a huge scientific exercise survived by the discovery of *Bacillus thuringiensis* Bt toxin. Cotton, therefore, has been firstly and successfully genetically manipulated to resist insect attack. The sustainability of GM crops has been subjected to heated debate in the world but Bt cotton has proven less controversial. This technology has solved to some extent the challenges posed to cotton farming in many countries.

Bt Cotton

Nnaemeka and Sun, 2021 [38, 42] and 2023 reviewed the Bt cotton situation in Nigeria, China and Africa and made possible improvement and sustainability recommendations. However, issues regarding the quality of the Bt cotton fiber have been approached because despite the recorded improvements, there are still limitations in terms of fiber quality which inspired our interest in working on the cotton plant *Gossypium hirsutum* "Gh" at Plant Genomics and Molecular Improvement of Coloured Fiber Lab, a key lab of Plant Secondary Metabolism and Regulation of Zhejiang Province Cotton Secondary Metabolism Research Centre at Zhejiang Sci-Tech University (ZSTU). We focused on combining traits of cotton to genetically improve the fiber quality of Gh which is the dominant species in both China and Africa (Nigeria). MYB308 gene one of our earlier tested genes showed a significant effect on the Gh cotton fiber development with measurable improvements. However, the use of naturally colored cotton (NC cotton) in textile industries is statistically low due to its inferior fiber quality e.g. Short fiber length (Table 1). The limited color choices of NC cotton have also greatly minimized their use in textile manufacture (Dutt *et al*., 2004) [8].

Naturally Coloured Cotton (Nc Cotton)

With the demand for a sustainable future, NC cotton (Figure 1) has regained attention as a solution for reducing the obnoxious impacts of the textile cycle. Studies on NC cotton have advanced understanding of the pigment composition and the molecular mechanisms underlying the pigmentation of NC cotton fiber and the genetic relationship between pigmentation and fiber development (Sun *et al*., 2021) [42]. Variable colors can be found in different wild cotton species (Figure 1), but for the cultivated species, only brown and green with different intensities are recorded. The formation of colors in cotton fiber involves certain biochemical and physiological processes usually through the flavonoid pathways. Flavonoids are known to plant secondary metabolites, which play important roles mainly in the color formation of most flowers, fruits, seeds, etc. (Chaves-Silva et al., 2018; Falcone Ferreyra et al., 2012; Tanaka and Brugliera, 2013) [2, 10, 44]. Anthocyanins, a major form of flavonoids particularly responsible for the red, purple, and blue colors of many flowers and fruits, are synthesized via the general phenylpropanoid pathway which has been well documented during the past decades (Falcone Ferreyra et al., 2012; Tanaka and Brugliera, 2013) [10, 44]. The phenylpropanoid and flavonoid pathways are active in NC cotton and white-coloured fiber (WCF). Proanthocyanidins (PAs) or their derivatives synthesized by the flavonoid pathway are the pigments responsible for the color of brown cotton fiber (BCF). PAs are polyphenols formed by oligomers or polymers of flavan-3-ol units. Most of these PA building blocks are present in cotton fiber but higher in BCF than in green cotton fiber (GCF) and white cotton fiber (WCF). The oxidation product of PAs "Quinones", also directly contributes to color formation in brown fibers. (Feng et al.,2013, 2014; Li et al., 2012; Liu et al., 2018a ; Sun et al., 2022) [11, 12, 24, 25, 43]. Apart from color formation, Quinones also endow fibers with antibacterial activity, mildew resistance, flame retardant, and UV protection (Chen and Cluver, 2010; Emiliani et al., 2013; Hinchliffe et al., 2016; Hustvedt, 2005) [3, 9, 18, 20]. Flavonoid pathway genes are known to be co-ordinately induced, and transcription factors that directly regulate the expression of the structural genes of the pathway have been identified in several species (Jaakola, 2013) [22]. For example, upregulation of Gh_A07G2341 activates genes of the phenylpropanoid and flavonoid pathways and leads to PA accumulation in BCF. Over-expressing Gh_A07G2341 in WCF produces light-brown fiber (Ke et al., 2022). In NC cotton, the main genes involved in anthocyanidin and PAs synthesis, including GhCHS, GhCHI, GhDFR, GhC4H, GhF3H, GhF30H, GhF3050H, GhANS, GhLAR, and GhANR, have been cloned and proved to contribute to the color formation (Feng et al., 2013, 2014; Gao et al., 2019; Gong et al., 2014) [11, 12, 13, 21]. However, the scarcity of germplasm resources and relatively poor understanding of the mechanisms underlying the color formation of NC cotton have greatly hindered the progress in the breeding of NC cotton with improved colors and fiber quality (Ke et al., 2022).

Table 1: Agronomic traits of NC cotton in China since 2000 (Li et al., 2020) [31].

Fiber Color	No. of Varieties	Fiber Length (mm)	Fiber Strength (CN/tex)	Micronaire	Lint (%)	Boll Weight (g)	Lint Yield (kg/ha)
Brown	43	28.94 ± 1.64	28.43 ± 3.28	4.07 ± 0.42	38.06 ± 3.65	5.38 ± 0.52	1546 ± 406
Brown*	10	30.83 ± 0.51	31.21 ± 0.65	4.22 ± 0.32	41.67 ± 1.52	5.82 ± 0.32	2262 ± 121
Green	18	27.62 ± 1.91	21.91 ± 3.45	2.80 ± 0.42	28.59 ± 3.44	4.64 ± 0.39	962 ± 235
White#	22	31.15 ± 0.83	32.19 ± 1.18	4.25 ± 0.23	41.68 ± 0.90	5.72 ± 0.28	2255 ± 81

Notes: *Brown*: Brown-color varieties released in Xinjiang since 2017.
#White#: Nationally authorized white-color varieties released in Xinjiang since 2017.

So far, it's notable that the need for further improvement of fiber quality, especially in NC and Bt cotton, can not be over-emphasized. Exploring fiber color and insect resistance with superior fiber quality by understanding the level of expression of genes in the necessary biosynthetic pathway is essential for better modification of the process mechanism towards molecular breeding of cotton for the desired trait.

Comparative Assessment of Nc and White Cotton

White cotton has been spun, woven, and dyed since prehistoric times; it clothed the people of ancient India, Egypt, and China, and remains today the most widely used textile fiber (Colleen, 2005) [5]. The fact that the cotton color we see today was not always off-white is not popular. Colored cotton was cultivated and utilized in South and Central America around 2300 BC (Nagarajan et al., 2022) [37]. Natural colors of cotton then were mocha, tan, gray, and red-brown. The NC cotton grew with some ancient civilizations, but priorities changed over time to push them into obscurity. Fabric products made of NC cotton appeared as fashion items during the 1990s. Soon after, it lost most of its market and commercial production due to poor fiber quality and limited shades of color (Sun et al., 2021) [42]. It was during the Industrial Revolution that the looms came. Since the NC cotton fibers were short, processing them on the looms became a hindrance. So, the long-fibered white cotton slowly and steadily replaced them on the looms. Subsequently, growing white cotton became an inevitable part of agriculture which made the colored cotton grow extinct for a long time. Climate change and environmental concerns have taken enormous significance currently. Therefore, there is now a great need to reflect, review, and modify all available to ascertain if the prevailing methods are consequential to the contemporary lifestyle or should be discontinued for the ancient procedure for the betterment of mankind. In the textile industry, this is very much a reality due to the way chemicals have entered into the use of dyes and coloring substances for fabrics. Their disposal after the coloring processes added worry because they are hazardous to the ecosystem. Evaluations were made by considering the production cycle: Energy, water, labor, electricity, chemical, auxiliary agent, dyestuff, etc were considered. As a result, all the flowcharts in the dye house were analyzed. It was found that treating the naturally colored cotton fabric is 2.9 times less expensive than treating white cotton fabric in the Dyehouse. Sally Fox's successful experiment in modifying colored cotton for machine suitability spurred several countries to increase interest in studying colored cotton. Today, genetic modification towards the development of new colored cotton with better quality is pursued in many countries. In the process, advancement has been made to have gray, black, mahogany, purple, orange, red, pink, blue, green, grey, and cream, as well. They are at various stages in the genetic experiments in different countries (Martha, 2005) [32].

Fig 1: NC and White cotton.

There is experimental evidence to demonstrate that naturally pigmented cotton, especially green cotton, has excellent sun protection properties when compared with unbleached white cotton that needs to be treated with dyes or finishes to obtain similar properties (Nagarajan *et al*., 2022) [37]. This gives rise to the hypothesis that pigments in naturally pigmented cotton fibers protect the embryonic cotton seeds from the UV radiation of the sun, so it can be assumed that fabrics from such fibers would also do the same for the human body. NC cotton is also resistant to change compared with conventional dyed white cotton. After laundering, the color becomes stronger and more intense, a characteristic documented during research studies at Texas Tech University. Some NC cotton darkens with exposure to the sun. However, green is seen as less stable and easily fades to tan when exposed to sunlight.

The low yield potential of NC cotton has acted as a barrier to the expansion of its cultivation. However, the problem being addressed, there is still a long way to go. There is a need to improve the fiber properties of NC cotton, particularly fiber strength to make it suitable for high-speed spinning. NC cotton genotypes currently available in the germplasm have limited lint colors. There are only two colors: brown in various shades and green. With only two colors, NC cotton can not compete with white cotton as varied treatments of colors and shades can be easily imparted to white cotton. Therefore, a call for continuous advancement of colored cotton is recommended.

G. Hirsutum and G. Barbadense in Fiber Quality Improvement

Cotton (Gossypium sp) belongs to the plant family Malvaceae and the genus Gossypium comprising 50 species. *Gossypium hirsutum* "Gh" (Upland cotton) and *Gossypium barbadense* "Gb" (Pima or Egyptian cotton) are the most cultivated species (Mehboob-ur-Rahman *et al*., 2011) [34]. About 90% of all cotton production globally are cultivars derived from Gh. Gh importance and demand contributed to its wide cultivation and research interest but Gb is characterized by its superior fiber quality (Wang M *et al*., 2019 and Hu Y *et al*., 2019) [19, 45]. Several Breeders have tried to develop high-quality cotton varieties with high yield, wide adaptability, and superior fiber properties by crossing *G. barbadense* with *G. hirsutum*, which is tedious and often inefficient owing to the complex trait segregation of hybrid progenies. A more economical and effective strategy is molecular breeding, including molecular marker-assisted selection, transgene, and gene editing. This strategy requires a wide identification of fiber development-related genes and accurate verification of their functions. Comparative transcriptome analysis of fiber tissues between Gh and Gb could also reveal the molecular mechanisms underlying high-quality fiber formation and identify candidate genes for fiber quality improvement (Liu *et al*., 2023) [28]. Liu *et al*., 2023 [28], further identified cotton fiber development-related genes, *G. barbadense* acc. Pima90-53 and Hai7124 with superior fiber properties and *G. hirsutum* acc. HY405 and ND13 with good fiber properties but inferior to *G. barbadense* were selected for comparative transcriptome analysis. Fiber quality analysis showed that the two *G. barbadense* accessions compared with the two *G. hirsutum* accessions possessed significantly higher fiber length (i.e., fiber upper-half mean length) and fiber strength and a lower fiber micronaire. *G. barbadense*, compared with *G. hirsutum*, possesses a higher rate of lint elongation and a prolonged elongation period, which gives rise to a greater length of mature fibers therefore, recommending the *G barbadense* integration in developing high-quality cotton varieties. In addition to revealing the genetic basis for high-quality cotton fiber formation, more importantly, they further identified elongation-related candidate genes for fiber quality improvement. For cotton fiber elongation the identified 42 genes among which Gbar_A05G037170 and Gbar_A05G020690 driven by a CaMV35S promoter were transferred independently into *A. thaliana* plants, and then their roles were determined by observing the changes in leaf trichomes and dark-grown hypocotyl cells. The two genes were considered following their potential as novel genes related to cotton fiber development. Also, transient expression analysis of Gbar_A05G037170 fused to a green fluorescent protein (GFP) confirmed its nuclear localization. Two transgenic T3 lines OE2 and OE5 were developed, in which the stable expression of Gbar_A05G037170 was confirmed by qRT-PCR (Liu *et al*., 2023) [28]. Arabidopsis leaf trichomes were often used because they partly share common regulatory mechanisms with cotton fiber cells (Guan X *et al*., 2007)

[16]. In addition, several genes were found in cotton fiber development-related gene families, such as Gbar_A03G019080 (nonspecific lipid-transfer protein) by Meng C *et al*., 2018 [35], Gbar_A07G007890 (α-expansin) by Lv *et al*., 2020 [27] and Gbar_D08G001620 (dirigent protein). Due to the reports that *G. barbadense* fibers have better properties, genes regulating fiber development in *G. barbadense* are recommended for consideration in the transformation of *G hirsutum*. Consequently, most of these genes are from the expansin gene family.

Expansins

Expansins are plant proteins that function in cell wall modification and growth. They are involved in the loosening of the cell wall structure thus, allowing the cell to expand and elongate. Expansins are majorly found in higher plants, bacteria, and fungi. Expansins may unlock the network of wall polysaccharides without lytic activity, permitting turgor-driven cell enlargement (Cosgrove, 2000, Sampedro, 2005) [6, 41]. Plant expansins are usually 250 to 275 amino acid residues long, and the majority have a signal peptide in the N terminus; the signal peptides are usually 20 to 30 amino acid residues long (Cosgrove, 2015, Sampedro, 2005) [7, 41]. Typical structures of plant expansins are torpedo-shaped proteins (Lv *et al*., 2020) [27]. *Gbar_A07G007890* an (α-expansin 13) have been predicted to be substantial in developing better fiber quality in *Gossypium barbadense* because of its contribution to the expansion of the fiber cell wall. Experimental evidence suggests that expansins loosen cell walls via a nonenzymatic mechanism that induces slippage of cellulose microfibrils in the plant cell wall (Sampedro, 2005) [41]. The Expansin gene family consists of various members, often denoted as EXP1 and EXP2, and so forth. Each member may have specific functions and can be expressed in different tissues or developmental stages. Expansins are crucial for various physiological processes in plants, including seed germination, root growth, and responses to environmental stimuli. However, the biological functions of this gene family in cotton are still not well explored (Li-Min *et al*., 2020) [31] despite some reported efforts. It was found from http://www.expansingenefamily.com/ that over 3000 Expansin genes in different plant species both monocot and dicot have been identified, sequenced, and reported. Lv *et al*., 2020 [27] identified 93 expansin genes in *Gossypium hirsutum.* These genes were classified into four subfamilies (Alpha Expansin EXPA, Beta Expansin EXPB, Alpha Expansin Like EXPLA, and Beta Expansin Like EXPLB gene), and they are divided into 15 subgroups). The 93 expansin genes are distributed in over 24 chromosomes. All GhEXP genes contain multiple exons, and each GhEXP protein has multiple conserved motifs. Transcript profiling and qPCR analysis revealed that the expansin genes have distinct expression patterns among different stages of cotton fiber development (Lv *et al*., 2020) [27]. The multiple sequence alignment results of 93 expansin proteins from *G. hirsutum* showed similar sequence characteristics (Lv *et al*., 2020) [27]. It has been confirmed that expansins play an important role in cotton fiber development for example: The *Gossypium hirsutum* GhEXPA8 fiber expansin gene was introduced into the local cotton variety NIAB84 using an Agrobacterium-mediated gene transformation. The data collected from the field performance of the transgenic cotton plants expressing GhEXPA8 showed significant improvement in the fiber lengths and micronaire values compared to the control *G. hirsutum* variety NIAB846 (Bajwa *et al*., 2015) [1]. Apart from GhEXPA8 and a few others, no extensive study has been conducted using GhEXPA13 in Bt and NC cotton. The cotton genome has been sequenced and re-sequenced in succession (Zhang *et al*., 2015, Li *et al*., 2014, Ma *et al*, 2018, and Wang *et al*., 2019) [30, 33, 45, 46]. Many countries are conserving genetic resources within and between species, implying that this valuable germplasm can be exchanged among countries for increasing productivity (Nnaemeka and Sun 2023) [39, 40]. There is a huge scope for pre-breeding work in cotton to combat biotic and abiotic stresses. Some countries, however, have maintained a wide list of cotton germplasm (Nnaemeka and Sun 2023) [39, 40], supporting the possibilities of transforming cotton for improved fiber quality.

Conclusion

Advances in Gene discovery, genome mapping, identification of linked molecular markers, and key functional genes contribute to fiber quality improvement and understanding of the fiber development process. The need for further improvement of cotton fiber quality for textile benefit contributed enormously to laying the foundation of this review, and recommendations for subsequent research. More hands towards improving these qualities are necessary. However, improved cotton yields and fiber quality have been realized through modern biotechnology-based plant breeding. It is, therefore, essential to improve NC and Bt cotton fiber quality. Towards actualizing this, we have considered and recommended the integration of an expansin gene named EXPA13 in Cotton FGD, and an MYB gene named MYB308 in Cotton FGD associated with cotton development. There are predictions that they have importance in Cotton fiber quality in the molecular and genetic engineering of cotton plants. Our interest is focused on genetically improving the fiber quality of *Gossypium hirsutum* L: A Nigeria Bt Cotton seed identified as MAHYCO C567 BG11 (with Bollgard 11 Technology) and known China-colored cotton fiber seeds, at Plant Genomics and Molecular Improvement of Colored Fiber Laboratory, College of Life Science and Medicine, Zhejiang Sci-Tech University, Hangzhou 310016, Zhejiang, China. The genes were identified by bioinformatics to elucidate further their influence on fiber quality improvement in different Chinese cotton seeds and Nigeria Bt (Mahyco C567 BG11) seeds. The gene's biotic and abiotic regulation influence will also be observed by contemporary molecular biology and genetic engineering techniques. The success of the research may bring about the integration of its approaches towards further improvement and sustainability of improved cotton fiber quality. These recommendations are possible because the current trend of cotton breeding has been successful, and these techniques have increased the productivity of cotton within the period of invention.

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