FIBER QUALITY-COLOR ASSOCIATIONS IN GOSSYPIUM HIRSUTUM L. HYBRIDS

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Abstract. The use of naturally colored cotton fiber, which eliminates the need for chemical dyes and reduces environmental pollution, is a requirement of modern sustainable textile production. However, fiber color is usually negatively correlated with fiber quality in cotton. The objective of this study was to determine the polymorphism of fiber quality and color traits in cotton varieties and F_4 hybrid combinations using SSR markers. As a result, hybrid combinations with high fiber quality stability (107 and 135 bp) and improved coloration, associated with a pleiotropic locus on chromosome 7 (genome A), were identified. Markers BNL1604 and NAU1043 demonstrated high informativeness for genotype identification, making them promising tools for molecular tagging of breeding material and accelerated selection for fiber quality and color traits. The use of SSR markers BNL1604 and NAU1043 enabled the identification of allelic combinations associated with improved fiber properties. The F_4 hybrids L-001 × 010444 and Bukhara-6 × 02408 are recommended for further breeding as donors of genes for high fiber quality. Marker-based identification confirmed the effectiveness of SSR technology for accelerating selection in cotton breeding programs. The results of this study have both scientific and practical significance for developing environmentally safe varieties of naturally colored cotton with improved fiber properties.

Keywords: cotton, fiber color, fiber quality, SSR markers, primers, polymorphism, molecular breeding.

GOSSYPIUM HIRSUTUM L. DURAGAYLARIDA TOLA SIFATI VA RANGINING BOG'LIQLIGI

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Annotatsiya: Tabiiy rangli paxta tolasi ishlab chiqarishda kimyoviy bo'yoqlardan foydalanishni kamaytirish va atrof-muhit ifloslanishini pasaytirish imkonini berishi sababli zamonaviy barqaror to'qimachilik sanoatining muhim talabi hisoblanadi. Biroq, paxtada tola rangi odatda uning sifat ko'rsatkichlari bilan salbiy korrelyatsiyada bo'ladi. Ushbu tadqiqotning maqsadi paxta navlari va F_4 duragay kombinatsiyalarida tola sifati va rang belgilari polimorfizmini SSR-markerlar yordamida aniqlashdan iborat edi. Natijada 7-xromosomada (A genomi) joylashgan pleiotrop lokus bilan bog'liq holda yaxshilangan rang va yuqori tola sifati barqarorligi (107 va 135 juft nukleotidli allellar)ga ega duragay kombinatsiyalar aniqlandi. BNL1604 va NAU1043 markerlari genotiplarni identifikatsiya qilishda yuqori informativlik ko'rsatdi, bu esa ularni seleksiya materiallarini molekulyar markirovkalash va tola sifati hamda rang belgilariga yo'naltirilgan tezlashtirilgan seleksiya uchun istiqbolli vosita sifatida tavsiya etish imkonini beradi. BNL1604 va NAU1043 SSR-markerlaridan foydalanish yaxshilangan tola xususiyatlari bilan bog'liq allel kombinatsiyalarini aniqlash imkonini berdi. F_4 duragaylari L-001 × 010444 va Bukhara-6 × 02408 yuqori tola sifati genlari donorlari sifatida keyingi seleksiya ishlari uchun tavsiya etildi. Molekulyar markerlar asosida o'tkazilgan identifikatsiya SSR texnologiyasining paxta seleksiyasi dasturlarida tanlash jarayonini tezlashtirishdagi samaradorligini tasdiqladi. Olingan natijalar tabiiy rangli paxtaning yaxshilangan xususiyatlarga ega, ekologik xavfsiz navlarini yaratishda ilmiy va amaliy ahamiyatga ega.

Kalit so'zlar: g'o'za, tola rangi, tola sifati, SSR-markerlar, praymerlar, polimorfizm, molekulyar seleksiya

ВЗАИМОСВЯЗЬ КАЧЕСТВА И ЦВЕТА ВОЛОКОН У GOSSYPIUM HIRSUTUM L. ГИБРИДЫ

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Аннотация: Использование натурального окрашенного хлопкового волокна, исключающее необходимость применения химических красителей и снижающее загрязнение окружающей среды, является требованием современного устойчивого текстильного производства. Однако цвет волокна обычно отрицательно коррелирует с качеством волокна в хлопке. Целью данного исследования было определение полиморфизма признаков качества и цвета волокна у сортов хлопка и гибридных комбинаций F4 с использованием SSR-маркеров. В результате были идентифицированы гибридные комбинации с высокой стабильностью качества волокна (107 и 135 п.н.) и улучшенной окраской, связанные с плейотропным локусом на хромосоме 7 (геном A). Маркеры BNL1604 и NAU1043 продемонстрировали высокую информативность для идентификации генотипа, что делает их перспективными инструментами для молекулярной маркировки селекционного материала и ускоренной селекции по качеству волокна и цветовым признакам. Использование SSR-маркеров BNL1604 и NAU1043 позволило идентифицировать аллельные комбинации, связанные с улучшенными свойствами волокна. Гибриды F4 L-001 × 010444 и Bukhara-6 × 02408 рекомендуются для дальнейшей селекции в качестве доноров генов высокого качества волокна. Идентификация на основе маркеров подтвердила эффективность технологии SSR для ускорения селекции в программах селекции хлопка. Результаты данного исследования имеют как научное, так и практическое значение для разработки экологически безопасных сортов натурально окрашенного хлопка с улучшенными свойствами волокна.

Ключевые слова: хлопок, цвет волокна, качество волокна, SSR-маркеры, праймеры, полиморфизм, молекулярная селекция.

INTRODUCTION

As the entire world moves toward environmentally friendly and organic textile production, naturally colored cotton fiber has become a new innovative breakthrough in the textile industry. The creation of new varieties of colored cotton with high fiber quality remains one of the most urgent challenges of the present time. The modern development of the textile industry is aimed at producing eco-friendly, organic, and sustainable materials. One of the most promising directions is the use of naturally pigmented cotton fiber, which eliminates the need for chemical dyes and thereby reduces environmental pollution (Rakhmonova et al., 2025). However, compared to white cotton, colored cotton usually shows a decrease in fiber yield and its mechanical characteristics, which limits its wide industrial application (Pavan M., Paschapur N.S., 2022).

Colored cotton is generally inferior to white cotton, especially with respect to fiber quality, including shorter and weaker fibers and lower micronaire. There is typically a negative correlation between fiber color and fiber quality traits, mainly due to the pleiotropic effects of fiber color genes (Wang L. et al., 2014).

SSR markers (Simple Sequence Repeats) are convenient for research due to their ability to demonstrate high polymorphism and relatively low cost. Molecular marker technologies (Marker-Assisted Selection, MAS) allow selection at early stages of ontogenesis, without waiting for the phenotypic traits to appear in the field (Wang H. et al., 2024). SSR markers have gained the widest application because of their informativeness, reproducibility, and ease of interpretation of results.

Genetic linkage analysis was carried out to map the fiber color loci *Lc1* and *Lc2* on two brown cotton cultivars with SSR and EST-SSR markers in the reference map by F_2 segregation populations (Wang L. et al., 2014).

This experiment used upland cotton green fiber germplasm line 1-4560 and the genetic inbred line TM-1. The lipid profiles of green fibers at 30 (white stage) and 35 days post-anthesis (DPA) (early greening stage), as well as those of TM-1 at the same stages, were analyzed. Among the 109 differential types of lipids (DTLs) unique to green fiber, the content of phosphatidylserine PS (16:0_18:3) was significantly different at 30 and 35 DPA. It is speculated that this lipid is crucial for the pigment accumulation and color formation process of green fibers. These findings provide a new theoretical basis for studying cotton fiber development and offer important insights into the specific mechanism of green fiber color formation (Li T. et al., 2024).

MATERIALS AND METHODS

The objects of the study were parental forms of cotton such as L-001, L-100, Bukhara-6, Bukhara-102, and S-01 with white fibers, as well as accessions 010444, 011283, 02408, 04511, and 08814 with colored fibers, and hybrid combinations of the fourth generation (F_4) obtained as a result of crossings between white- and colored-fiber varieties of *G. hirsutum* L. In total, 20 genotypes were studied: 10 parental forms and 10 hybrids.The research was conducted in 2023–2025 at the Scientific Research Institute of Cotton Breeding, Seed Production, and Agrotechnology (Tashkent, Uzbekistan).

The reagents used in DNA extraction, PCR, and electrophoresis included: Tris, EDTA, STAB, NaCl, boric acid, ethyl alcohol (70%), agarose, potassium acetate, sodium acetate, RNase, ethidium bromide, bromophenol blue, BZA, dNTP (deoxyribonucleotide triphosphates), isoamyl alcohol, isopropyl alcohol, xylene cyanol, Metaphor agarose, RNase, magnesium chloride, Taq polymerase, liquid nitrogen, 2×CTAB buffer, 10×CTAB buffer, chloroform/isoamyl alcohol (24:1), TE buffer, DNA markers, and pairs of primers.

The equipment used included a PCR thermocycler (Heal Force), pipettes (single-channel and 8-channel, Labnet, USA), centrifuge (Biobase), thermoshaker (ZHICHENG 2102C), vortex mixer (Vortex), mortar and pestle, tubes (1500 μL), rubber gloves, pipette tips (100 μL, 200 μL, and 1000 μL), electrophoresis apparatus, vacuum concentrator, microwave oven (Artel), analytical balance (AE323), and a gel documentation system (I BRIGHT 900).

Genomic DNA was extracted from the leaf tissues of parental forms and F_4 hybrid plants using the CTAB method (Paterson A.H. et al., 1993; Zhang J. et al., 2000).

RESULTS AND DISCUSSION

After the DNA samples extracted from the parental forms and F4 hybrids were adjusted to a uniform concentration of 50 ng/μL, screening PCR analyses were performed using 10 SSR primers consisting of a set of microsatellite markers. From this set, the DNA markers **BNL1604** and **NAU1043**, which are genetically associated with fiber quality and color and exhibit high polymorphism, were selected, and PCR analyses were carried out.

The SSR marker BNL1604, genetically linked to fiber quality, was amplified by PCR in 20 cotton genotypes, revealing clear polymorphism (Fig. 1). Three distinct allele sizes were observed at this locus: approximately 107 bp, 123 bp, and 135 bp, as confirmed by the band patterns on the gel. Each genotype produced between one and three PCR fragments, indicating that BNL1604 amplifies multiple loci.

The presence of multiple alleles (e.g., in 04511, 08814) suggests that BNL1604 targets at least two homologous loci, which is consistent with previous studies, as this SSR marker locus is located on more than one chromosome in cotton (homologous chromosomes 7, 16, and 17) (Wang H. et al., 2011).

The hybrid combinations and their frequencies in the panel were as follows: 107, 123, and 135 bp were observed in genotypes 11, 12, and 15 (11 – F_4 L-001 × 010444, 12 – F_4 010444 × L-001, 15 – F_4 Bukhara-6 × 02408), while 107 and 123 bp were detected in all other genotypes. The 123 bp allele was present in nearly every genotype (20 out of 20 genotypes, 100%), the 107 bp allele was present in 17 genotypes (85%), and the 135 bp allele was present in 5 genotypes (25%).

The 123 bp allele is likely fixed at a single subgenomic locus in the cotton genotypes, whereas the 107 bp and 135 bp fragments segregate among genotypes, reflecting different genetic backgrounds. Hybrid combinations F_4 L-001 × 010444, F_4 010444 × L-001, and F_4 Bukhara-6 × 02408, which carry the 107 bp and 135 bp alleles, were identified as materials with high fiber quality.

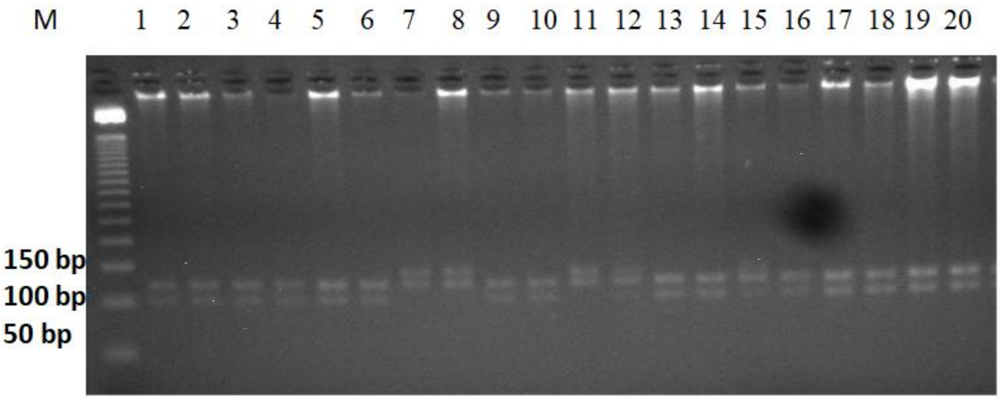

Fig. 1. PCR analysis of parental forms and F_4 hybrids using the BNL1604 marker.

The BNL1604 marker, associated with fiber quality, exhibited a high level of polymorphism. Three alleles were identified: 107 bp, 123 bp, and 135 bp. The 123 bp allele was present in all genotypes (100%), the 107 bp allele in 85%, and the 135 bp allele in 25%.

The F_4 combinations L-001 × 010444 and Bukhara-6 × 02408 showed a stable combination of the 107 bp and 135 bp alleles, indicating their high fiber quality.

Overall, the observed polymorphism and multilocus profile confirm that the BNL1604 marker is highly specific and precise for the identification of these cotton genotypes.

To identify new loci of quantitative traits (QTLs) responsible for fiber color and to understand the relationship between fiber color and its quality, white- and colored-fiber cotton varieties and accessions, as well as F_4 hybrids obtained from their crosses, were analyzed using PCR screening with the SSR marker NAU1043, which is genetically linked to fiber color (Fig.2).

According to the results of the study, among the NAU1043 markers on chromosome 7 (A genome), a locus with multiple effects was identified, and the QTLs qLC-7-1 and qFC-7-1 had a positive influence on this locus.

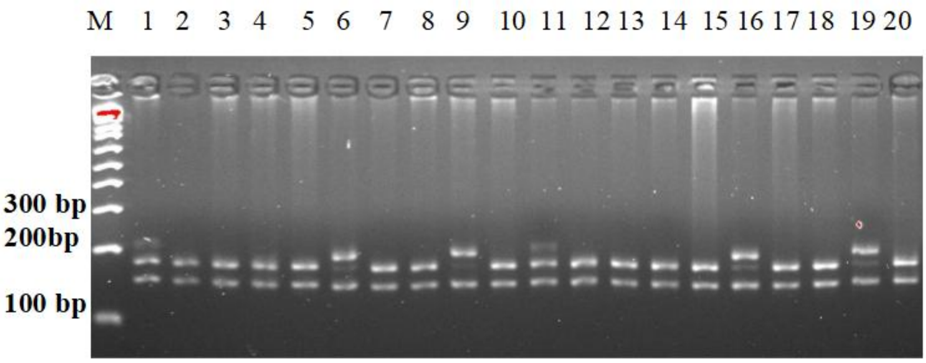

Fig. 2. PCR analysis of parental forms and F_4 hybrids using the NAU1043 marker.

For the NAU1043 marker, associated with fiber color, three alleles were identified: 155 bp, 180 bp, and 200 bp. The greatest effect on color expression was observed in the hybrids 11 – F_4 L-001 × 010444, 16 – F_4 02408 × Bukhara-6, and 19 – F_4 S-01 × 08814.

Among the 10 F_4 hybrid combinations, alleles of 155 bp, 180 bp, and 200 bp were observed in hybrids 11, 16, and 19, whereas alleles of 155 bp and 180 bp were detected in the hybrid combinations located in lanes 12, 13, 14, 15, 17, 18, and 20.

Based on PCR screening using the SSR marker NAU1043, genetically linked to fiber color, dominance for fiber color was observed in hybrids 11 – F_4 L-001 × 010444, 16 – F_4 02408 × Bukhara-6, and 19 – F_4 S-01 × 08814, which carried all three allele sizes. This confirms that fiber color inheritance in the progeny depends on the parental genotype.

To identify new loci of quantitative traits (QTLs) responsible for fiber color and to understand the relationship between fiber color and quality, white- and colored-fiber cotton varieties and accessions, as well as F_4 hybrids obtained from their crosses, were analyzed using PCR screening with the SSR marker NAU1043, which is genetically linked to fiber color.

According to the results, among NAU1043 markers on chromosome 7 (A genome), a locus with multiple effects was identified, and the QTLs qLC-7-1 and qFC-7-1 had a positive influence on this locus, improving fiber color in the progeny (Hongjie Feng et al., 2015).

Analysis showed that the qLC-7-1 locus (chromosome 7, A genome) exerts a multiplicative effect, positively influencing fiber color intensity while maintaining fiber quality.

The presence of multiple alleles indicates the polygenic nature of traits associated with fiber quality and color. The combined use of the BNL1604 and NAU1043 markers provides reliable genetic identification and selection.

The results obtained are consistent with the findings of Feng et al. (2015) and Wang et al. (2024), confirming the negative correlation between color intensity and fiber strength, while also demonstrating the possibility of improving both traits through proper selection of parental material.

CONCLUSION

The study revealed a high level of genetic polymorphism for fiber quality and color traits in F_4 cotton hybrid combinations. The use of SSR markers **BNL1604** and **NAU1043** allowed the identification of allele combinations associated with improved fiber properties. The **135 bp allele** (BNL1604) and the **qLC-7-1 locus** (NAU1043) had a significant influence on fiber quality and color, respectively.

The F_4 hybrids L-001 × 010444 and Bukhara-6 × 02408 are recommended for further breeding work as donors of high-quality fiber genes. Marker-assisted identification demonstrated the effectiveness of SSR technologies in accelerating selection in cotton breeding programs.

The results of this study provide both scientific and practical value for programs aimed at developing environmentally friendly colored cotton varieties with improved fiber properties.

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