2024年第7期总8期

棉花遗传育种专题

本期导读

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政策法规

1．HudsonAlpha researchers create valuable genomic tools for the cotton industry(HudsonAlpha生物技术研究所基因组测序中心研究人员为棉花产业创造了有价值的基因组工具)

简介：We live in an ever-changing and growing world. Changing climates, emerging pests, and other environmental stressors put pressure on the cash crops that feed and fuel the world. As we race to meet the growing demand for sustainable and high-quality food and fiber crops, genomics is emerging as a powerful tool in the fight. By understanding plants' genetic codes, researchers and breeders can develop crops with increased yields, improved resistance to pests and diseases, and greater adaptability to environmental challenges. Genome-informed breeding primarily benefits crops with existing high-quality genomic resources, like rice and wheat. However, crops with less mature genomic resources must continue to rely on traditional breeding methods, which sometimes suffer due to a lack of genomic diversity within the breeding populations. Cotton, a vital cash crop worldwide, lacks robust genomic resources. The cotton industry is big business, with a global economic impact of $600 billion and providing jobs for more than 250 million people. Successful cotton production relies on cotton varieties with desirable characteristics like high yield, good fiber quality, pest and disease resistance, and drought tolerance. "Cotton breeders have improved fiber yield and quality over the years using traditional breeding methods," says Jeremy Schmutz, co-director of the HudsonAlpha Genome Sequencing Center, who has been working on cotton genomics for over a decade. "Achieving additional improvements may be difficult for them due to the lack of genetic variation across modern domesticated cotton. Creating new genomic tools for the industry will help take cotton improvements to the next level." Scientists at the HudsonAlpha Institute for Biotechnology Genome Sequencing Center (GSC) and other collaborators set out to create high-quality genome sequences for three important cotton varieties, providing necessary genome resources for cotton breeders. The results were recently published in Nature Plants. "Cotton research has relied heavily on one reference genome, 'TM1', a variety of cotton that is no longer widely used in breeding programs," says Avinash Sreedasyam, PhD, first author of the manuscript. "In order for molecular breeding to benefit the cotton industry, many, varied genomes must exist to represent the diversity of cotton varieties. This study generated high-quality reference genomes for three modern upland cotton cultivars and updated the 'TM-1' cotton genetic standard reference." Initial analysis of the new reference genomes produced important information about fiber quality. The highly accurate and complete genome assemblies were used to identify genetic material from Pima cotton (known for superior fiber quality) within modern cotton varieties. Small segments of each genome were compared to both Pima and the reference cotton genome. Segments that matched Pima more closely than the reference cotton were classified as potential introgressions, suggesting Pima DNA had been incorporated into the modern cotton's genetic makeup. Knowledge of these Pima introgressions will help breeders to efficiently select progeny with these fiber-quality linked genetic markers in their breeding programs. "Leveraging relatively inexpensive low-pass sequencing alongside these genomes empowers breeders to select progeny rapidly," says Sreedasyam. "This will not only save time but also reduce costs associated with traditional fiber phenotyping, a laborious process usually requiring hundreds to thousands of samples per breeding cycle." These findings highlight the significance of using detailed genome assemblies to uncover genetic variations that can improve cotton breeding programs. The more these new, high-quality genomes are used for comparative studies, the more information about economically important cotton traits will emerge. The genomic resources described in this study represent a valuable addition to the cotton breeding toolkit and will reap benefits for years to come. Collaborators on this project include Don C. Jones, Cotton Incorporated, NC; Peng W. Chee, University of Georgia, Tifton, GA; Warwick N. Stiller, CSIRO, Cotton Research Unit, Australia; and Fred Bourland, University of Arkansas, Keiser, AR.This work is supported by Cotton Incorporated (Award 18-753) and the intramural research program of the US Department of Agriculture, National Institute of Food and Agriculture Foundational and Applied Science Program Award 2022-67013-36899. The findings, conclusions, or recommendations expressed here have not been formally disseminated by the US Department of Agriculture and should not be construed to represent any agency determination or policy.

来源：HUDSONALPHA INSTITUTE FOR BIOTECHNOLOGY

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学术文献

1. Pyramiding effects of favorable haplotypes of loci on major fiber yield and quality traits in Upland Cotton (Gossypium hirsutum L.)(陆地棉主要纤维产量和品质性状的有利单倍型聚合效应)

简介：Enhancing both fiber yield and quality is crucial in cotton breeding programs. Typically, a negative correlation exists between fiber yield and quality, and the strategy to decouple this relationship remains obscure. In this study, we generated three multi-parent intercross populations, encompassing 2,852 F2:3 lines, which represent fiber yield potential (FY), fiber quality potential (FQ), and a combination of both potentials (FYQ), derived from sixteen diverse Upland cotton cultivars. We utilized twenty-four kompetitive allele specific PCR (KASP) molecular markers, based on previously identified loci associated with fiber quality (FL5/FS1, FL3/FS2, and FL2) and fiber yield (BW1, BW2, and LP1), for genotyping purposes. The pyramiding of favorable haplotypes from three fiber quality-associated loci resulted in an increase exceeding 10 % in fiber length (FL5+FL3+FL2+LP1) and in an increase exceeding 17 % in fiber strength (FL5+FL3+FL2). Notably, our findings suggest that the combination of FL3+FL2+BW1+LP1 alleles has the potential to disrupt the negative correlation between fiber yield and quality. Lines harboring these alleles simultaneously exhibited an elevated lint percentage (LP), as well as enhanced fiber length and quality. This study offers robust molecular markers for the efficient selection of superior progeny with desired phenotypes in the early stages of cotton breeding. Furthermore, the allelic combination that concurrently improves fiber quality and yield sheds light on for further exploration the molecular basis of breaking unfavorable linkages in crop breeding.

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2. Transcriptome analysis reveals potential of down-regulated genes in cotton fiber improvement(转录组分析揭示下调基因在棉花纤维改良中的潜力)

简介：Cotton fiber serves as the predominant source material for operations within the textile industry, and enhancements in fiber quality are likely to yield substantial economic benefits. Nonetheless, the efforts of the cotton genomics community have been predominantly concentrated on up-regulated genes that transcriptionally contribute to fiber quality, revealing a limited pool of genetic resources for the application of gene knock-out technology. To expand the genetic resources available for knock-out strategies in cotton molecular breeding, we assembled two accessions characterized by divergent fiber lengths for comparative transcriptome analysis. Transcriptional abundance analysis identified 1604 up-regulated and 1059 down-regulated genes during the course of fiber development. We further identified the gene regulatory network of the down-regulated genes through weighted gene co-expression network analysis (WGCNA) and selected 17 genes as the final downregulated gene pannel by simulating genomic selection based on a previous cotton cohort. An array of these genes had transcription regulation enriched to sugar metabolism. Our preliminary experiments, where GhUXS5 was overexpressed in Arabidopsis thaliana, significantly inhibited both trichome formation and root elongation. This suggests it may also inhibit cotton fiber elongation. Our approach provided new genetic resources for discovering fiber quality related genes. GhUXS5 and the other 16 down-regulated genes could be used as the potential target genes for genetic engineering of cotton fiber improvement induced by gene knock-out technology.

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3. Insights into genetic diversity and functional significance of the bHLH genes in cotton fiber development(bHLH基因在棉花纤维发育中的遗传多样性及其功能意义)

简介： This study investigated the identification and distribution of single nucleotide polymorphisms (SNPs) within basic Helix-Loop-Helix (bHLH) transcription factors (TFs) across diverse cotton (Gossypium hirsutum) accessions, including wild, landrace, and improved varieties. Comprehensive genomic analyses revealed substantial SNP diversity in wild cotton, distinct from that observed in landraces and improved accessions. Comparative analysis showed notable variations in SNP abundance and impact levels across different accessions, with missense SNPs being prevalent. Nucleotide substitution patterns highlighted the dominance of G>A and A>G substitutions. Population genetic analyses unveiled significant genetic diversity within wild accessions and distinct clustering between wild and improved accessions. Temporal expression profiling of bHLH genes during cotton fiber development demonstrated dynamic expression patterns, emphasizing their roles in fiber initiation, elongation, and cellulose biosynthesis. Moreover, association analysis identified SNPs significantly associated within bHLH genes, particularly GhbHLH149, with fiber quality traits, indicating their potential functional significance. Population genetic analyses further revealed evidence of positive selection on GhbHLH149 during cotton improvement. This study provides a comprehensive understanding of SNP diversity, gene expression dynamics, and genetic selection in bHLH genes, offering valuable insights for future cotton breeding efforts aimed at improving fiber quality traits in cotton.

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4. GhCKX1 is an important genetic target for improving fiber strength in cotton(GhCKX1是提高棉花纤维强力的重要遗传靶标)

简介：Cotton is a widely grown crop to produce natural textile fiber, and improving the fiber strength (FS) is one of the main targets that cotton breeders focus on. The long-term natural selection and domestication have produced abundant germplasm resources of Gossypium hirsutum, and exploring genetic underpinnings underlying these FS innovation in elite collections is crucial. PCAMP is proposed as a most optimized NGS based bulked segregant analysis (NGS-BSA) for the high-resolution identification of markers linked to specific genomic regions through pairwise comparing multiple BSA bulks. In this study, we firstly applied PCAMP to resolved the FS genetic architecture in G. hirsutum cv. CCRI127. As an extension for PCAMP approach, graded bulks were constructed using F-2 segregants with the FS phenotype revalidated by F-2:3 lines, and then, a major QTL was eventually narrowed to 2.47 Mb from 8.14 Mb generated by traditional BSA approaches. Subsequently, through a saturated genetic map constructed in this locus, an novel FS gene, GhCKX1, predicted to produce a cytokinin (CTK) oxidase was isolated. It can negatively modulate the CTK signaling circuit via irreversible degradation of CTKs, resulting in an additional cell wall thickness to xylem tracheary elements in transgenic lines of Arabidopsis thaliana. Thus, the GhCKX1 gene will be an potential genetic target, with which, we can genetically manipulate the secondary wall synthesis in unicellular cotton fibers.

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5. Deepening genomic sequences of 1081 Gossypium hirsutum accessions reveals novel SNPs and haplotypes relevant for practical breeding utility(深化1081份陆地棉种质的基因组序列发现与育种实用性相关的新SNP和单倍型)

简介：Fiber quality is a major breeding goal in cotton, but phenotypically direct selection is often hindered. In this study, we identified fiber quality and yield related loci using GWAS based on 2.97 million SNPs obtained from 10.65x resequencing data of 1081 accessions. The results showed that 585 novel fiber loci, including two novel stable SNP peaks associated with fiber length on chromosomes At12 and Dt05 and one novel genome regions linked with fiber strength on chromosome Dt12 were identified. Furthermore, by means of gene expression analysis, GhM_A12G0090, GhM_D05G1692, GhM_D12G3135 were identified and GhM_D11G2208 function was identified in Arabidopsis. Additionally, 14 consistent and stable superior haplotypes were identified, and 25 accessions were detected as possessing these 14 superior haplotype in breeding. This study providing fundamental insight relevant to identification of genes associated with fiber quality and yield will enhance future efforts toward improvement of upland cotton.

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6. A cotton mitochondrial alternative electron transporter, GhD2HGDH,

induces early flowering by modulating GA and photoperiodic pathways(棉花线粒体替代电子转运体GhD2HGDH通过调控GA和光周期途径诱导早花)

简介： D-2-hydroxyglutarate dehydrogenase (D2HGDH) is a mitochondrial enzyme containing flavin adenine dinucleotide FAD, existing as a dimer, and it facilitates the specific oxidation of D-2HG to 2-oxoglutarate (2-OG), which is a key intermediate in the tricarboxylic acid (TCA) cycle. A Genome-wide expression analysis (GWEA) has indicated an association between GhD2HGDH and flowering time. To further explore the role of GhD2HGDH, we performed a comprehensive investigation encompassing phenotyping, physiology, metabolomics, and transcriptomics in Arabidopsis thaliana plants overexpressing GhD2HGDH. Transcriptomic and qRT-PCR data exhibited heightened expression of GhD2HGDH in upland cotton flowers. Additionally, early-maturing cotton exhibited higher expression of GhD2HGDH across all tissues than delayed-maturing cotton. Subcellular localization confirmed its presence in the mitochondria. Overexpression of GhD2HGDH in Arabidopsis resulted in early flowering. Using virus-induced gene silencing (VIGS), we investigated the impact of GhD2HGDH on flowering in both early- and delayed-maturing cotton plants. Manipulation of GhD2HGDH expression levels led to changes in photosynthetic pigment and gas exchange attributes. GhD2HGDH responded to gibberellin (GA3) hormone treatment, influencing the expression of GA biosynthesis genes and repressing DELLA genes. Protein interaction studies, including yeast two-hybrid, luciferase complementation (LUC), and GST pull-down assays, confirmed the interaction between GhD2HGDH and GhSOX (Sulfite oxidase). The metabolomics analysis demonstrated GhD2HGDH's modulation of the TCA cycle through alterations in various metabolite levels. Transcriptome data revealed that GhD2HGDH overexpression triggers early flowering by modulating the GA3 and photoperiodic pathways of the flowering core factor genes. Taken together, GhD2HGDH positively regulates the network of genes associated with early flowering pathways.

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7．Evaluating Water Stress Adaptation in Cotton: Multivariate Analysis in F6-F7 Generations for Yield, Fibre Quality and Variety Selection(棉花水分胁迫适应性评价：F6-F7代产量、纤维品质及品种选择的多元分析)

简介： The impact of drought stress on productivity of cotton (Gossypium hirsutum L.) is a well-known challenge in agricultural production, and concurrently, the question of whether using the same or different selection criteria in well-watered and water-deficit conditions to select drought-tolerant cotton varieties remains unclear. This study aimed to comprehensively assess the single plant progeny lines within the F6 and F7 generations for determine response to DS and select the tolerant lines within the F7 generation. Single plant progeny rows were established, with the deficit water condition comprising 108 and 136 single plants for the F6 and F7 generations, respectively, and the WW condition consisting of 120 and 156 single plants for the F6 and F7 generations, respectively, with four blocks in Augmented experimental design. These progeny rows have length of 12 m, incorporate five control varieties (Karizma, Gloria, Carla, Candia and Claudia) to facilitate a comprehensive comparison. The study findings showed that fibre length, boll number and lint percentage were identified as the most crucial selection criteria under both WW and deficit irrigation conditions through principal component analysis. These indicators are highly beneficial for evaluating cotton's drought tolerance and screening potential drought-tolerant lines under both irrigation scenarios. According to the decision tree analysis, FL and BN have emerged as the most critical decision-making parameter in both irrigation conditions. Furthermore, the analysis revealed that each selection criterion has different impact in the comprehensive selection process. Also, as a result of all statistical analysis results and breeder observations, a total of 10 cotton lines were selected in the F7 generation. These selected genotypes hold promise for future cotton breeding programmes, providing an avenue to enhance drought tolerance and elevate cotton yield and productivity.

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8．Grafting based DNA methylation alteration of snoRNAs in upland cotton (Gossypium L.)(基于嫁接的陆地棉snoRNAs DNA甲基化变化)

简介: The effects of grafting in response to various biotic and abiotic stressors have been studied, however, the methylation status of small nucleolar RNA (snoRNA) genes in heterograft and homograft cotton needs investigation. This study was undertaken to determine grafting effects on DNA methylation of snoRNA genes in Upland cotton. Rootstocks used were Pima 3-79 (Gossypium barbadense acc. Pima 3-79) and Texas Marker-1 (G. hirsutum acc. TM-1), representing two different species with different fiber properties, adaptations, and morphologies. The methylation ratio and differently methylated cytosines (DMCs) of 10935 snoRNA genes in mature seeds of heterograft and homograft cotton samples were studied using the whole genome bisulfite sequencing method. Seedling vigor and seed weight were studied to investigate phenotype alterations that might be associated with altered methylation levels among grafts. Statistically significant DMC differences among gene elements of snoRNA genes and between homograft and heterograft cotton samples were identified in the absence of DNA sequence alterations. DNA methylation alterations of snoRNA genes associated with seedling vigor and 100 seed weight. The majority of snoRNA genes showed higher numbers of (m)CG + (m)CHG-DMCs with increased methylation levels in heterograft, while there were higher numbers of (m)CG + (m)CHG-DMCs with decreased methylation levels in homograft. Since snoRNAs regulate essential genes for plant growth and development and plant adaptation to different habitats or extreme environments, their altered methylation levels should be related with plant physiology.

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9．Genetic parameters and selection index in intraspecific cotton lines in a Brazilian semi-arid region(巴西半干旱区棉花种内品系的遗传参数和选择指数)

简介: The climatic and edaphic conditions of the semi-arid Brazilian Northeast region limit the production of several annual crops, including cotton. The aim of this study was to estimate the genetic parameters and select water stress tolerant cotton lines based on yield and fiber quality traits. Twenty cotton lines were evaluated in Alagoinha-PB over two years under rainfed conditions. Individual and joint analysis of variance was performed on the data. Genetic parameters were determined, and lines were selected using

the selection index. SA 2019-165 were selected for further tests in the region for recommendation of new cultivars. These lines can form new blocks of crosses, together with the BRS 286 check cultivar, as they have the best mean yield and fiber quality values, with the expectation of significant genetic gains.
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10．GhFAD3-4 Promotes Fiber Cell Elongation and Cell Wall Thickness by Increasing PI and IP3 Accumulation in Cotton(GhFAD3-4通过增加PI和IP3积累促进棉花纤维细胞伸长和细胞壁厚度)

简介：The omega-3 fatty acid desaturase enzyme gene FAD3 is responsible for converting linoleic acid to linolenic acid in plant fatty acid synthesis. Despite limited knowledge of its role in cotton growth, our study focused on GhFAD3-4, a gene within the FAD3 family, which was found to promote fiber elongation and cell wall thickness in cotton. GhFAD3-4 was predominantly expressed in elongating fibers, and its suppression led to shorter fibers with reduced cell wall thickness and phosphoinositide (PI) and inositol triphosphate (IP3) levels. Transcriptome analysis of GhFAD3-4 knock-out mutants revealed significant impacts on genes involved in the phosphoinositol signaling pathway. Experimental evidence demonstrated that GhFAD3-4 positively regulated the expression of the GhBoGH3B and GhPIS genes, influencing cotton fiber development through the inositol signaling pathway. The application of PI and IP6 externally increased fiber length in GhFAD3-4 knock-out plants, while inhibiting PI led to a reduced fiber length in GhFAD3-4 overexpressing plants. These findings suggest that GhFAD3-4 plays a crucial role in enhancing fiber development by promoting PI and IP3 biosynthesis, offering the potential for breeding cotton varieties with superior fiber quality.
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11．Genome resources for three modern cotton lines guide future breeding efforts(三个现代棉花品系的基因组资源指导今后的育种工作)

简介：Cotton (Gossypium hirsutum L.) is the key renewable fibre crop worldwide, yet its yield and fibre quality show high variability due to genotype-specific traits and complex interactions among cultivars, management practices and environmental factors. Modern breeding practices may limit future yield gains due to a narrow founding gene pool. Precision breeding and biotechnological approaches offer potential solutions, contingent on accurate cultivar-specific data. Here we address this need by generating high-quality reference genomes for three modern cotton cultivars (‘UGA230’, ‘UA48’ and ‘CSX8308’) and updating the ‘TM-1’ cotton genetic standard reference. Despite hypothesized genetic uniformity, considerable sequence and structural variation was observed among the four genomes, which overlap with ancient and ongoing genomic introgressions from ‘Pima’ cotton, gene regulatory mechanisms and phenotypic trait divergence. Differentially expressed genes across fibre development correlate with fibre production, potentially contributing to the distinctive fibre quality traits observed in modern cotton cultivars. These genomes and comparative analyses provide a valuable foundation for future genetic endeavours to enhance global cotton yield and sustainability.

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12. Application of chitosan in the cultivation of colored fiber cotton 'BRS Jade' under water restriction(壳聚糖在彩色纤维棉“BRS玉”水分限制栽培中的应用)

简介：In the semi-arid region of Northeastern Brazil, temporal and spatial variations in rainfall are common, resulting in water limitations that significantly impact production, especially of cotton. In this context, chitosan may serve as a strategy to minimize the effects of water deficits by enhancing water and nutrient absorption. This study aimed to evaluate the effectiveness of different concentrations of chitosan as a mitigator of water restriction in the cultivation of the naturally colored fiber cotton variety 'BRS Jade'. The plants were cultivated in drainage lysimeters under greenhouse conditions. A completely randomized design was implemented using a 2 x 4 factorial arrangement, which included two levels of irrigation (100% and 50% of the water requirement of the crop) and four chitosan concentrations (0.0, 0.25, 0.50, and 0.75 g L -1), with three replications and one plant per plot. Water restriction at 50% of the required amount reduced the relative water content, the synthesis of photosynthetic pigments, and seed cotton weight. However, foliar application of chitosan at concentrations between 0.25 and 0.50 g L -1 alleviated the detrimental effects of water restriction on the chlorophyll b content; carotenoid content; stem diameter; leaf area; 100 -seed weight; average boll weight; seed cotton weight; total boll dry biomass; and dry biomass of stems, leaves, and shoots of the 'BRS Jade' colored fiber cotton plant.

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13. Suppressing a mitochondrial calcium uniporter activates the calcium signaling pathway and promotes cell elongation in cotton(抑制线粒体钙单转运蛋白激活钙信号通路促进棉花细胞伸长)

简介：Mitochondrial calcium uniporter (MCU) is a conserved calcium ion (Ca 2+) transporter in the mitochondrial inner membrane of eukaryotic cells. How MCU proteins regulate Ca 2+ flow and modulate plant cell development remain largely unclear. Here, we identified the gene GhMCU4 encoding a MCU protein that negatively regulates plant development and fiber elongation in cotton (Gossypium hirsutum). GhMCU4 expressed constitutively in various tissues with the higher transcripts in elongating fiber cells. Knockdown of GhMCU4 in cotton significantly elevated the plant height and root length. The calcium signaling pathway was significantly activated and calcium sensor genes, including Ca 2+ dependent modulator of interactor of constitutively active ROP (GhCMI1) , calmodulin like protein (GhCML46) , calciumdependent protein kinases (GhCPKs), calcineurin B-like protein (GhCBLs), and CBL-interacting protein kinases (GhCIPKs), were dramatically upregulated in GhMCU4 - silenced plants. Metabolic processes were preferentially enriched, and genes related to regulation of transcription were upregulated in GhMCU4 - si- lenced plants. The contents of Ca 2+ and H 2 O 2 were significantly increased in roots and leaves of GhMCU4 - silenced plants. Fiber length and Ca 2+ and H 2 O 2 contents in fibers were significantly increased in GhMCU4 - silenced plants. This study indicated that GhMCU4 plays a negative role in regulating cell elongation in cotton, thus expanding understanding in the role of MCU proteins in plant growth and development.

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14．'Pink cotton candy'-A new dye-free cotton(新型无染料棉“粉红棉花糖”)

简介：White cotton is the dominant natural fibre, accounting for a $USD 36 billion share of the $USD 1.5 trillion global textile industry and, for many decades, has been dyed post-production. Modern cotton ginning and spinning processes require longer and stronger fibres, favouring superior white cotton varieties, which are more amenable to post-harvest dyeing. However, large quantities of synthetic dyes from textile dyeing released into the environment/waterways are harming the health of humans and other organisms. Eco-friendly alternatives are urgently needed to reduce pollution and save water; coloured dye-free cotton could be a solution. While naturally coloured cotton has been known for more than 5000 years and occurs in all four species of cultivated cotton, that is Gossypium (G.) hirsutum, G. barbadense, G. herbaceum and G. arboretum, these coloured varieties generally have low yield, poor fibre quality and variable and unstable colours. Although conventional breeding has improved the properties of some coloured cotton, quality and yield remain low compared with white cotton and colour range is limited.Betalains are tyrosine-derived pigments found naturally in the order Caryophyllales of flowering plants, and fungi and bacteria. Betalains comprise two classes of compounds, that is yellow-orange betaxanthins and red-violet betacyanins. These compounds are synthesized through a series of enzymatic steps including hydroxylases, dioxygenases and glucosyl transferases that produce visible colours. As the Malvaceae typically do not produce betalains, we embarked on genetically engineering the betalain pathway in G. hirsutum, the world's largest plant-based fibre commodity.We designed constructs that included the coding sequences (CDS) of BvDODA1 (Beta vulgaris, GeneBank ID HQ656027.1), BvCYP76AD1 (HQ656023.1) and MjcDOPA5GT (Mirabilis jalapa, AB182643.1; Polturak et al., 2017; Timoneda et al., 2019). The CDSs were optimized for Arabidopsis and synthesized by GeneArt . Constructs (pAGM4723 vector) were assembled via Golden Gate cloning with a 2 × 35 S-driven kanamycin resistance gene. The betalain genes were driven by either a 2 × 35 S constitutive promoter or a ltp3/8K12 (LTP) mid-late-stage cotton fibre-specific promoter. Transgenic plants were generated through tissue culture transformation (Murray et al., 1999). Coker 315-11 was used as the recipient of transformation by infection with Agrobacterium tumefaciens strain AGL1 containing different constructs. Transgenic and wild-type (controls) cotton plants were grown in a greenhouse at 28°C/20°C (day/night) with natural light.Despite strong betalain accumulation during fibre development in the fibre-specific lines, the colour faded to light brown/pink in the final days of boll maturation, when the bolls dried and opened (transgenic lines: 5560 DPA vs wild type: 55 DPA). This suggests that vacuole-located betalain was degraded during the final maturation stage. Cotton bolls were collected at around 10, 15, 20, 25, 30, 40, 50 and 60 DPA, and boll coats were either cut open or removed entirely followed by 48 h of freeze-drying, and the pink colour was retained.Mature fibre or freeze-dried immature fibre (>46 DPA) from controls and five independent T0 plants with the fibre-specific betalain expression were measured by Cottonscope for fibre quality. The results suggested the transgenic lines have the potential to present similar maturity ratio and fineness as the wild type (Line 44). The seed numbers and fibre yield were less in the T0 compared with wild type, which is common to see in T0 generation. Subsequent generations of transgenic plants could potentially retain wild-type-like yield and fibre quality alongside strong pigment accumulation, noting that Coker 315 can be introgressed into modern elite varieties for yield and quality. New colours may be generated by crossing the betalain lines with existing naturally coloured genotypes (Ke et al., 2022).In conclusion, we present a novel example of introducing the exogenous multi-gene betalain pathway to generate a plant-made pink cotton fibre which remains pink until the very late stages of fibre development. Using the betalain pathway is advantageous because the pigment is stable over a wide pH range (Jackman and Smith, 1996), potentially improving colour stability and consistency. Future research could investigate reducing pigment degradation (potentially via cross-linking) and introducing new colours.

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