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棉花遗传育种专题

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前沿资讯

1. G-Star Raw Explores the Potential of Greenhouse-Grown Cotton(G-Star Raw探索了温室种植棉花的潜力)

简 介： G-Star Raw unveiled the “Homegrown Denim” project on Wednesday, the first greenhouse-grown cotton initiative in collaboration with Wageningen University & Research and Dutch Cotton. It explores how cotton grown in greenhouses can minimize the impact of growing the resource-intensive fiber worldwide.With Homegrown Denim, the Amsterdam-based denim brand aims to eliminate many of the challenges with growing cotton. Though it is an easy crop to grow, cotton is needy. In addition to using lot of land, G-Star reports that it requires up to 10,000 liters of water to yield just one kilo of fiber. Plus, considering that land must be situated in a warm climate, sourcing cotton fields marks the beginning of a long, complex supply chain—one that is increasingly asked to be more responsible and traceable.A six-month experiment studied a small group of greenhouse-grown cotton at a research facility in Bleiswijk, the Netherlands. The research examined quality, yield and fiber properties, comparing its environmental footprint to traditional methods. Strategies like precision irrigation and renewable energy were also explored to reduce impact, and economic viability and market potential were analyzed as well.This research discovered that growing cotton in a controlled and protected environment boosts crop productivity, quality and sustainability while lowering the risks associated with outdoor growing. The top benefits found included increased yield, with plants growing up to four meters tall and producing between five to 23 times more cotton, with the controlled environment enabling cotton harvesting for longer than usual. Greenhouse-grown cotton was cleaner and whiter with minimal contamination as it was shielded from weather damage. The enclosed environment naturally deters pests and diseases, eliminating the need for synthetic pesticides, the research found.Greenhouse systems can also save up to 95 percent of water per kilo of cotton by using recycled rainwater for irrigation, while potted cultivation minimizes soil erosion, thus preserving fertility.“G-Star’s curiosity and drive for innovation led us to our partnership with Wageningen University & Research to study the feasibility of growing cotton in a greenhouse,” Rebecka Sancho, head of sustainability at G-Star, said. “This groundbreaking experiment could revolutionize cotton production by severely decreasing water consumption and lead use, eliminating the use of chemical pesticides, improving the quality of cotton and drastically shortening the supply chain.”With the greenhouse-grown cotton in place, G-Star partnered with local suppliers—including Spinning Jenny, Liberty Threads and Blueprint Amsterdam—to create the first-ever 100 percent locally sourced and manufactured pair of jeans. Every aspect of cotton processing and production was completed in the Netherlands. G-Star also used electric vehicles between suppliers to create the shortest supply chain possible.At this stage, no products will be available for purchase using the greenhouse-grown cotton. Rather, the experiment wanted to investigate the feasibility and potential of growing cotton in a greenhouse. Now that the potential has been proven, a second phase will explore scaling the innovation. G-Star and Wageningen University & Research have partnered with Inno Growers to transition the research efforts into “practical cultivation” and scale-up production. The goal is to enhance cotton yield per square meter, increasing from 1.2kg to up to 2.5kg. “This research allows us to rethink the way the entire industry is set up,” Willeke Hendriks, chief product officer of G-Star, said. “And that is exactly what we need to do to find new and effective ways to

improve our impact together. Therefore, getting involved in this project was an absolute must for G-Star, to support solutions for the future of denim."

来源：SOURCING JOURNAL

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2．新疆搭建棉花抗逆生物育种平台

简介：记者从自治区科学技术厅获悉：新疆将投资1500万元搭建棉花抗逆生物育种平台，提升新疆棉花重大品种的抗逆性，以应对盐碱、高温、冷害等逆境胁迫。植物的抗逆性是指植物具有的抵抗冷害、干旱、盐碱、病虫害等不利环境的特性。植物的优良抗逆性状，在自然条件下很难转移到其他种类的植物体内，需借助基因工程改良等生物育种技术将优良基因导入其他作物中，进而提升抗逆性。记者了解到，目前新疆棉花的主栽品种以及即将大面积推广的品种综合性能优良，具备了高产、优质等特性，但在抗逆性等方面有待进一步提升。在极端天气气候事件频发多发背景下，提升新疆棉花品种的抗逆性显得更为迫切。为提升棉花品种整体性能，新疆农业科学院经济作物研究所经过反复论证，策划设计"棉花品种重大农艺性状解析与分子设计育种"项目。该项目为新疆今年发布的5个"揭榜挂帅"项目之一，项目涉及金额1500万元，实施周期为3年。中国农业科学院西部农业研究中心为该项目揭榜方。未来双方将在人才培养、平台建设、生物育种创新体系建立等方面加强合作，为新疆棉花生物育种创新奠定基础。作为项目主要参与者，中国农业科学院西部农业研究中心研究员葛晓阳说，该项目将以新疆棉花重大品种源棉8号或其他重大棉花品种为载体，挖掘其他耐逆植物中的耐高温、耐冷或者耐盐碱等优异基因，并通过基因工程改良技术导入棉花，提升棉花品种的整体性能，以有效应对各种极端逆境。依托该项目，中国农业科学院西部农业研究中心、新疆农科院经济作物研究所等单位将共同搭建新疆棉花抗逆生物育种平台。该平台集生物育种技术、基因挖掘、芯片开发、材料创制等实验技术和经验方法于一体，将棉花育种当中从优异基因挖掘到优异材料创制的周期缩短至1—2年，有效提升新疆棉花生物育种效率。葛晓阳说，该平台将研发出通用的生物技术体系等，源于棉花但不局限于棉花，还可为提高其他作物抗逆性提供参考。

来源：新疆日报

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3．Ginning up a Market for U.S. Cotton in Bangladesh(美国棉花在孟加拉国开拓市场)

简介：For almost 50 years, Bangladesh required U.S. cotton be fumigated because of concerns about the boll weevil. Collaboration between USDA agencies and the Bangladesh Ministry of Agriculture resulted in amended import requirements, exempting the United States from the list of countries required to fumigate cotton upon arrival.This is a significant trade win for American cotton as Bangladesh is the fifth-largest export market for U.S. cotton, with export values exceeding $339 million in 2023. This decision gins up a new chapter for U.S. cotton growers to expand their market access to Bangladesh. As one of the world's top import markets for cotton, Bangladesh is a growth market with great potential for American cotton for years to come. FAS worked diligently to improve perceptions of U.S. cotton and provide evidence that the boll weevil is not a serious threat to imports. Momentum spun up when FAS provided significant technical evidence on the near total eradication of

the boll weevil back in 2021 to ease Bangladesh's concerns over the pest. Following that, FAS spent two years meeting with Bangladesh officials, including a High-Level Economic Consultation and an Agriculture and Ease of Business meeting. The Cotton Council International (CCI) continued bilateral efforts, bringing a Bangladesh delegation to visit U.S. cotton facilities and farmers in November 2022. The delegation witnessed the effectiveness of the Boll Weevil Eradication Program. Also, during the visit, the delegation learned about modern cotton harvesting and standardized ginning techniques while touring cotton fields, gins, and warehouses in Mississippi, Tennessee, and Texas. This is an example of American farmers showcasing high quality agricultural products to an overseas market a crucial element to growing American exports. U.S. cotton farmers not only utilize the Animal and Plant Health Inspection Service (APHIS) Boll Weevil Eradication Program to eliminate the pest, but the program also helps thousands of U.S. cotton growers become more competitive. Additionally, the CCI receives FAS Market Access Program funds to help grow overseas markets for American cotton growers.This success is a testament to the continued efforts and nearly 22 years of engagement among the U.S. cotton industry, FAS, APHIS, and Agricultural Research Service officials, and the Government of Bangladesh to advocate for fair and open trade practices that benefit American farmers and businesses.

来源：U.S.DEPARTMENT OF AGRICULTURE

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学术文献

1．Genomic insights into local adaptation of upland cotton in China and Pakistan(中国和巴基斯坦陆地棉地方适应性的基因组学研究)

简介：Key messageDifferent kinship and resistance to cotton leaf curl disease (CLCuD) and heat were found between upland cotton cultivars from China and Pakistan. 175 SNPs and 82 InDels loci related to yield, fiber quality, CLCuD, and heat resistance were identified. Elite alleles found in Pakistani accessions aided local adaptation to climatic condition of two countries.AbstractAdaptation of upland cotton (Gossypium hirsutum) beyond its center of origin is expected to be driven by tailoring of the genome and genes to enhance yield and quality in new ecological niches. Here, resequencing of 456 upland cotton accessions revealed two distinct kinships according to the associated country. Fiber quality and lint percentage were consistent across kinships, but resistance to cotton leaf curl disease (CLCuD) and heat was distinctly exhibited by accessions from Pakistan, illustrating highly local adaption. A total of 175 SNP and 82 InDel loci related to yield, fiber quality, CLCuD and heat resistance were identified; among them, only two overlapped between Pakistani and Chinese accessions underscoring the divergent domestication and improvement targets in each country. Loci associated with resistance alleles to leaf curl disease and high temperature were largely found in Pakistani accessions to counter these stresses prevalent in Pakistan. These results revealed that breeding activities led to the accumulation of unique alleles and helped upland cotton become adapted to the respective climatic conditions, which will contribute to elucidating the genetic mechanisms that underlie resilience traits and help develop climate-resilient cotton cultivars for use worldwide.

来源：THEORETICAL AND APPLIED GENETICS

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2．Gland-specific GhVQ22 negatively regulates gland size and affects secondary metabolic accumulation in cotton(棉花腺体特异性GhVQ22负调控腺体大小并影响次生代谢积累)

简介：Cotton (Gossypium spp.) has evolved pigment glands (PGs) that accumulate toxic terpenoids, such as gossypol, which serve as a defence mechanism against pests (Gadelha et al., 2014). Laboratory experiments and field trials have confirmed that PGs are essential for tolerance to chewing pests in cotton (Benedict et al., 1977; Mao et al., 2007). Altering the ability of PGs to synthesize and accumulate secondary metabolites is a promising strategy for pest resistance. The discovery of PG development-related genes, such as Gl2/Gl3, CGF1 and CGF2, has preliminarily revealed the genetic mechanisms involved in PG biogenesis and the gossypol synthesis pathway (Janga et al., 2019; Ma et al., 2016). However, few studies have focused on the regulation of PG size. Here, we isolated a PG-specific valine glutamine (VQ) gene, GhVQ22 (GH_A12G0470/GH_D12G0482), regulates PG size and affects the composition and content of secondary metabolites in PGs. GhVQ22 has potential applications in novel anti-pest strategies for cotton.Comparative transcriptome analyses between the PG tissues (PGT) and the PG-adjacent tissues (PGAT) in embryos at 18 days post-anthesis (DPA) were performed through laser-capture microdissection (Figure S1). We detected 506 differentially expressed genes (DEGs) in PGT compared with PGAT (Figure 1a). The 20 DEGs with the highest fold changes were selected for preliminary gland phenotype screening by virus-induced gene silencing (Table S1). GhVQ22-silenced plants (TRV2:GhVQ22) showed significantly enlarged PGs compared to the wild-type (WT) (Figure S2a). GhVQ22 expression was barely detected in PGAT (Figure 1b) and glandless cotton Z12YW (gl2gl2gl3gl3) (Figure 1c), β-Glucuronidase (GUS) reporter expression driven by the GhVQ22 promoter was limited to PGs in stable transgenic G. hirsutum (Figure 1d). These results confirmed that GhVQ22 expression is PG-specific.The Ghvq22 mutant exhibited significant increase in PG size across most tissues (Figure 1e), and PG diameter was approximately 2.7 times larger as compared to WT (Figure S3a). In true leaves of the Ghvq22 mutant, the PG size was significantly larger than that of the WT across the pseudo-developmental trajectory (Figure 1f, Figure S3b). In Ghvq22 mutant, mature PGs exhibited 35 sheath cell layers, whereas in WT, there were only 13 sheath cell layers (Figure 1g, Figure S3c). Similarly, the cavity diameter was correspondingly increased (Figure 1h, Figure S3c), meanwhile its PG density was half as compared to WT (Figure 1i). But the PG diameter and density were significantly reduced in gl2gl2Gl3Gl3 and Gl2Gl2gl3gl3 mutants with lower GhVQ22 expression (Figure S4bd).The total secondary metabolites extracted from Ghvq22 leaves were significantly different from the WT (Figure S5). Liquid chromatographymass spectrometry revealed that 2193 and 1879 metabolites were up-regulated and down-regulated, respectively, in Ghvq22 mutant compared with the WT (Figure 1k). The gossypol content was decrease approximately 50% as compared to WT (Figure 1j). The kaempferol and catechin content was significantly increased and decreased compared with the WT, respectively (Figure 1l,m, Figures S6 and S7). These results suggested that GhVQ22 might regulate secondary metabolite synthesis in PGs.The regulatory relationship between GhVQ22 and PG development-related genes was investigated to determine how GhVQ22 regulates PG

development. As PG development progressed with embryo development from 13 to 30 DPA, GhVQ22 expression lagged behind that of Gl2/Gl3 (Figure 1n). Gl2 and Gl3, the core factors that redundantly regulate PG development, activate target gene expression by binding to the G-box (5′-CACGTG-3′) cis-regulatory element (Lin et al., 2023). The GhVQ22 promoter contains a G-box at −234 to −229 bp (Figure S8). Through conducting an electrophoretic mobility shift assay (EMSA) (Figure 1o) and yeast one-hybrid analysis (Figure S9), we confirmed that Gl2 can bind to the GhVQ22 promoter. In addition, Gl2 could activate its transcriptional activity as indicated by a dual-luciferase reporter assay in leaves of Nicotiana benthamiana (Figure 1p). The comparative transcriptome of 18 DPA embryos revealed that 1464 and 1370 genes were up-regulated and down-regulated, respectively, in Ghvq22 (Figure S10). Gene Ontology (GO) enrichment analysis demonstrated that the up-regulated DEGs enriched in cell division, secondary metabolite and flavonoid biosynthesis (Figure 1q). The expression level of PG development-related genes, such as Gl2/Gl3, CGF1, CGF2, JUB1 and ERF105, was significantly enhanced in Ghvq22 mutant (Figures S11 and S12a). These results suggested that GhVQ22 regulation of PG development might depend on the genetic networks of Gl2/Gl3 and are involved in cell division and secondary metabolite pathway. Our hypothesis suggests that Gl2/Gl3 not only activates the expression of genes involved in PG development but also triggers the expression of the negative regulator GhVQ22 (Figure 1r). These two opposing mechanisms form a delicate balance in regulating PG development and secondary metabolite synthesis.Our study revealed that GhVQ22 as a downstream target of Gl2/Gl3, negatively regulates PG size and affects secondary metabolic accumulation. We speculate that the significant changes in PG development-related genes and gland morphogenesis might affect secondary metabolic synthesis. We also observed the stem trichome density of the Ghvq22 mutant was significantly lower than that of the WT (Figure S13b), suggesting that the PGT and PGAT might influence each other by the signal molecular communications. In general, the present results lay a foundation for further research on the regulation of PG development.

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3. Drought response modelling of leaf photosynthetic parameters in two Gossypium species(两种棉花叶片光合参数的干旱响应模拟)

简介：Cotton is well adapted to dry areas, but progressive water deficits can lead to declines in net photosynthesis (A), ultimately reducing yield. However, the exact mechanism responsible for this decline in net photosynthesis (stomatal or non-stomatal) is not fully understood under field conditions, partially due to limitations in the ability to collect critical data. To our knowledge, no other study has compared the drought responses of Pima and upland cotton using both CO2 response and chlorophyll fluorescence under field conditions. To this end, a field study was conducted to quantify the impact of progressive mild drought, as measured by midday stomatal conductance to water vapour (g(s)), on cotton leaf metabolism in Pima and upland cotton. Survey gas exchange and rapid photosynthetic CO2 response (RACiR) were conducted during flowering on the same leaf. The study observed decline in A as g(s) declined for both species. Correlation analysis indicated typical relationships with A and parameters associated with stomatal limitations such as decreased CO2 inside the leaf and at the site of

carboxylation; however, it was found that while Pima exhibited a strong relationship between maximum electron transport rate (J(max)) and electron transport rate (ETR), upland cotton did not. Furthermore, when ETR is broken down into proportions contributing to net photosynthesis and photorespiration (ETRA, ETRP, respectively), we found that a greater proportion of ETR is being shuttled to the photorespiratory pathway in upland, relative to Pima as g(s) decreases. Our results fill critical knowledge gaps that can be useful for modellers and breeders when preparing for future climate change scenarios.

来源：JOURNAL OF AGRONOMY AND CROP SCIENCE

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4. iJAZ-based approach to engineer lepidopteran pest resistance in multiple crop species(基于iJAZ的多作物鳞翅目害虫抗性工程方法)

简介：The fall armyworm (FAW) poses a significant threat to global crop production. Here we showed that overexpression of jasmonate ZIM-domain (JAZ) protein GhJAZ24 confers resistance to cotton bollworm and FAW, while also causing sterility in transgenic cotton by recruiting TOPLESS and histone deacetylase 6. We identified the NGR motif of GhJAZ24 that recognizes and binds the aminopeptidase N receptor, enabling GhJAZ24 to enter cells and disrupt histone deacetylase 3, leading to cell death. To overcome plant sterility associated with GhJAZ24 overexpression, we developed iJAZ (i, induced), an approach involving damage-induced expression and a switch from intracellular to extracellular localization of GhJAZ24. iJAZ transgenic cotton maintained fertility and showed insecticidal activity against cotton bollworm and FAW. In addition, iJAZ transgenic rice, maize and tobacco plants showed insecticidal activity against their lepidopteran pests, resulting in an iJAZ-based approach for generating alternative insecticidal proteins with distinctive mechanisms of action, thus holding immense potential for future crop engineering.

来源：NATURE PLANTS

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5. A dominant negative mutation of GhMYB25-like alters cotton fiber initiation, reducing lint and fuzz(GhMYB25类显性负性突变改变棉纤维起始，减少皮棉和绒毛)

简介：Cotton (Gossypium hirsutum) fibers, vital natural textile materials, are single-cell trichomes that differentiate from the ovule epidermis. These fibers are categorized as lint (longer fibers useful for spinning) or fuzz (shorter, less useful fibers). Currently, developing cotton varieties with high lint yield but without fuzz remains challenging due to our limited knowledge of the molecular mechanisms underlying fiber initiation. This study presents the identification and characterization of a naturally occurring dominant negative mutation GhMYB25-like_AthapT, which results in a reduced lint and fuzzless phenotype. The GhMYB25-like_AthapT protein exerts its dominant negative effect by suppressing the activity of GhMYB25-like during lint and fuzz initiation. Intriguingly, the negative effect of GhMYB25-like_AthapT could be alleviated by high expression levels of GhMYB25-like. We also uncovered the role of GhMYB25-like in regulating the expression of key genes such as

GhPDF2 (PROTODERMAL FACTOR 2), CYCD3; 1 (CYCLIN D3; 1), and PLD (Phospholipase D), establishing its significance as a pivotal transcription factor in fiber initiation. We identified other genes within this regulatory network, expanding our understanding of the determinants of fiber cell fate. These findings offer valuable insights for cotton breeding and contribute to our fundamental understanding of fiber development.A dominant negative mutation of GhMYB25-like inhibits fuzz initiation but only slightly reduces lint initiation by compensating with higher wild-type protein level during lint cell differentiation.

来源：PLANT CELL

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6. Study of Ethiopian genetically modified and organic cotton fiber quality compared with conventional cotton(埃塞俄比亚转基因有机棉与常规棉纤维品质比较研究)

简介： In Ethiopia, investigation of fiber quality and commercialization is limited to conventional cotton and there is no fiber property analysis for Organic and genetically modified cotton. The objective of this study is to characterize organic and genetically modified cotton and compare them to conventional cotton varieties. Conventional varieties with chemically treated and untreated seed cotton; organic and genetically modified cotton samples were collected using random sampling methods from different regions of Ethiopia. Cotton fiber qualities are tested on High-Volume Instruments based on the ASTM standard. The tested data is experimentally designed and analyzed using SPSS software version 22. The model is significant for the effect of fiber quality parameters on the similar and different cotton varieties $p < 0.05$. The JKCH 1947 cotton has a maximum spinning consistency index, Uniformity index, and maturity ratio of 151, 86.6, and 0.83, respectively. The organic cotton cultivated MRC (270) has a maximum fiber strength of 29.29 g/tex and elongation of 7.44 mm, minimum short fiber content of 4, and maturity of 4. Deltapine (DP) 90 untreated seed Forgena cotton has a maximum micronaire value of 4.24 and a minimum fiber length of 27.17 mm. Acala SJ2 cotton variety has a maximum fiber length of 28.3 mm, minimum micronaire of 3.69, and elongation of 4.44 mm. It was revealed there is little improvement in qualities of the organic and genetically modified cotton. Chemical treatment has a significant effect on the quality of cotton. Cotton parameters have a significant effect on varieties with strong and weak correlations.

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7. Gossypium purpurascens genome provides insight into the origin and domestication of upland cotton(紫花棉基因组研究为陆地棉的起源和驯化提供了新思路)

简介： Introduction: Allotetraploid upland cotton (Gossypium hirsutum L.) is native to the Mesoamerican and Caribbean regions, had been improved in the southern United States by the

mid-eighteenth century, was then dispersed worldwide. However, a Hainan Island Native Cotton (HIC) has long been grown extensively on Hainan Island, China. Objectives: Explore HIC's evolutionary relationship and genomic diversity with other tetraploid cottons, its origin and whether it was used for YAZHOUBU (Yazhou cloth, World Intangible Cultural Heritage) weaving, and the role of structural variations (SVs) in upland cotton domestication. Methods: We assembled a high-quality genome of one HIC plant. We performed phylogenetic analysis, divergence time estimation, principal component analysis and population differentiation estimation using cotton assemblies and/or resequencing data. SVs were detected by whole-genome comparison. A F2 population was used for linkage analysis and to study effects of SVs. Buoyancy and salt water tolerance tests for seeds were conducted. Results: We found that the HIC belongs to G. purpurascens. G. purpurascens is best classified as a primitive race of G. hirsutum. The potential for long range transoceanic dispersal of G. purpurascens seeds was proved. A set of SVs, selective sweep regions between G. hirsutum races and cultivars, and quantitative trait loci (QTLs) of eleven agronomic traits were obtained. SVs, especially large-scale SVs, were found to have important effects on cotton domestication and improvement. Of them, eight large-scale inversions strongly associated with yield and fiber quality have probably undergone artificial selection in domestication. Conclusion: G. purpurascens including HIC is a primitive race of G. hirsutum, probably disperse to Hainan from Central America by floating on ocean currents, may have been partly domesticated, planted and was likely used for YAZHOUBU weaving in Hainan much earlier than the Pre-Columbian period. SV plays an important role in cotton domestication and improvement.
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8. Enhanced phenylpropanoid metabolism underlies resistance to Fusarium oxysporum f. sp. vasinfectum race 4 infection in the cotton cultivar Pima-S6 (Gossypium barbadense L.)(Pima-S6棉花品种对尖孢镰刀菌4号生理小种侵染的抗性与苯丙烷代谢增强)

简 介： Introduction: Fusarium oxysporum f. sp. vasinfectum (FOV) race 4 (FOV4) is a highly pathogenic soil-borne fungus responsible for Fusarium wilt in cotton (Gossypium spp.) and represents a continuing threat to cotton production in the southwest states of the United States, including California, New Mexico, and Texas. Pima (G. barbadense L.) cotton, which is highly valued for its fiber quality, has been shown to be more susceptible to this pathogen than Upland (G. hirsutum L.) cotton. Still, some Pima cultivars present resistance to FOV4 infection.Methods: To gain insights into the FOV4-resistance mechanism, we performed comparative transcriptional and metabolomic analyses between FOV4-susceptible and FOV4-resistant Pima cotton entries. FOV4-resistant Pima-S6 and FOV4-susceptible Pima S-7 and Pima 3-79 cotton plants were infected with FOV4 in the greenhouse, and the roots harvested 11 days post-infection for further analysis.Results: We found that an enhanced root phenylpropanoid metabolism in the resistant Pima-S6 cultivar determines FOV4-resistance. Gene-ontology enrichment of phenylpropanoid biosynthesis and metabolism categories correlated with the accumulation of secondary metabolites in Pima-S6 roots. Specifically, we found esculetin, a coumarin, an inhibitor of Fusarium's growth, accumulated in the roots of Pima-S6 even under non-infected conditions. Genes related to the phenylpropanoid biosynthesis and metabolism, including

phenylalanine ammonia-lyase 2 (PAL2) and pleiotropic drug resistance 12 (PDR12) transporter, were found to be upregulated in Pima-S6 roots.Discussion: Our results highlight an essential role for the phenylpropanoid synthesis pathway in FOV4 resistance in Pima-S6 cotton. These genes represent attractive research prospects for FOV4-disease resistance and breeding approaches of other cotton cultivars of economic relevance.

来源：FRONTIERS IN GENETICS

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9．Single-cell transcriptome atlas identified novel regulators for pigment gland morphogenesis in cotton(单细胞转录组图谱鉴定棉花色素腺体形态发生的新调控因子)

简介：Cotton (Gossypium spp.) is a leading economic crop that is grown in more than 50 countries. The cottonseeds, once regarded as the by-product of fibre production, contain a rich supply of unsaturated fatty acids, proteins and vitamins. To date, the annual production of cottonseeds has the potential to meet the protein requirements for 550 million people globally, which shows great potential as a food resource amidst a growing food shortage (Janga et al., 2019). However, the utilization of cottonseed for food purposes is limited owing to the presence of ‘pigment glands’, which contains gossypol and its derivatives that are toxic to humans (Gao et al., 2020).To study how pigment gland cells differentiate and to reveal the gene regulatory network in gland morphogenesis, scRNA-Seq was performed using a pair of NILs (gland cotton ‘CCRI12’ and glandless cotton ‘CCRI12gl’). The 1-week-old cotyledons were enzymatically digested, and the purified protoplasts were labelled with a 10x genomics barcode for high-throughput sequencing (Figure 1a). A total of 9186 individual cells, including 4790 cells from ‘CCRI12’ and 4396 cells from ‘CCRI12gl’, were obtained after cell filtering process (Figure S1, Table S1) and were divided into 12 clusters based on highly variable genes (Figure 1b, Figure S2).To verify and correct cell group classifications, the expression profile of reported marker genes in 12 cell clusters of cotton cotyledons was studied. The clusters 0, 1 and 4 were identified as spongy mesophyll cells (SMC) due to the enrichment of a photosynthesis-related gene LHCB, and clusters 3 and 6 were identified as palisade mesophyll cells (PMC) due to the high expression of RBCS. The dominantly expressed GSTF9 marked cluster 5 as epidermal cells (EPC), and GSTL3 identified cluster 10 as the primordial cells (PRC) that could differentiate. In addition, cell type that specifically expressed MYB44, PXY, LTP and CYP82A3 marked the clusters 2, 7, 8 and 11 as guard cells (GC), xylem cells (XC), parenchyma cells (PAC) and phloem cells (PHC), respectively (Figures 1c,d, Table S2).No well-known marker gene for pigment gland cells has been reported to date. GoPGF is the key factor that controls the biogenesis of pigment glands (Ma et al., 2016). However, the expression of GoPGF in different cell types has not been studied. Cluster 9 was identified in the cotyledons of gland cotton ‘CCRI12’ but not glandless cotton ‘CCRI12gl’, and GoPGF was specifically detected in the cells of cluster 9. This led to the tentative annotation of cluster 9 of the cotyledon cells as pigment gland cells (PGC; Figure 1c).A pseudotime analysis was performed to uncover the differentiation relationships of cotyledon cell types. The study of the individual cell distribution and trajectory revealed that the PRCs originated earlier than the PGCs, suggesting that the PGCs could have differentiated from the PRCs (Figure 1e, Figure S3). In addition, four representative

genes were selected to show their distribution and expression levels in PRCs and PGCs (Figure 1f).To explore the potential regulators of gland development of cotton, the highly expressed genes in each cell cluster were studied. A total of 9325 DEGs were obtained with 1430 DEGs preferentially expressed in PGC, while the other cell clusters contained a range from 572 to 1704 (Table S3). Other than GoPGF, the previously reported GhERF105 (Wu et al., 2021), which determines the biogenesis of pigment glands in cotton leaves, was also identified as PGC-specific gene in our scRNA-Seq data. These results suggested the reliability of scRNA-Seq analyses in pigment gland cells and confirmed the accuracy of our classification of cell types.To date, the regulators involving in pigment gland biogenesis that have been identified are all TFs, including CGF1, CGF2, GoPGF/CGF3, GaGRAS/GoSPGF and GhERF105. Therefore, this study focused on the TFs that were preferentially expressed in PGCs (Figure 1g, Table S4). qPCR revealed that most of the identified TFs were highly expressed in gland cells, while they were expressed at very low levels in the mesophyll cells (Figure S4). In addition, five candidate genes, including GoPGF, were selected for RNA in situ hybridization. These results showed that these genes have strong hybridization signals in the glandular structure (Figure 1h).A 1.5-kb promoter upstream of the GoPGF initiation codon was cloned to drive the expression of GUS in the cotton gland cultivar ‘Coker312’. The transgenic lines that expressed GUS were obtained and used for histochemical staining. As shown in Figure 1i, a strong and clear GUS staining was restricted to the pigment glands. To our knowledge, this study is the first to use GUS staining to demonstrate that the transcription of GoPGF is restricted to gland cells.Virus-induced gene silencing was utilized to quickly screen the candidate genes that controlled the formation of pigment glands. The results suggested that knock down of some candidate genes, including ERF13 and MYB14, mildly reduced the gland density (Figure S5). Notably, the GH_A05G3906 could modulate the contents of gossypol without changing the number of pigment glands, which suggested a possible biosynthetic pathway of sesquiterpene metabolism that is independent of pigment gland biogenesis (Figure S5). Among all the candidates, JUNGBRUNNEN 1 (GhJUB1) is of particular interest. Knock down of GhJUB1 inhibits gland biogenesis and the accumulation of gossypol.The GhJUB1-silenced plants (TRV:JUB1) exhibited dramatically reduced pigment glands in newly growing tissues (Figure 1jl). In addition, GhJUB1-silenced cotton plants exhibited gossypol levels of 15% in the leaves and 18% in the stems compared with those of the control plants (Figure 1m). These results revealed that GhJUB1 regulates gland morphogenesis, which was similar to that of GoPGF. To study the relation between GhJUB1 and GoPGF, the expression of GhJUB1 was studied in the GoPGF-silenced cotton (TRV:PGF), and the results showed that the expression of GhJUB1 dramatically decreased to an undetectable level (Figure 1n), which suggests that GhJUB1 could be downstream of GoPGF to control the biogenesis of pigment glands.The pigment gland of cotton is a highly distinctive structure, which provides an ideal system to study cell differentiation and organogenesis. Our study indicates that the initiation of cell differentiation of pigment glands is highly correlated with the specific expression of key genes. One of the major constraints in the study of glandular development of cotton is the lack of natural glandless mutants. The scRNA-Seq data that we provide is invaluable for producing novel glandless mutants, which will greatly accelerate the breeding of commercially desired cotton varieties with glandless seeds.

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10．VILLIN2 regulates cotton defense against Verticillium dahliae by modulating actin cytoskeleton remodeling(Villin 2通过调节肌动蛋白细胞骨架重塑调节棉花对黄萎病的防御)

简介： The active structural change of actin cytoskeleton is a general host response upon pathogen attack. This study characterized the function of the cotton (Gossypium hirsutum) actin-binding protein VILLIN2 (GhVLN2) in host defense against the soilborne fungus Verticillium dahliae. Biochemical analysis demonstrated that GhVLN2 possessed actin-binding, -bundling, and -severing activities. A low concentration of GhVLN2 could shift its activity from actin bundling to actin severing in the presence of Ca2+. Knockdown of GhVLN2 expression by virus-induced gene silencing reduced the extent of actin filament bundling and interfered with the growth of cotton plants, resulting in the formation of twisted organs and brittle stems with a decreased cellulose content of the cell wall. Upon V. dahliae infection, the expression of GhVLN2 was downregulated in root cells, and silencing of GhVLN2 enhanced the disease tolerance of cotton plants. The actin bundles were less abundant in root cells of GhVLN2-silenced plants than in control plants. However, upon infection by V. dahliae, the number of actin filaments and bundles in the cells of GhVLN2-silenced plants was raised to a comparable level as those in control plants, with the dynamic remodeling of the actin cytoskeleton appearing several hours in advance. GhVLN2-silenced plants exhibited a higher incidence of actin filament cleavage in the presence of Ca2+, suggesting that pathogen-responsive downregulation of GhVLN2 could activate its actin-severing activity. These data indicate that the regulated expression and functional shift of GhVLN2 contribute to modulating the dynamic remodeling of the actin cytoskeleton in host immune responses against V. dahliae.The regulated expression and subsequent functional shift of VILLIN2 from actin bundling to severing contribute to the modulation of cotton immune responses against Verticillium dahliae.

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11．Attenuation of ethylene signaling increases cotton resistance to a defoliating strain of Verticillium dahliae(乙烯信号减弱提高棉花对一株落叶型棉花黄萎病的抗性)

简介： The severity of Verticillium wilt on cotton caused by defoliating strains of Verticillium dahliae has gradu-ally increased and threatens production worldwide. Identification of the molecular components of leaf defoliation may increase cotton tolerance to V. dahliae. Ethylene, a major player in plant physiological processes, is often associated with senescence and defoliation of plants. We investigated the cotton-V. dahliae interaction with a focus on the role of ethylene in defoliation and defense against V. dahliae. Cotton plants inoculated with V. dahliae isolate V991, a defoliating strain, accumulated more ethylene and showed increased disease symptoms than those inoculated with a non-defoliating strain. In cotton with a transiently silenced ethylene synthesis gene (GhACOs) and signaling gene (GhEINs) during cot-ton-V. dahliae interaction, ethylene produced was derived from

cotton and more ethylene increased cot-ton susceptibility and defoliation rate. Overexpression of AtCTR1, a negative regulator in ethylene signaling, in cotton reduced sensitivity to ethylene and increased plant resistance to V. dahliae. Collectively, the results indicated precise regulation of ethylene synthesis or signaling pathways improve cotton resistant to Verticillium wilt.

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12．GhWRKY41 forms a positive feedback regulation loop and increases cotton defence response against Verticillium dahliae by regulating phenylpropanoid metabolism(GhWRKY41形成正反馈调节环，通过调节苯丙素代谢提高棉花对大丽轮枝菌的防御反应)

简介：Despite the established significance of WRKY proteins and phenylpropanoid metabolism in plant immunity, how WRKY proteins modulate aspects of the phenylpropanoid pathway remains undetermined. To understand better the role of WRKY proteins in plant defence, we identified a cotton (Gossypium hirsutum) protein, GhWRKY41, that is, universally and rapidly induced in three disease-resistant cotton cultivars following inoculation with the plant pathogenic fungus, Verticillium dahliae. We show that overexpression of GhWRKY41 in transgenic cotton and Arabidopsis enhances resistance to V. dahliae, while knock-down increases cotton more susceptibility to the fungus. GhWRKY41 physically interacts with itself and directly activates its own transcription. A genome-wide chromatin immunoprecipitation and high-throughput sequencing (ChIP-seq), in combination with RNA sequencing (RNA-seq) analyses, revealed that 43.1% of GhWRKY41-binding genes were up-regulated in cotton upon inoculation with V. dahliae, including several phenylpropanoid metabolism master switches, receptor kinases, and disease resistance-related proteins. We also show that GhWRKY41 homodimer directly activates the expression of GhC4H and Gh4CL, thereby modulating the accumulation of lignin and flavonoids. This finding expands our understanding of WRKY-WRKY protein interactions and provides important insights into the regulation of the phenylpropanoid pathway in plant immune responses by a WRKY protein.

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