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Brief Communication

Development of an eco-friendly pink cotton germplasm by engineering betalain biosynthesis pathway

Xiaoyang Ge[1,2,†], Peng Wang[2,†], Ye Wang[2,†], Xi Wei[3], Yanli Chen[2] and Fuguang Li[1,2,*]

[1]*Zhengzhou Research Base, State Key Laboratory of Cotton Biology, School of Agricultural Sciences, Zhengzhou University, Zhengzhou, China*
[2]*State Key Laboratory of Cotton Biology, Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang, China*
[3]*Henan Normal University Research Base of State Key Laboratory of Cotton Biology, Xinxiang, China*

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**Correspondence* (Tel +8603722562204; fax +8603722562256; email aylifug@caas.cn
[†]These authors made equal contributions.

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The vast majority of the world's cotton varieties produce white fibre, which is then coloured with synthetic dyes during textile processing to fulfil the diverse needs of consumers. This results in potential environmental pollution and is detrimental to human and animal health. Although there exists naturally coloured cottons, mostly in brown and green, their poor agronomic performance and suboptimal fibre quality have severely hampered their widespread applications in cotton breeding program. Therefore, there is an impetus to create multicoloured fibre through synthetic biology biotechnology by leveraging the biochemical processes of colour formation and associated regulatory genes in cotton.

Pigment accumulation typically results in colourful phenotypes in various plant tissues. Proanthocyanidins or lignin are the pigments responsible for the brown and green cotton fibre. Betalain and anthocyanin are two primary groups of red pigments that are found in the vacuoles of plant cells. The former, present in dragon fruit (*Hylocereus undulatus Britt*) and sugar beet (*Beta vulgaris*), is derived from the tyrosine synthesis pathway through three sequential catalytic steps (Polturak and Aharoni, 2018), whereas the latter requires the engineering of additional 13 or 14 genes (Zhu *et al*., 2017). In Arabidopsis, rice, and tobacco, co-expression of a P450 oxygenase (CYP76AD1), L-DOPA 4,5-dioxygenase (DODA), and Glucosyl Transferase (GT) resulted in considerable betalain accumulation, which results in vibrant red colour in various tissues of transgenic plants, respectively (He *et al*., 2020). However, it remains unknown if the betalain pathway can be engineered into cotton plants to alter the colour of cotton fibre without compromising fibre quality.

To generate cotton plants with colourful fibre, three genes, including *CYP76AD1*, *DODA*, and *GT* from *B. vulgaris* were cotton codon optimized. Three transgene expression cassettes (Figure 1a) termed "Ruby" by He *et al*. (2020), each driven by either a 2x35S *CaMv* constitutive promoter (hereafter referred to as 35 S-RUBY) or a fibre-specific E6 promoter (hereafter referred to as E6-RUBY), are separated by two 2A self-cleavage peptide-encoding genes (Sharma *et al*., 2012; Figure 1b). Multiple independent transgenic upland cotton (*Gossypium hirsutum* L.) plants overexpressing Ruby were generated using a high-yielding and high-fibre-quality modern elite variety, Zhongmian49, as a donor plant (Ge *et al*., 2022; Figure 1c). PCR analysis detected that gene expression level varies among different T_0 plants, and two 35 S-RUBY lines and one E6-RUBY line with higher expression level of GT in immature fibres were selected to perform DNA blotting analysis, showing the stable integration of transgene (Figures 1d,e). Segregation analysis indicated that the transgenes could be inherited into the following generation (Figure 1f). Single copy T_2 homozygous transgenic plants with high gene expression levels, together with wild-type Zhongmian49 (WT), were grown under field conditions in a confined environment to evaluate the agronomic performance of transgenic lines (Figure 1g). All of the transgenic plants accumulated betalain, but the accumulation patterns were different depending on whether the plants were co-overexpressing 35 S CaMv-driven betalain genes or E6-driven betalain genes. Whereas the WT control showed a normal phenotype, the leaf, stem, boll, bract, flower, anther, and seed were all purple or pink in the 35 S-RUBY plants (Figure 1h). Notably, light pink colour was discernible in immature fibres at 12, 27, and 42 days post-anthesis (DPA), but it faded away when fibre completely matured (Figures 1h,i). The colour phenotype of the E6-RUBY plants remained unchanged from that of the WT, apart from the immature fibres, which showed pink in colour at 12, 27 and 42 DPA. (Figures 1g,h). Although the pink colour did not persist in mature fibres in both E6-RUBY and 35 S-RUBY transgenic lines due to the lower level of betalain in mature fibre compared to leaves and seeds (Figure 1j), colour fibres were observed in near-maturity transgenic cotton plants at 45 DPA after freeze (−40 to −50 °C) or high temperature treatment (~ 40 °C), and the pink colour was stably inherited from T_0 to T_2 generation (Figures 1k,l). As illustrated in Figures 1m,n,p,t, the fibre length and strength of the transgenic fibres at 45 DPA and mature stage were comparable to those of the WT fibre. Moreover, there was no difference in the weight of cotton bolls between transgenic plants and control (Figure 1o). It is hence apparent that there are no quality trade-offs in the transgenic lines overexpressing the betalain genes in either a constitutive or fibre-specific manner. Considering the superior fibre yield and quality of Zhongmian49, these transgenic plants with an intrinsically vibrant pink colour maintained at near-maturity may have the potential to be used for commercial exploration.

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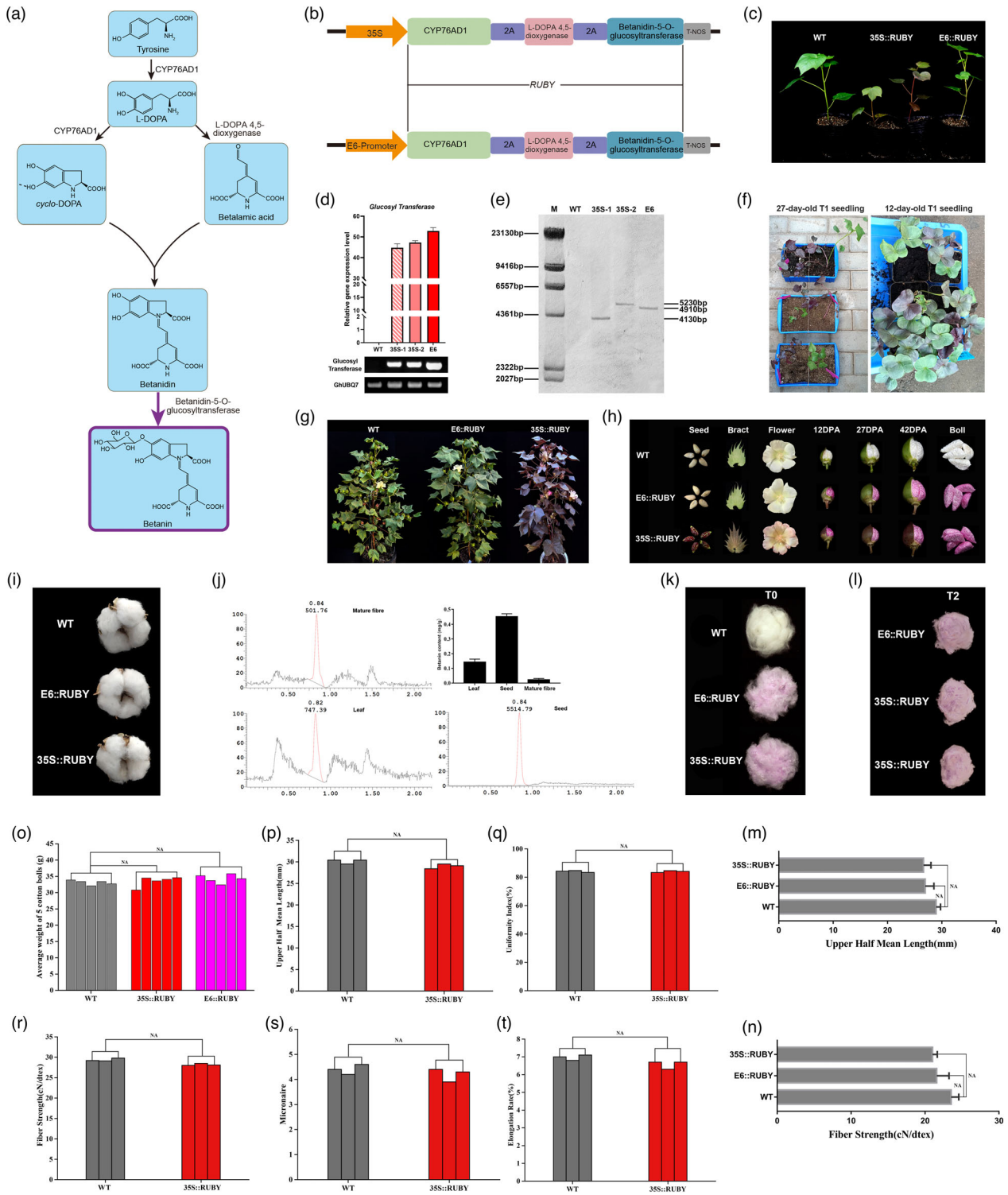

Figure 1 Accumulation of heterologous betalains results in pink cotton fibre. (a) The introduced betalain biosynthesis pathway. (b) Three key betalain genes, each driven by a 35 S *CaMv* promoter or an E6 promoter, were co-expressed in *Gossypium hirsutum*. (c) Phenotype of the seedlings of WT and T_0 transgenic lines. (d) Expression level of GT gene in the immature fibres of T_0 transgenic lines. (e) DNA blot analysis of T_0 transgenic lines. (f) Transgene inheritance to offspring. (g) Phenotypes of WT, 35 S-RUBY or E6-RUBY transgenic plants. (h) Betalain accumulation in seed, bract, flower, and boll in developing cotton plants. (i) Phenotype of mature fibre of WT and transgenic lines. (j) Betalain content in the leave, seed, and mature fibre of 35-RUBY plant. The colour, fibre length and strength of freeze-dried fibre at 45 DPA in WT, T_0 and T_2 transgenic plants (k–n).Cotton boll weight (o), mature fibre length (p), uniformity index (q), fibre strength (r), micronaire values (s) and elongation rate (t) were measured in the transgenic lines and WT plants.

As betalains are most stable in the pH range of 4.0–6.0, they can be used in textile processing without additional treatment to improve colour stability and uniformity. Future research could be directed to elucidate the regulatory processes that underpin the degradation of betalain in fully mature fibres and develop alternative strategies, e.g., increasing the deposition of betalain in fibre secondary cell walls (Huang *et al*., 2021). Moreover, the development of a robust synthetic biology biotechnology for cotton is envisaged to expedite the generation of pure inbred lines with multiple desirable features, bypassing the lengthy procedure of repeated crossing and backcrossing in conventional breeding. Crossing HS2 with naturally coloured fibres led to enrichment and diversification in fibre colours (Ke *et al*., 2022). It is plausible to assume that more vibrant or versatile colours may be attainable by crossing E6-RUBY with the naturally coloured fibres. Overall, our findings demonstrate for the first time that it is possible to produce natural pink colour fibre by engineering key genes involved in betalain synthesis without compromising fibre yield and quality in cotton, providing an environmentally and health-friendly alternative to synthetic dye for producing coloured cotton fibre.

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Author contributions

F-G. L., X-Y. G designed the studies and wrote the manuscript. P.W., Y.W., Y-L. Chen and X.W performed the experiments.

Conflict of interest

The authors declare no conflict of interest.

References

Ge, X.Y., Xu, J.T., Yang, Z.E., Yang, X.F., Wang, Y., Chen, Y.L., Wang, P. *et al*. (2022) Efficient genotype-independent cotton genetic transformation and genome editing. *J. Integr. Plant Biol*. https://doi.org/10.1111/jipb.13427

He, Y.B., Zhang, T., Sun, H., Zhan, H.D. and Zhao, Y.D. (2020) A reporter for noninvasively monitoring gene expression and plant transformation. *Hortic. Res.* **7**, 152.

Huang, J.F., Chen, F., Guo, Y.J., Gan, X.l., Yang, M.M., Zeng, W., Persson, S. *et al*. (2021) GhMYB7 promotes secondary wall cellulose deposition in cotton fibres by regulating GhCesA gene expression through three distinct cis-elements. *New Phytol.* **232**, 1718–1737.

Ke, L.P., Yu, D.L., Zheng, H.L., Xu, Y.H., Wu, Y.Q., Jiao, J.Y., Wang, X.L. *et al*. (2022) Function deficiency of GhOMT1 causes anthocyanidins over-accumulation and diversifies fiber colors in cotton (*Gossypium hirsutum*). *Plant Biotechnol. J.* **20**, 1546–1560.

Polturak, G. and Aharoni, A. (2018) "La vie en rose": Biosynthesis, sources, and applications of betalain pigments. *Mol. Plant* **11**, 7–22.

Sharma, P., Yan, F., Doronina, V.A., Escuin-Ordinas, H., Ryan, M.D. and Brown, J.D. (2012) 2A peptides provide distinct solutions to driving stop-carry on translational recoding. *Nucleic Acids Res.* **40**, 3143–3151.

Zhu, Q.L., Yu, S.Z., Zeng, D.C., Liu, H.M., Wang, H.C., Yang, Z.F., Xie, X.R. *et al*. (2017) Development of "purple endosperm rice" by engineering anthocyanin biosynthesis in the endosperm with a high-efficiency transgene stacking system. *Mol. Plant* **10**, 918–929.

