

Supplementary Materials:

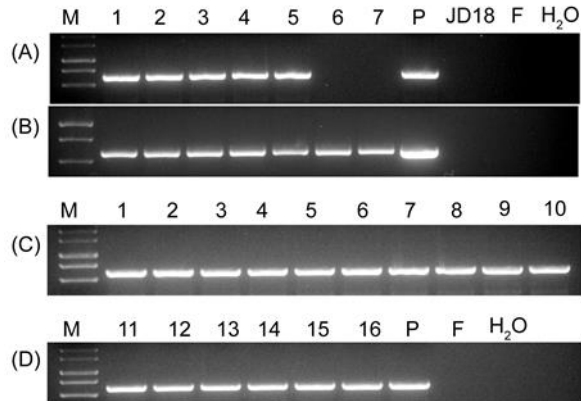


Figure S1. Detection of transgenic wheat plants with *ZmC1* and *ZmR* genes by PCR. **A:** PCR amplification for *ZmC1*; **B:** PCR amplification for *ZmR*; 1-5: transgenic plants transformed with expression vector pWMB202; 6-7: transgenic plants transformed with expression vector pWMB197; P: positive control of vector pWMB202; JD18 and F: negative control of wild type Jingdong18 and Fielder, respectively; H₂O: empty control of sterile water. **C and D:** PCR amplification for *ZmC1*; 1-16: transgenic plants transformed with expression vector pWMB196; P: positive control of vector pWMB196; F: negative control of wild type Fielder; H₂O: empty control of sterile water.

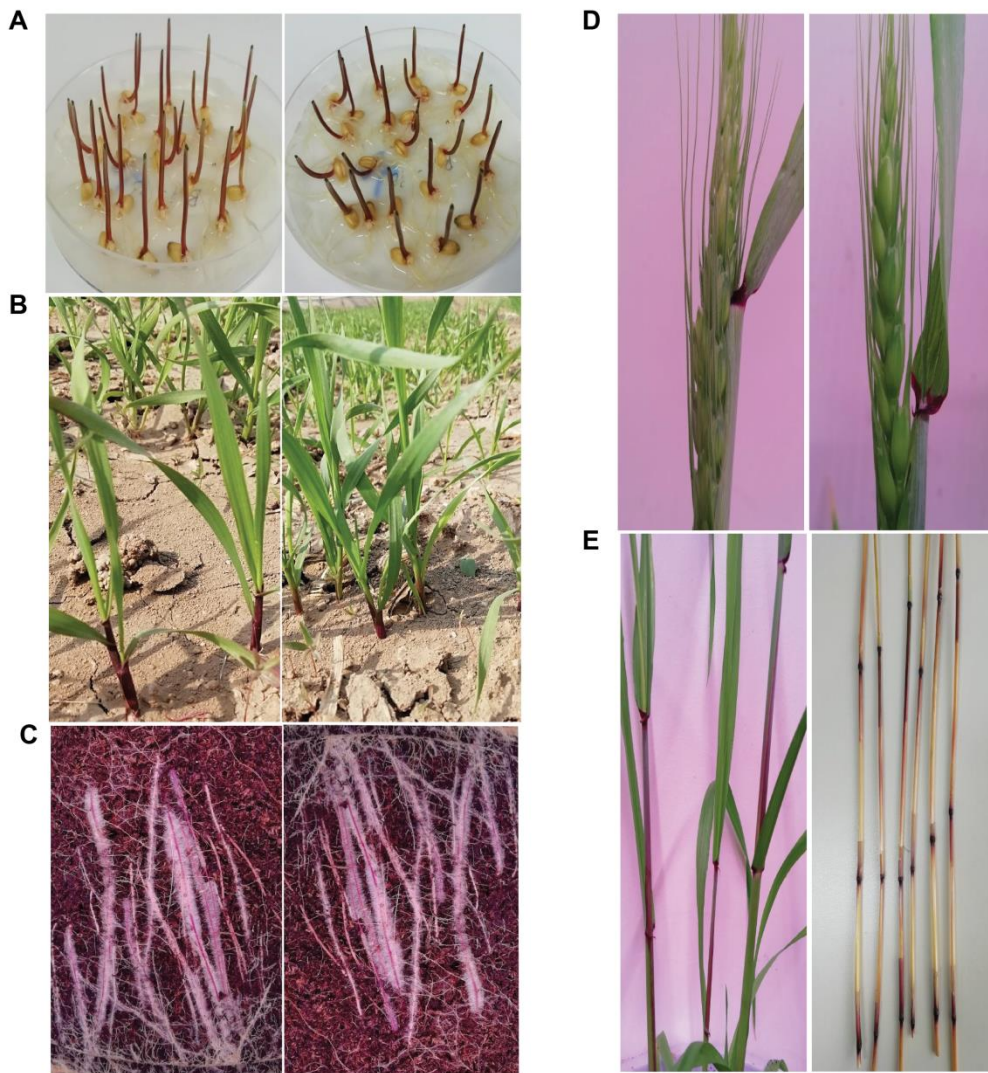


Figure S2. Anthocyanin production in the vegetative tissues of transgenic line AL-31. **A:** coleoptiles; **B:** seedlings; **C:** roots; **D:** auricles; **E:** stems.

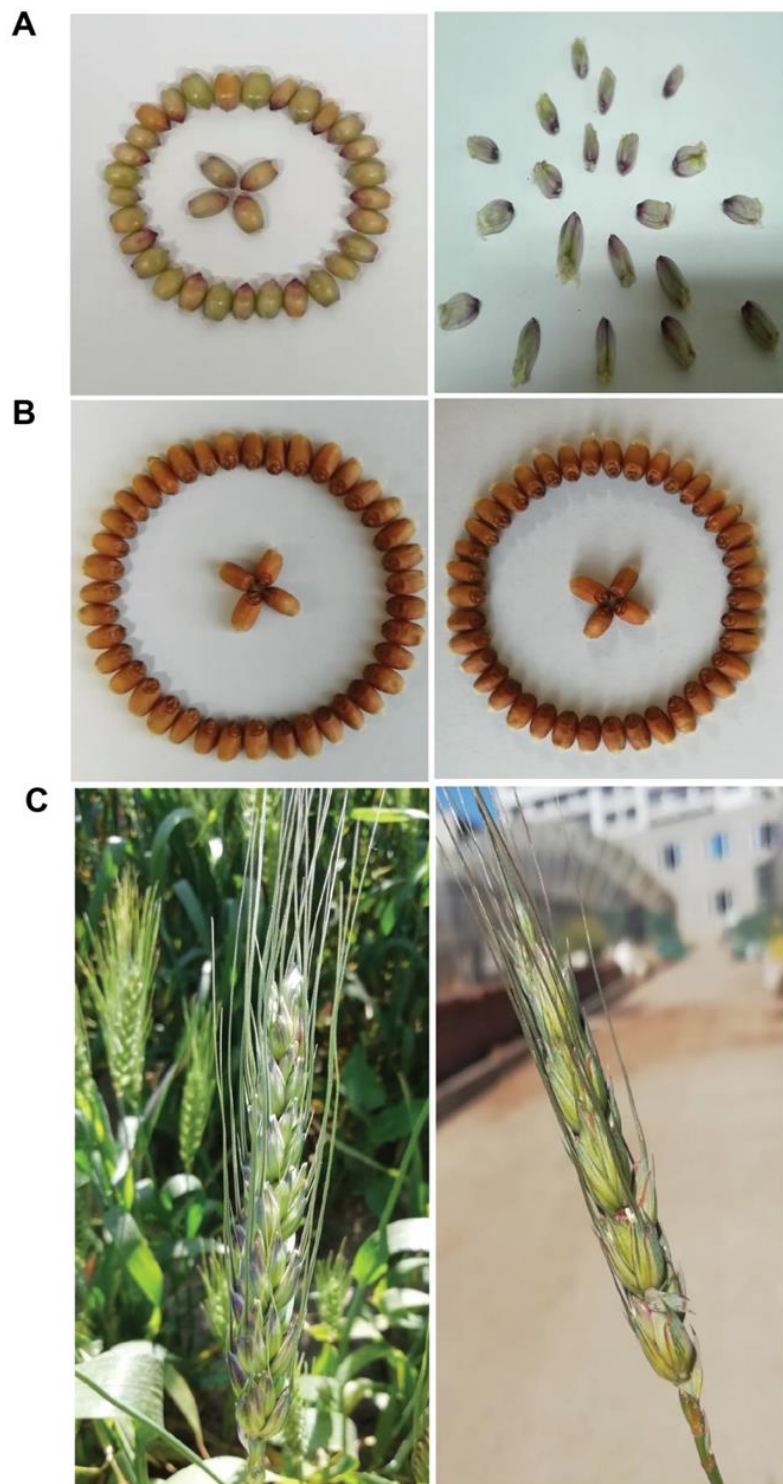


Figure S3. Anthocyanin production in the reproductive tissues of transgenic line AL-41. **A:** immature seeds; **B:** mature seeds; **C:** spikes.



Figure S4. Anthocyanin production in the tissues of transgenic line AL-44. **A:** seedlings; **B:** leaves; **C:** stems; **D:** immature seeds; **E:** mature seeds; **F:** germinated roots; **G:** spikes.

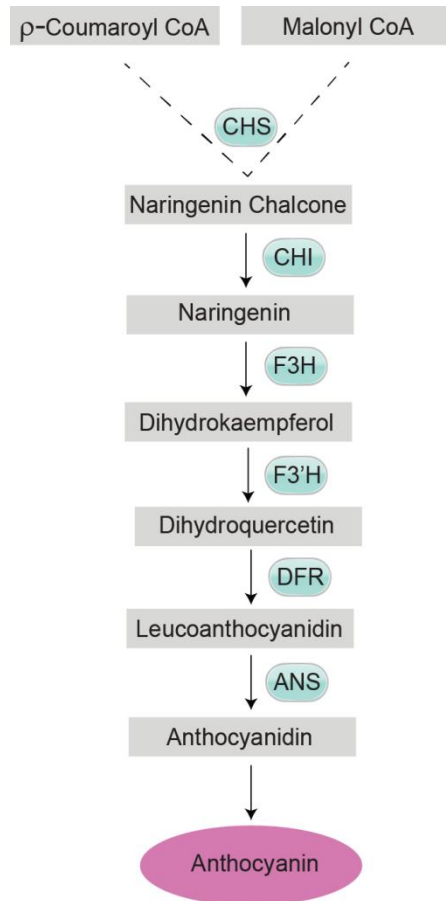
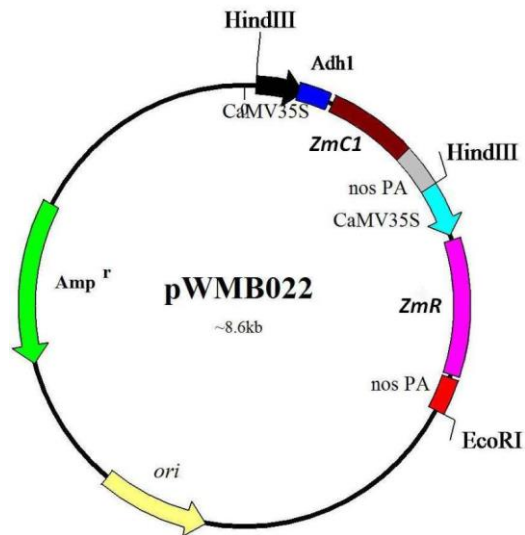


Figure S5. Structural genes involved in anthocyanin biosynthesis pathway in wheat.

A



B

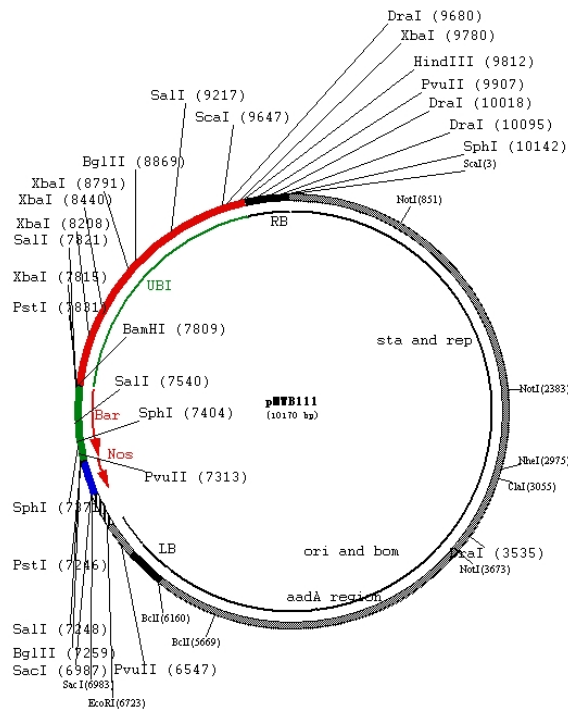
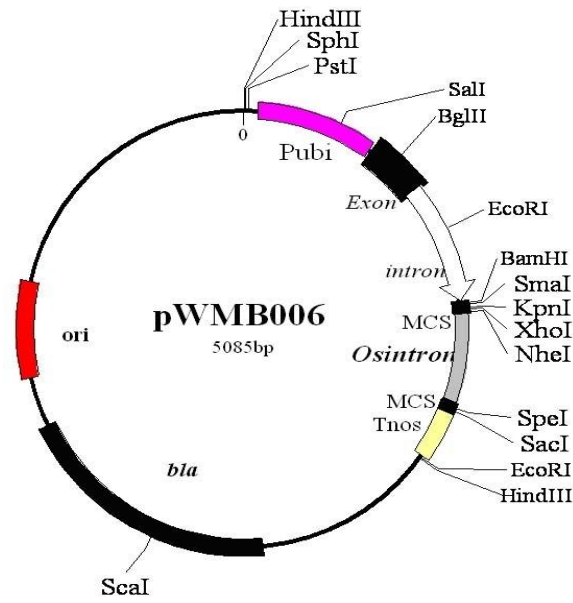


Figure S6. Plasmids pWMB022, pWMB006, and pWMB111 used in this study for constructing the expression vectors containing *ZmC1* and *ZmR* genes which are involved in anthocyanin biosynthesis pathway for wheat transformation. A: pWMB022; B: pWMB006; C: pWMB111 carrying *Bar* gene expression cassette.

Table S1. Determination of the anthocyanin contents in the transgene wheat lines.

| Transgenic lines | Concentration anthocyanin contents ($\mu\text{g/g}$) | |
|------------------|--|---------------------------------|
| | Seeds | Leaves |
| AL-44 | 10.177 \pm 0.180 ^b | 96.517 \pm 3.328 ^b |
| AL-45 | 12.954 \pm 0.840 ^a | 102.2 \pm 8.029 ^a |
| AL-30 | 0.0206 \pm 0.009 ^c | 0.0307 \pm 0.005 ^c |
| AL-31 | 0.2283 \pm 0.0237 ^c | 0.0678 \pm 0.036 ^c |
| AL-31 | 0.1541 \pm 0.023 ^c | 0.0159 \pm 0.006 ^c |
| AL-40 | 0.054 \pm 0.070 ^c | 0.8205 \pm 0.082 ^c |
| AL-41 | 0.2385 \pm 0.012 ^c | 0.5608 \pm 0.041 ^c |
| Fielder | 0.0861 \pm 0.032 ^c | 0.1465 \pm 0.016 ^c |
| Jingdong18(JD18) | 0.0243 \pm 0.008 ^c | 0.0579 \pm 0.026 ^c |

^{a, b, c} Different letters indicate statistically significant differences ($P < 0.05$).

Table 1. Primers used in this study for PCR and quantitative real-time PCR analysis.

| Name | Forward (5'-3') | Reverse (5'-3') |
|------------------|---------------------------|---------------------------|
| <i>ZmC1</i> | GAAGGCGTTAAGAGAGGGGC | CCTGCTTCTCCTCCTCCTCCT |
| <i>ZmR</i> | CGACATAGAGGCGATGAC | ACTTGAGGACGAGGAACA |
| <i>TaADP-RT</i> | GCTCTCCAACAACATTGCCAAC | GCTTCTGCCTGTCACATACGC |
| <i>ZmC1-RT</i> | GTGGACGAGCAAGGAGGACGAT | CCTGTGGAGGCGGATGATGAGA |
| <i>ZmR-RT</i> | GGTTCTACAACGCGAGGTGAA | CGGAAGGCGTAGGTCATGGAGA |
| <i>TaC1-RT</i> | GGTTGAGAGAGGGAGAGGGGAT | CGCAGACCAGCTCGCTGC |
| <i>TaR-RT</i> | CGGCCTGAGCAGTGTCA | GTGTCACGCTTCAACAACATCC |
| <i>TaCHS-RT</i> | TGGAAAACAACCTACTACATACAGC | CAGACTAACAGGAGATCGAATGCAT |
| <i>TaCHI-RT</i> | CGTCCTCGTATTTGTCCGCTG | GAACCATAGTCACATATCACGAGG |
| <i>TaF3H-RT</i> | TGATTGATGCGTGGTGGGA | CAGAAACCAGTACGAAATATACGC |
| <i>TaF3'H-RT</i> | TAGCTCTCCAGTCTTCCTTGCC | GCCGTCGGCGTACACGA |
| <i>TaDFR-RT</i> | GCTCATCACAGGGAATGAAGC | GTCGTCGACGCCTGCGA |
| <i>TaANS-RT</i> | GAGGACGACCTGCTGCTGAA | GCCGTTGGTGAGGATGAAGGA |

*RT: Used for quantitative real-time PCR.