The gene TaWOX5 overcomes genotype dependency in wheat genetic transformation

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Although great progress has been achieved regarding wheat genetic transformation technology in the past decade1–3, genotype dependency, the most impactful factor in wheat genetic transformation, currently limits the capacity for wheat improvement by transgenic integration and genome-editing approaches. The application of regeneration-related genes during in vitro culture could potentially contribute to enhancement of plant transformation efficiency4–11. In the present study, we found that overexpression of the wheat gene TaWOX5 from the WUSCHEL family dramatically increases transformation efficiency without resulting in aberrant phenotypes12,13. The TaWOX5 chimera construct was used to generate transgenic plants, which achieved an average transformation efficiency of 65% (with a range of 27–96%) in two tetraploid wheat genotypes14. Recently, a co-overexpression of genes BBM and WUS2 produced high transformation frequency in several previously transformation-recalcitrant inbred maize lines11. Moreover, maize embryos and leaf tissues were used to generate transgenic plants with these two morphogenic regulators, and co-overexpression of ZmBBM and ZmWUS2 clearly enhanced the transformation efficiency of certain recalcitrant genotypes of sorghum (Sorghum bicolor (L.) Moench), indica rice and sugarcane (Saccharum officinarum L.). However, overexpression of WUS2 and BBM in cereal crops resulted in many negative effects including callus necrosis, compromised differentiation of shoots and roots, decreased fertility of transgenic plants and a variety of aberrant, stunted and twisted phenotypes11.

There are also reports of using the promoter of maize phospholipid transferase protein (PLTP) to drive BBM expression and an auxin-inducible promoter to drive WUS2 to increase transformation efficiency without resulting in aberrant phenotypes12,13. The PLTP promoter, incorporated with three viral enhancer elements, enhanced WUS2 expression and precluded the regeneration of cells with WUS2 integration, while strong transient expression of WUS2 stimulated somatic embryo formation in cells without WUS2 integration in the tissue, which was designated ‘altruistic transformation’ and can be used to obtain transgenic plants without WUS2 (ref. 14). Recently, a GRF4-GIF1 chimera construct was used to generate transgenic plants, which achieved an average transformation efficiency of 65% (with a range of 27–96%) in two tetraploid wheat varieties (Desert King and Kronos), even reaching 9–19% in two previously non-transformable common wheat varieties, Hahn and Cadenza11. In the present study, we report that overexpression of the wheat gene TaWOX5 dramatically improves the transformation frequency of wheat and five other cereal species, with less genotype dependence and no obvious negative effects on callus differentiation or plant phenotype.

Results and Discussion

The WUS gene is an important regulator of somatic embryogenesis in Arabidopsis10,16. Based on the Arabidopsis WUS sequence, two wheat homologous genes (TaWOX5 and TaWUS) were obtained. TaWOX5 is more closely related to Arabidopsis WOX3 (Extended Data Fig. 1) containing a WUS-related homeobox domain according to the description of the AtWUS protein structure7, which belongs to the WOX5 type in the WUS gene family and is specifically expressed in the root tip9. In contrast, TaWUS is more

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closely related to AtWUS (Extended Data Fig. 1). Furthermore, six different sequences corresponding to TaWOX5 were amplified by PCR from the common wheat line CB037 (Triticum aestivum, AABBDD, 2n = 42), and three TaWOX5 sequences were obtained from Triticum monococcum accession CItrl3961 (AA, 2n = 14) and Aegilops speltoides accession PI554241 (SS, 2n = 14)), the two diploid species most closely related to common wheat (Supplementary Table 1). Three homoeologous TaWUS genes
(TaWUS-A, TaWUS-B and TaWUS-D) were also obtained from CB037 (Supplementary Table 1).

The genomic sequences of TaWOX5 (MN412513), TaWUS-A, TaWUS-B and TaWUS-D were cloned into the pWMB111 expression vector under the control of the maize ZmUbi promoter and Nos terminator (Fig. 1a). Following the stable delivery of pWMB111-TaWOX5 into wheat genotypes Fielder and CB037 by Agrobacterium-mediated transformation (PureWheat), we detected transformation efficiencies significantly higher than those from the transformation experiments using the control (empty) pWMB111 vector (100% versus 50% efficiency, \( P < 0.05 \); Fig. 1b). Notably, transformation efficiencies obtained using the constructs for separate expression of TaWUS-A, TaWUS-B and TaWUS-D were lower than those using the pWMB111 control vector (Fig. 1b), because many of the regeneration shoots from those TaWUS transformation experiments did not produce roots and were therefore excluded from calculations regarding transformation efficiency. The shoots recovered from experiments using TaWOX5 were normal and developed healthy roots.

To further examine the impacts of TaWOX5 and TaWUS on transformation, expression vectors pWMB111, pWMB111-TaWOX5 and pWMB111-TaWUS-D were transformed into several widely commercialized wheat cultivars including Zhongmai895, Jimai22, Jing411, Luxxuan987 and Zhengmai9023. TaWUS-D improved the transformation efficiency of all tested wheat cultivars except Zhengmai9023. TaWOX5 significantly improved the transformation efficiency of Zhongmai895, Jimai22, Luxxuan987 and Jing411 (\( P < 0.05 \)) and increased the efficiency of Zhengmai9023 (42 ± 3.1% versus 11.7% for the control vector; Fig. 1c).

Over the past 5 years we have utilized TaWOX5 in the successful transformation of 29 common wheat varieties, including several known to be transformation recalcitrant (Fig. 1d and Supplementary Table 2). Wheat variety Jimai22 is the most widely cultivated in China, with an annual planting area exceeding 2 million hectares, and the calli derived from Jimai22 immature embryos are very poor in quality. However, overexpression of TaWOX5 dramatically improved the quality of Jimai22 calli (Fig. 2). The transformation efficiency of this cultivar was significantly increased to 55.4 ± 17.9% from 5.8 ± 3.3% following the application of TaWOX5 (\( P < 0.001 \); Fig. 1d and Supplementary Table 2). Moreover, when TaWOX5 was not used in the transformation, wheat variety Ningchun4 did not regenerate green shoots, Jimai22 and Kenong199 generated only one to three green shoots and Fielder showed fewer than ten green shoots per immature embryo (Fig. 2b). When using TaWOX5, all tested varieties produced more than ten green shoots per immature embryo (Fig. 2d). Similarly, notable improvement in green shoot number was also observed in other wheat cultivars (Extended Data Fig. 2).

The application of TaWOX5 significantly (\( P < 0.001 \)) increased the transformation efficiency of readily transformed wheat varieties CB037, Fielder and Kenong199 (with transformation efficiency >20% using control vector), to 96.2±2.9%, 94.5±9.4% and 75.7±17.8% from 39.9±9.9%, 45±13.0% and 17.7±9.7%, respectively (Fig. 1d and Supplementary Table 2). With the application of TaWOX5, the transformation efficiency of difficult-to-transform
varieties Zhongmai895, Sumai3 and Jing411 (transformation efficiency <8% using a control vector) was significantly increased, to 82.7 ± 14.4%, 57.4 ± 13.0% and 17.5 ± 5.3% (P < 0.05 or 0.001), respectively. The use of TaWOX5 also significantly improved the transformation efficiency of previously non-transformable varieties including Bs366, Ningchun4, Aikang58, Xinong979 and Sunstate, to 83.5 ± 15.6%, 29.3 ± 13.6%, 21.8 ± 3.8%, 16.7 ± 3.1% and 9.1 ± 5.4% (P < 0.05 or 0.001), respectively. In addition, transgenic plants were also successfully generated from other important wheat varieties or germplasms including Zhengmai6694, Zhengmai9170, Zhongmai175, Cang6005 and Luohanmai (a landrace genotype) using TaWOX5, with efficiency ranging from 6.1% to 97.8%.

In the course of our transformation work over the past few years we have used TaWOX5 in the transformation of 3,290 Fielder immature embryos and 5,459 Jimai22 immature embryos, the numbers of immature embryos used in these experiments being 1,000 greater than for each genotype among the majority of commercial wheat varieties examined in our study (Supplementary Table 2). Note that genotyping of transgenic plants overexpressing TaWOX5 was variously performed using PCR, droplet digital PCR (ddPCR) and QuickStix analyses (Extended Data Fig. 3 and Supplementary Table 3). The data from ddPCR showed that transgenic wheat plants recovered in TaWOX5 experiments contained one or more copies of the transgene (Supplementary Table 3). TaWOX5 was inherited by the T1 generation based on Mendelian pattern (Supplementary Table 4). Collectively, our data with these large sample sizes and diverse common wheat genotypes demonstrate that the application of TaWOX5 greatly improves transformation efficiency.

It is well known that Agrobacterium-mediated plant transformation depends on both the infection efficiency of Agrobacterium and the regeneration ability of host cells. The application of TaWOX5 was able to enhance the ability of host tissues in callus induction and regeneration. In this case, the infection efficiency of Agrobacterium will determine the final transformation efficiency of wheat tissues. According to our evaluation in a previous study19, the ranked order of regeneration capacity for five widely cultivated Chinese commercial wheat varieties was Zhongmai895, Ningchun4, Aikang58, Xinong979 and Jimai22. However, the transformation efficiency of Jimai22 using TaWOX5 (55.4 ± 17.9%) was higher than that for Aikang58, Xinong979 and Ningchun4 (Supplementary Table 2). To explore this phenomenon, we introduced maize genes ZmR and ZmC1, involved in anthocyanin biosynthesis20, into three groups of wheat genotypes of varying transformation efficiency: a ‘high’ group with an efficiency of 80–100% (Fielder and Zhongmai895), a ‘medium’ group (40–80%, Jimai22) and a ‘low’ group (0–50%, Ningchun4, Aikang58 and Sunstate). When calculating transient transformation efficiency based on visualization of areas where anthocyanin expression exceeded 50% of the entire immature embryo, efficiency order from high to low was Fielder (87%), Zhengmai895 (73%), Jimai22 (54%), Ningchun4 (14%), Aikang58 (4%) and Sunstate (0%) (Extended Data Fig. 4), which is consistent with the stable transformation efficiency of these genotypes using TaWOX5 (Fig. 1d and Supplementary Table 2). While regeneration capacity remains an underlying requirement for successful transformation of all tested varieties, it appears that transient transformation efficiency is roughly correlated with final transformation efficiency when TaWOX5 is used.

Fig. 3 | Regeneration improvement in immature embryos of poor physiological status by application of TaWOX5 in two wheat genotypes. a, Shoot regeneration in wheat embryos transformed with control vector. b, Shoot regeneration in wheat embryos transformed with vector containing TaWOX5. c, The enlarge view of b. d, Flag leaves of transgenic wheat plants with and without overexpression of TaWOX5 in Fielder. Calli on plates were somewhat overcrowded.
The physiological status of wheat immature embryos greatly affects successful transformation. High temperature during the growth period of wheat mother plants, especially at the grain filling stage, negatively affects production and differentiation of embryonic calli derived from immature embryos, which can often result in transformation failure. In standard PureWheat methods, the application of TaWOX5 can enable the production by wheat of between five and ten shoots per callus. These results demonstrate efficacy as high as 33.8% for CB037.

Table 1 | Overexpression of TaWOX5 increased transformation efficiency in T. monococcum, barley, S. cereale and triticale at ICS-CAAS, and wheat and maize at JT. Data were tested using Student’s t-test (two-sided)

| Variety       | Species                | Control vector | TaWOX5 vector | P value |
|---------------|------------------------|----------------|---------------|---------|
|               | No. of experiments     | No. of explants transformed | No. of positive plants | Transformation efficiency (%) | No. of experiments | No. of explants transformed | No. of positive plants | Transformation efficiency (%) |         |
| Pl428182      | T. monococcum          | 2              | 125           | 0        | 0       | 4              | 273           | 258           | 94.5 ± 8.2 | 0.0001 |
| Dwarf Polish  | T. polonicum           | 2              | 142           | 0        | 0       | 2              | 166           | 25            | 15.1 ± 4.1 | 0.0429 |
| Vlamingh      | H. vulgare             | 2              | 138           | 14       | 10.1 ± 4.3 | 2              | 159           | 125           | 78.6 ± 9.8 | 0.0123 |
| Buloke        | H. vulgare             | 2              | 105           | 0        | 0       | 2              | 82            | 10            | 12.2 ± 1.8 | 0.0069 |
| Baudin        | H. vulgare             | 2              | 123           | 0        | 0       | 2              | 159           | 28            | 17.6 ± 2.8 | 0.0109 |
| Zhepi8        | H. vulgare             | 2              | 168           | 0        | 0       | 2              | 128           | 26            | 20.3 ± 5.0 | 0.0307 |
| Supi3         | H. vulgare             | 2              | 231           | 0        | 0       | 2              | 85            | 21            | 24.7 ± 4.6 | 0.0198 |
| Linfen45      | Triticale              | 2              | 86            | 0        | 0       | 2              | 75            | 40            | 53.3 ± 5.1 | 0.0042 |
| ZS3297        | Triticale              | 1              | 35            | 0        | 0       | 1              | 51            | 21            | 41.2       | -       |
| ZS1257        | Triticale              | 1              | 43            | 0        | 0       | 1              | 56            | 11            | 19.6       | -       |
| ZS3224        | Triticale              | –              | –             | –        | –        | 1              | 67            | 11            | 16.4       | -       |
| Lanzhou Heimai | S. cereale             | 4              | 253           | 0        | 0       | 1              | 51            | 4             | 7.8        | -       |
| Fielder       | T. aestivum            | 3              | 95            | 71       | 74.7 ± 11.4 | 3              | 88            | 86            | 97.7 ± 2.5 | 0.0155 |
| Norin61       | T. aestivum            | 2              | 86            | 0        | 0       | 2              | 77            | 22            | 28.6 ± 4.1 | 0.0026 |
| Chinese Spring| T. aestivum            | 2              | 104           | 12       | 11.5 ± 7.2 | 2              | 111           | 28            | 25.2 ± 6.6 | 0.1853 |
| B73           | Z. mays                | 3              | 203           | 0        | 0       | 5              | 578           | 134           | 23.2 ± 4.0 | 0.0032 |
| A188          | Z. mays                | 2              | 145           | 9        | 6.2 ± 2.3  | 2              | 125           | 41            | 32.8 ± 5.6 | 0.0236 |

TaWOX5 has merits in the promotion of transformation efficiency. First, it dramatically increased the transformation efficiency of wheat varieties/lines to a high level in the present study. The transformation efficiency of selected wheat varieties, including Fielder, CB037, Zhouma18 and Zhengmai6994, was as high as 100% and 90% in small- and large-scale studies, respectively, when using TaWOX5 in many repeated experiments conducted over the past 5 years (Supplementary Table 2). Second, wheat varieties/lines previously recalcitrant to transformation, including Dwarfing Polish, BS366, Aikang58, Sunstate, Ningchun4 and Xinong979, were readily transformed with TaWOX5. Third, TaWOX5 proved efficient in the transformation of T. monococcum, barley, rye, triticale and maize. Fourth, transgenic regeneration shoots
overexpressing TaWOX5 developed into normal plants with healthy roots, and therefore TaWOX5 can be used by itself to increase transformation efficiency without the use of special promoters, which is convenient for improvement of transformation efficiency. Lastly, TaWOX5 can be used as a marker to differentiate transgenic from non-transgenic plants. Although overexpression of TaWOX5 had no negative effect on shoot differentiation or root development (Extended Data Fig. 5), it led to distinct phenotypes in transgenic wheat plants, including wide, short flag leaves and thick stems (Fig. 3d and Supplementary Table 5), thus helping us to recognize TaWOX5 transgenic wheat plants.

TaWOX5 has considerable potential applications in genetic transformation and genome editing for cereal crops. We incorporated TaWOX5 into our vector containing a double TDNA region (pWMB248) and the CRISPR-associated protein9 (Cas9) expression cassette (pWMB110-Cas9), in which TaWOX5 was linked with the Bar selection marker or Cas9, and TaWOX5 can be removed together with Bar or Cas9 in the progenies of transgenic or edited plants. A total of 51 and 33 wheat mutant plants for the TaQ gene were confirmed in 112 and 75 T₀ plants from Fielder and Jimai22, respectively, by PCR–restriction enzyme (PCR–RE). The editing efficiency of TaQ in Fielder was 45.5% with a control vector, and in Jimai22 was 44.0% with a TaWOX5 vector. Our results demonstrated that TaWOX5 is useful in the recovery of Cas9-edited events. Even though T₀ plants did not grow to maturity, we can assume that the tDNA locus would readily segregate from the edited locus in the next generation. Although the application of TaWOX5 cannot directly contribute to frequency improvement in the generation of marker-free or Cas9 cleavage plants, it is useful in regard to exclusion of most non-targeted candidate plants in segregating generations for obtaining marker-free or mutant plants, which can help to reduce workload by observing the TaWOX5 phenotype. For this workflow, we first selected plants without the phenotype of the wide flag leaf in the T₁ or T₂ generation then identified them for the absence of Bar and the presence of a target gene or edited sequence by PCR or PCR–RE and sequencing.

Conclusions

In summary, 31 common wheat cultivars were successfully transformed and developed into transgenic plants using TaWOX5. Overexpression of TaWOX5 also notably increased the transformation efficiency of T. monococcum, triticale, rye, barley and maize. The application of TaWOX5 can enhance the efficiency of the genetic transformation and genome editing of wheat and other crops and improve cost effectiveness by improving plant regeneration, reducing the requirement for embryo quality and identifying marker-free transgenic or transgene-free edited plants based on the visible botanical phenotypes associated with TaWOX5 overexpression.

Methods

Plant materials and cultivation conditions. Common wheat cultivars/lines Zhengmai7698, Zhengmai342, Zhengmai8160 and Zhengmai6694 were provided by W. Xu at the Wheat Research Institute, Henan Academy of Agricultural Sciences, Zhengzhou, China; Zhongmai895 and Zhongmai175 by Y. Zhang at the Institute of Crop Sciences, Chinese Academy of Agricultural Science (ICAS)-CAAS), Beijing, China; Luohannma by M. Hao at Sichuan Agricultural University, Chengdu, China; and Cang6005 and JS265 by X. Guo and H. Li at the Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang, China. Jimai22, Aikang58, Xinong979 and Jing411 were requested from the National Germplasm Bank at ICS-CAAS. Fielder and Chinese Spring were obtained from Yokohama City University, Yokohama, Japan, and Norin61 from Kyoto University, Kyoto, Japan. Three other wheat genotypes, CB037, Ningchun4 and Kenong199, and rye accession Lanzhou Heimai (RR, 2n = 14) were maintained in our laboratory. Tetraploid wheat (AABB, 2n = 28) line Dwarfing Polish was provided by Y. Wang at China Agricultural University, Chengdu, China. Barley (HH, 2n = 14) cultivars Flamingh, Supi3, Zhepi8, Baudin and Buloke were provided by Y. Xu at Yangtze University, Jingzhou, China. T. monococcum (AA, 2n = 14) accessions P242182 and Ctrl3961 and Ae. speltoides (SS, 2n = 14) accession P5554241 were provided by Y. Yan at Capital Normal University, Beijing, China, and triticate (AABBRR, 2n = 42) genotypes ZS3297, ZS1257, ZS3224 and Linfen45 were provided by Z. Wang at ICS-CAAS and F. Han at the Institute of Genetics and Developmental Biology of Chinese Academy of Sciences, Beijing, China. Maize inbred lines A188 and B73 were obtained from the National Agriculture and Food Research Organization, Tsukuba, Japan.

Cloning of TaWOX5. The coding sequence of Arabidopsis WUS (A012130) in NCBI was used as a query to search the homologous gene in common wheat (T. aestivum) via tblastn, resulting in only one sequence, FN564413. Based on this sequence, primer pair TaWOX5F (5′-GTGTCAATGGAGGCGCTGAGCG-3′) and TaWOX5R (5′-ATGCGTGCGTGCGACGTTGATT-3′) was designed to amplify TaWOX5 from the genomic DNA of wheat line CB037, T. monococcum accession Ctrl3961 and Ae. speltoides accession P5554241.

Because TaWOX5 is not an A1WUS orthologue, the protein sequence of A1WUS was used as a query for tblastn in IWGSC, with slight modifications. According to contig sequences, a pair of specific primers (5′-ATGGCAAGAACAGGCTGC-3′ and 5′-TACAGGAAATGCAAGGACGACT-3′) was designed to amplify the sequence, designated as TaWUS.

Vector construction. The primers CB1SmaF: 5′-AACCCCGGATGAGG GCCGTGACGGGG-3′ and CB1KpnR: 5′-AAAGGTACCTTACGACGATAC-3′ were used to perform PCR amplification using pMD-18T-TaWOX5 as a template with a high-fidelity enzyme KOD (Toyobo, KOD-401). The PCR product and pWMB003 vector (containing ZmUbi promoter and Nos terminator) were then digested with KpnI and Smal to obtain a 773-base-pair (bp) product and a 4,353-bp vector backbone. Next, the target PCR product and vector backbone were used to generate intermediate expression vector pWMB003-TaWOX5. Vectors pWMB003-TaWOX5 and pWMB111 (containing a Bar expression cassette controlled by ZmUbi promoter MZAS8107) were digested with HindIII to produce a 3,033-bp TaWOX5 expression cassette and a 10,170-bp vector backbone, respectively. Finally, the two enzyme-digested products were ligated to generate the target expression vector pWMB111-TaWOX5 (Extended Data Fig. 6a) for the transformation of wheat, T. monococcum, rye, triticale and barley. Vector pWMB202, containing anthocyanin biosynthesis-related genes ZmR and ZmC1 (ref. 1), was used to detect transient transformation efficiency in different wheat genotypes. The TaWOX5 expression cassette was inserted into the vector pLC41 (GenBank accession no. LC215638) containing a Bar expression frame controlled by Nos promoter and Nos terminator for transformation of wheat (varieties Chinese Spring and Norin61) and maize (inbred lines B73 and A188).

Vectors pWMB111-TaWOX5 and pWMB202 were introduced into Agrobacterium strain CS8C1, and vector pLC41-TaWOX5 was introduced into Agrobacterium strains EHA105 and LB4404 carrying pWG9, for wheat and maize transformation, respectively. pWG9 (SEQ ID 1 US10268385 B2) was a helper plasmid for plant transformation and contained bar, virR, virC, virD, virG and virJ from plasmid pTiB542 (GenBank accession no. NC_010929.1). The plasmids pLC41-TaWOX5 and pWG9 can be accessed for academic purposes with an MTA. According to our previously published methods1,6, a single-guide RNA (sgRNA) of TaWOX5 was constructed by primer TaQ-TaWOX5-5′-ATGGCAAGAACAGGCTGC-3′ (Fig. 3f) and vector SpCas9-TaQ for editing TaQ in the wheat cultivar Fielder. The whole expression cassette of TaWOX5 (ZmUbi-TaWOX5-NOS) was amplified from plasmid pWMB003-TaWOX5 and inserted onto vector pWMB110-SpCas9 to generate vector TaWOX5-SpCas9. The sgRNA of TaQ was introduced into TaWOX5-SpCas9 to generate vector TaWOX5-SpCas9-TaQ (Extended Data Fig. 6b) for editing TaQ in Jimai22.

Plant transformation. Wheat. Wheat spikes were sampled at 14 days post anthesis (DPA) and immature grains were carefully collected. Under aseptic conditions, grains were surface sterilized with 70% ethanol for 1 min and 5% sodium hypochlorite for 15 min and rinsed five times with sterile water. Fresh immature embryos were isolated and underwent Agrobacterium-mediated transformation to obtain transgenic plants following the protocol described by Ishida et al.1, with slight modifications. In brief, immature embryos were incubated with Agrobacterium for 5 min in co-cultivation WLS medium (1/10 Linsmaier and Skoog (LS) salts, 1/10 Murashige and Skoog (MS) vitamins, glucose 10 g l−1, 2-(N-morpholino)ethanesulfonic acid (MES) 0.5 g l−1 and acetylsyringone (AS) 100 μM, pH 5.8) at room temperature, and co-cultivated for 2 days on co-cultivation medium (WLS liquid medium plus AgNO₃, 0.85 mg l−1, CuSO₄·SH₂O 1.25 mg l−1 and agarose 8 g l−1), with the scutellum facing upwards, at 25°C under darkness. After co-cultivation, embryonic axes were removed with a scalpel and remaining source tissue was transferred to plates containing callus induction medium (LS salts, MS vitamins, 2.4–0.5 mg l−1 picloram 2.2 mg l−1, AgNO₃, 0.85 mg l−1, ascorbic acid 100 mg l−1, carbenicillin 250 mg l−1, cefotaxime 100 mg l−1, MES 1.95 g l−1 and agarose 5 g l−1) for delay culture for 5 days under the same conditions. Afterwards, tissues were cultured on selection medium (callus induction medium plus 4 mg l−1 picloram) for 4 weeks to induce further callus induction. Two weeks later, callus were placed on selection medium containing PPT 10 mg l−1 for 3 weeks for embryonic callus induction under darkness. Embryonic calli were then differentiated on 1/2 MS medium containing...
Barley. Barley transformation was performed following previously published protocols24, with a slight modification. Immature embryos were isolated after elongation and root formation. Plantlets with well-developed root systems were transferred for 21 days. Calli were cut into pieces of diameter 3–5 mm and transferred onto a shoot medium (first selection medium plus PPT 5 mg l−1). DNA was extracted following a standard CTAB method26. Total genomic DNA was amplified using a primer pair with a Bar gene was used to detect the transgene copy number in transgenic wheat plants. Duplexing a FAM-ACTTCCGTACCGAGCCG-MGB) for the TaWaxy-D primer pair increased the accuracy of ddPCR assays. The test for each sample was performed in duplicate.

DNA was calculated using QuantaSoft v.1.7.4.0917 (Bio-Rad). A no-template negative control was adopted in all ddPCR assays. The test for each sample was performed in duplicate.

Statistical analysis. Transformation efficiency is expressed as mean ± s.d., and data were analysed using SPSS 17.0. All data were tested using Student’s t-test (two-sided), in which P < 0.05 was considered statistically significant.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Accession numbers and gene names are available from the phylogenetic tree in Extended Data Fig. 1. The accession numbers of genes identified in this study are available in Supplementary Table 1, and their sequences are provided in the Supplementary sequence file. The accession number of pWMB11 is MZ458107. Raw data for experiments are available in Supplementary Tables 2 and 3. Transgenic lines and plasmids generated are available from the corresponding authors on request. Source data are provided with this paper.

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**Author contributions**

K.W. contributed to funding acquisition, experimental design, vector construction, wheat and barley transformation, data analysis and manuscript writing. L.S. contributed to gene identification, vector construction and transgenic detection. X.L. performed medium modification and wheat transformation. P.Z. was involved in gene identification and sequence analysis. W.W. was involved in barley transformation and manuscript writing. J.L. performed transformation of *T. monococcum* and rye. Y.C. performed transformation of triticale. Y.H. performed transformation of maize. C.Y. contributed to vector construction. L.D. contributed material management and medium preparation. Y.I. contributed to experimental design, wheat transformation and manuscript editing. X.Y. conceived the study, supervised experiments, conducted formal analysis and contributed to project administration, funding acquisition and manuscript editing.

**Competing interests**

K.W., X.Y. and L.D. (all ICS-CAAS) are co-inventors in Chinese patent application no. ZL201710422896.6. X.Y., K.W., L.S. and L.D. (all ICS-CAAS) and Y.I. and C.Y. (both JT) are co-inventors in international patent application no. PCT/CN2018/090239, in which ICS-CAAS and JT had shared ownership; the share of the latter was assigned to Kaneka Corporation, a Japanese chemical company, on 29 January 2021. The remaining authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41477-021-01085-8.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41477-021-01085-8.

Correspondence and requests for materials should be addressed to Ke Wang, Yuji Ishida or Xingguo Ye.

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Extended Data Fig. 1 | Phylogenetic relationships among TaWOX5 and TaWUS proteins from wheat, and WOX proteins from Arabidopsis. The phylogenetic tree was constructed based on the sequences of TaWOX5 and TaWUS proteins in wheat and WOX proteins in Arabidopsis in MEGA X using the neighbor-joining approach with 1,000 bootstrap replicates. Scale plate and legend in left display tree scale and bootstrap value. AtWUS: NP_565429; AtWOX1: NP_188428; AtWOX2: NP_200742; AtWOX3: NP_180429; AtWOX4: NP_175145; AtWOX5: NP_187735; AtWOX6: NP_565263; AtWOX7: NP_196196; AtWOX8: NP_199410; AtWOX9: NP_180944; AtWOX10: NP_173494; AtWOX11: NP_187016; AtWOX12: NP_197283; AtWOX13: NP_195280; AtWOX14: NP_173493. TaWOX5: MN412513; TaWUS-A: MW452946; TaWUS-B: MW452947; TaWUS-D: MW452945.
Extended Data Fig. 2 | Shoot regeneration of the immature embryos of different wheat genotypes promoted by the TaWOX5 gene. a: Shoot regeneration of the wheat embryos transformed with control vectors. b: Shoot regeneration of the wheat embryos transformed with TaWOX5 gene containing vector. The calli on plates were some overcrowding.
Extended Data Fig. 3 | Detection of transgenic wheat plants by QuickStix Kit and PCR. a: QuickStix Kit assay for the Bar protein; 1-21: transgenic plants; 22: wild-type Fielder. b: PCR detection for Bar gene, this testing experiment being repeated at least three times with similar results; 1: plasmid of TaWOX5 vector; 2: wild-type Fielder; 3-24: transgenic plants.
Extended Data Fig. 4 | Comparison of the transient infection efficiency of different wheat varieties by expressing anthocyanin biosynthesis genes ZmR and ZmC1 as visible markers.
Extended Data Fig. 5 | Normal growth of the regeneration shoots and roots derived from a transformed immature embryo of Fielder using the *Towox* gene in three experimental replicates. a: The growth status of regeneration shoots; b: the growth status of the transgenic plants with healthy shoots and roots.
**Extended Data Fig. 6** | The plasmids map of *pWMB111-TaWOX5* and *TaWOX5- SpCas9-ToQ*. 
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Data collection
The efficiencies were determined by counting the number of positive plants / total number of inoculated embryos. The QuantaSoft Version 1.7.4.0917 was used to collect the fluorescence signal of ddPCR.

Data analysis
The transformation efficiencies were expressed as the mean standard deviation (SD), and the data were analyzed using SPSS 17.0 (SPSS Inc, Chicago, IL, USA). All the data were tested using Student's t-test.

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| Data exclusions | No data was excluded from the analyses. |
| Replication | The number of replication are indicated in Fig. 1b, Fig.1c, Table1, Table 2, and Supplementary Tables 2 and 3. |
| Randomization | All experiments were randomized |
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Sampling strategy
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| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology and archaeology |
| ☒ | Animals and other organisms |
| ☐ | Human research participants |
| ☒ | Clinical data |
| ☒ | Dual use research of concern |

Methods

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|-----|-----------------------|
| ☒ | ChIP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

Antibodies

Antibodies used
Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.

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Policy information about cell lines

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|    | Enable evasion of diagnostic/detection modalities |
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ChIP-seq

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Magnetic resonance imaging

Experimental design

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Imaging type(s)

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Field strength

- Specify in Tesla

Sequence & imaging parameters

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☐ Used

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Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

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Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

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Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

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| [ ] | Graph analysis |
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