nature plants

LETTERS

https://doi.org/10.1038/s41477-021-01085-8

The gene *TaWOX5* overcomes genotype dependency in wheat genetic transformation

Ke Wang[1]✉, Lei Shi[1,4], Xiaona Liang[1,4], Pei Zhao[1], Wanxin Wang[1], Junxian Liu[2], Yanan Chang[2], Yukoh Hiei[3], Chizu Yanagihara[3], Lipu Du[1], Yuji Ishida[3]✉ and Xingguo Ye[1]✉

Although great progress has been achieved regarding wheat genetic transformation technology in the past decade[1–3], 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 efficiency[4–11]. In the present study, we found that overexpression of the wheat gene *TaWOX5* from the *WUSCHEL* family dramatically increases transformation efficiency with less genotype dependency than other methods. The expression of *TaWOX5* in wheat calli prohibited neither shoot differentiation nor root development. Moreover, successfully transformed transgenic wheat plants can clearly be recognized based on a visible botanic phenotype, relatively wider flag leaves. Application of *TaWOX5* improved wheat immature embryo transformation and regeneration. The use of *TaWOX5* in improvement of transformation efficiency also showed promising results in *Triticum monococcum*, triticale, rye, barley and maize.

Transformation of cereal crops including wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), barley (*Horderum vulgare* L.) and *Indica* rice (*Oryza sativa* L. ssp. *indica*) is not easy. Recently, a major advance in *Agrobacterium tumefaciens*-mediated wheat transformation efficiency (known as the PureWheat technique) was reported by Japan Tobacco Inc. (JT). This technique is based on a modified tissue culture medium and transformant selection regime, and it achieved transformation efficiency for the model wheat genotype Fielder as high as 50–90% (ref. [1]). Richardson et al.[2] employed this technique and obtained a transformation efficiency of around 6–45% for six Australian wheat cultivars. Moreover, 15 commercial Chinese common wheat cultivars were transformed with efficiency ranging from 2.9% to 22.7% using this technique[3]. However, the PureWheat technique still has limitations for transformation of many commercial wheat cultivars—for example, the transformation efficiency of Aikang58 and Jing411 still shows strong genotype dependency[3]. Therefore, methods of overcoming genotype dependence in wheat transformation need to be improved.

Although different tissue culture media and physical treatments can confer some improvement in the genetic transformation efficiency of cereal crops, genotype restriction strictly limits its success. Many studies have attempted to identify plant genes that exert effects on in vitro regeneration, and several candidate genes affecting somatic embryogenesis have been characterized—for example, *somatic embryogenesis receptor kinase* (SERK[4]), *LEAFY COTYLEDON1* (*LEC1* (ref. [5])), *LEAFY COTYLEDON2* (*LEC2* (ref. [6])), *NiR*[7], *BABY BOOM* (*BBM*[8]) and *WUSCHEL* (*WUS*[9,10]). In particular, co-overexpression of genes *BBM* and *WUS2* produced high transformation frequency in several previously transformation-recalcitrant inbred maize lines[11]. Moreover, mature 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 phenotypes[11].

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 phenotypes[12,13]. 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 Cadenza[15]. 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 *Arabidopsis*[10,16]. Based on the *Arabidopsis WUS* sequence, two wheat homologous genes (*TaWOX5* and *TaWUS*) were obtained. *TaWOX5* is more closely related to *Arabidopsis WOX5* (Extended Data Fig. 1) containing a WUS-related homeobox domain according to the description of the AtWUS protein structure[17], which belongs to the WOX5 type in the *WUS* gene family and is specifically expressed in the root tip[18]. In contrast, *TaWUS* is more

[1]Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China. [2]College of Life Science, Capital Normal University, Beijing, P. R. China. [3]Plant Innovation Center, Japan Tobacco Inc., Iwata, Japan. [4]These authors contributed equally: Lei Shi, Xiaona Liang. ✉e-mail: wangke03@caas.cn; yuji.Ishida@kaneka.co.jp; yexingguo@caas.cn

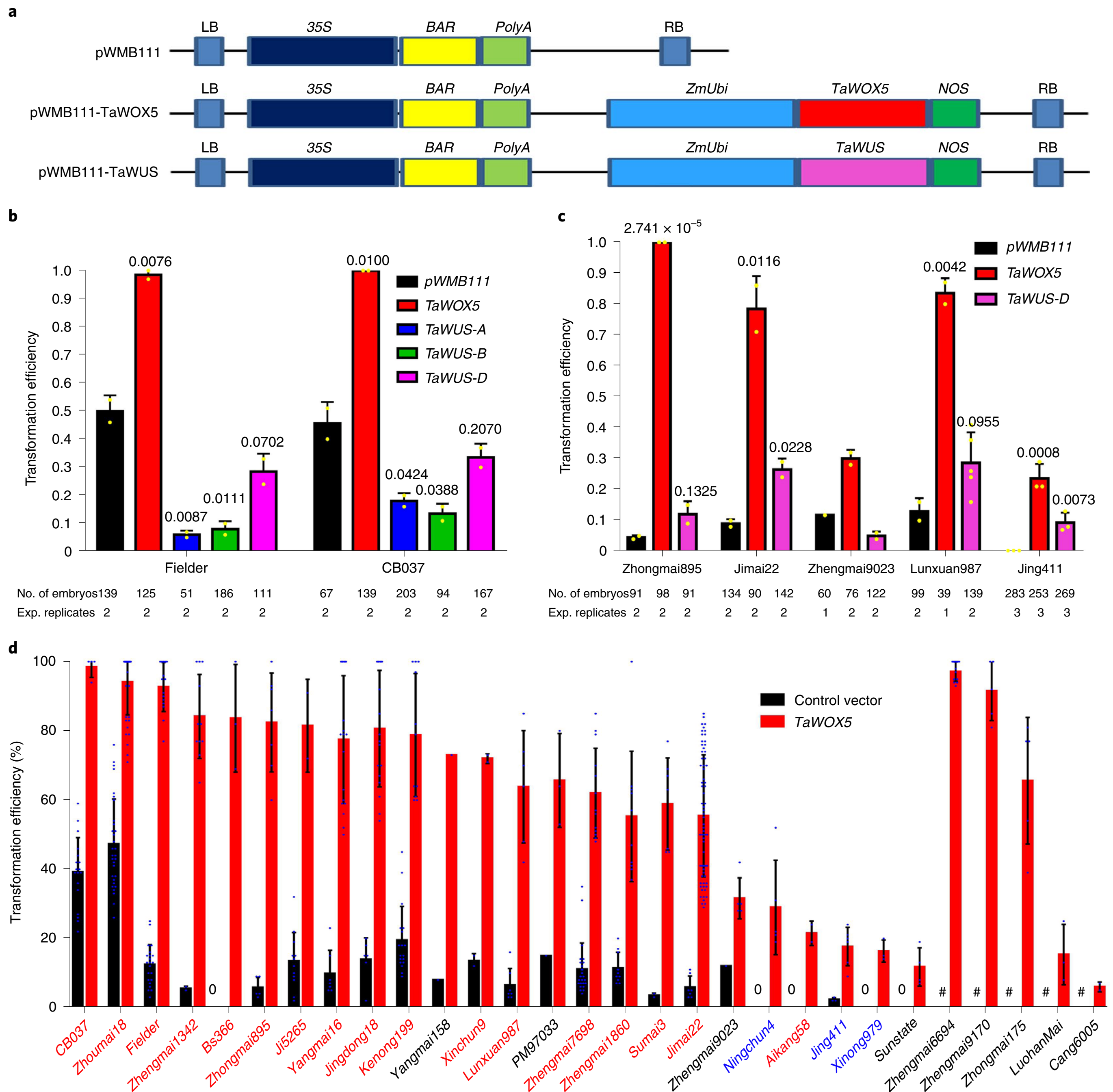


Fig. 1 | Effects of *TaWOX5* and *TaWUS* on transformation efficiency of selected wheat genotypes. a, Structure of the tDNA region on plasmids pWMB111, pWMB111-*TaWOX5* and pWMB111-*TaWUS*. **b**, Transformation efficiency of Fielder and CB037 using vectors containing *TaWOX5* and *TaWUS*, as well as control vector *pWMB111*. Data were tested using Student's *t*-test (two-sided), mean ± s.d. was plotted with all individual data points and exact *P* values are shown at the top of boxes. *n* (experimental (exp.) replicates) = 2 independent experiments. **c**, Transformation efficiency of Zhongmai895, Jimai22, Zhengmai9023, Lunxuan987 and Jing411 using vectors containing *TaWOX5* and *TaWUS-D*, as well as control vector *pWMB111*. Data were tested using Student's *t*-test (two-sided), mean ± s.d. was plotted with all individual data points, exact *P* values are shown at the top of boxes and *n* (exp. replicates) is listed at the bottom of the figure. **d**, Transformation efficiency of 29 wheat varieties using vector containing *TaWOX5* and control vector, in which different colours for selected varieties on the *x* axis represent different levels of *P* value significance (red, $P < 0.001$; blue, $P < 0.05$; black, no *P* value). Data were tested using Student's *t*-test (two-sided) and mean ± s.d. was plotted with all individual data points. Detailed information, including the number of embryos, exact *P* values and *n*, are shown in Supplementary Table 2. 0, transformation efficiency 0%; #, no data available.

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

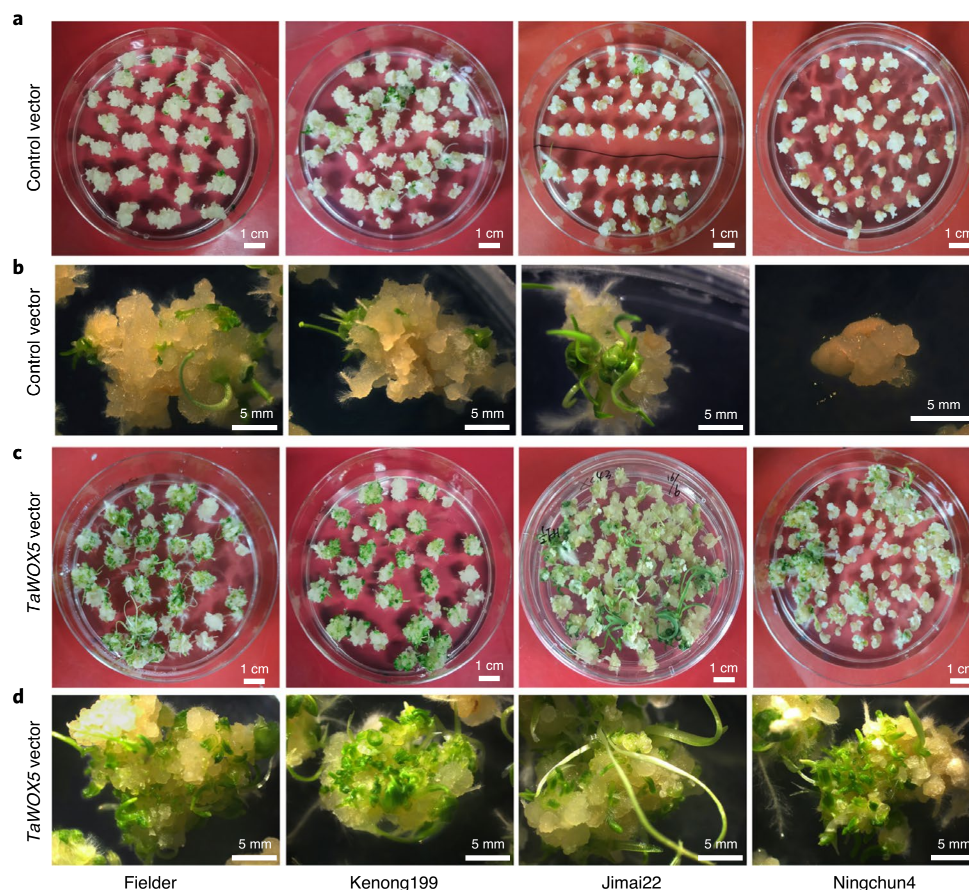


Fig. 2 | Shoot regeneration in different wheat genotypes at increased frequency using *TaWOX5*. **a**, Shoot regeneration of wheat immature embryos transformed with control vector. **b**, The enlarge view of **a**. **c**, Shoot regeneration of wheat immature embryos transformed with vector containing *TaWOX5*. **d**, The enlarge view of **c**. Calli on plates were overcrowded.

(*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, Lunxuan987 and Zhengmai9023. *TaWUS-D* improved the transformation efficiency of all tested wheat cultivars except Zhengmai9023. *TaWOX5* significantly improved the transformation efficiency of Zhongmai895, Jimai22, Lunxuan987 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

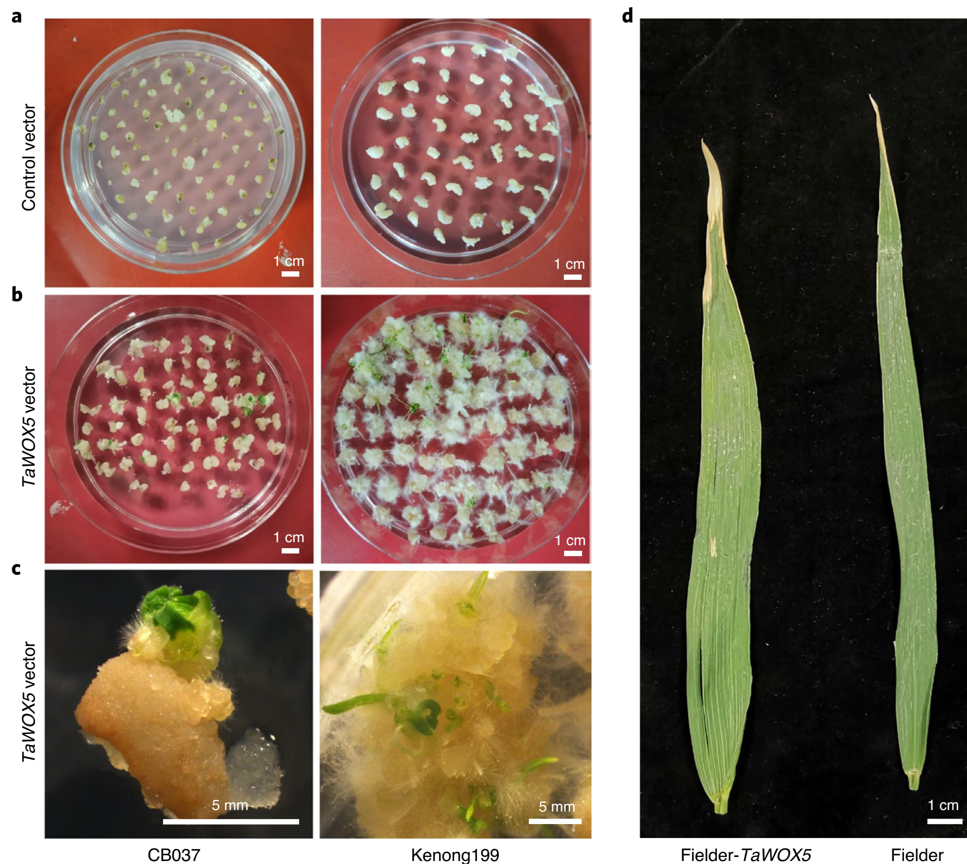


Fig. 3 | Regeneration improvement in immature embryos of poor physiological status by application of *TaWOX5* in two wheat genotypes. a, Shoot regeneration in wheat embryos transformed with control vector. **b**, Shoot regeneration in wheat embryos transformed with vector containing *TaWOX5*. **c**, 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.

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 T_1 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 study[19], 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 biosynthesis[20], 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%), Zhongmai895 (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.

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 (%)	
PI428182	*T. monococcum*	2	125	0	0	4	273	258	94.5 ± 8.2	0.0001
Dwarf Polish wheat	*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

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[21], which can often result in transformation failure[21,22]. In standard PureWheat methods, the physiological status of wheat immature embryos is also addressed to affect regeneration ability and transformation efficiency. When wheat mother plants are subjected to biotic or abiotic stress, the physiological state of immature embryos deteriorates. Typically, immature embryos from stressed wheat mother plants cannot generate embryonic calli[21]. There were no good-quality calli or transgenic shoots produced from the transformed immature embryos of CB037 and Kenong199 plants showing early senescence of the flag leaf in the transformation experiments with a control vector (Fig. 3a). However, when *TaWOX5* was used in transformation, immature embryos from stressed CB037 plants produced brown calli and generated between one and three green shoots per callus (Fig. 3b,c) while those from stressed Kenong199 plants generated between five and ten shoots per callus. These results demonstrate that the application of *TaWOX5* can enable the production by wheat immature embryos of poor status of transgenic plants with efficiency as high as 33.8% for CB037.

We also examined the effect of *TaWOX5* driven by the maize ubiquitin promoter on transformation of other cereal crop species, including *T. monococcum* line PI428182 and tetraploid wheat line Dwarfing Polish (*Triticum polonicum*); their transformation efficiency was significantly increased, to 94.5 ± 8.2% and 15.1 ± 6.0%, respectively ($P < 0.05$ or 0.001; Table 1). Barley (*Hordeum vulgare* L.) cultivar Vlamingh yielded a transformation frequency of 78.6 ± 9.8% using the *TaWOX5* vector, which was significantly higher than that with control vector (10.1 ± 4.3%; $P < 0.05$). Several commercial barley varieties (for example, Supi3, Zhepi8, Baudin and Buloke) known as being recalcitrant to transformation also showed significantly improved transformation frequency using *TaWOX5* ($P < 0.05$; Table 1). Additionally, *TaWOX5* increased transformation efficiency for rye (*Secale cereale* L.) accession Lanzhou Heimai (7.8%) and triticale (*Triticosecale* Wittmack) genotypes Linfen45, ZS3297, ZS1257 and ZS3224 (16.4–53.3%; Table 1).

To verify the transformation effects of *TaWOX5* on additional wheat genotypes and cereal plant species, *TaWOX5* was also tested at JT. *TaWOX5* significantly increased the transformation efficiency of Fielder, to 97.7 ± 2.5% from 74.7 ± 11.4% ($P < 0.05$); that of wheat variety Norin61 was significantly increased, to 28.6 ± 4.1% from 0 ($P < 0.001$); and that of wheat line Chinese Spring was also enhanced, to 25.2 ± 6.6% from 11.5 ± 7.2% (Table 1). In maize the transformation efficiency of inbred lines B73 and A188 was significantly increased, to 23.2 ± 4.0% and 32.8 ± 5.6% from 0% and 6.2 ± 2.3%, respectively, when using *TaWOX5* ($P < 0.05$) (Table 1).

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, Zhoumai18 and Zhengmai6694, 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 protein 9 (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 T_0 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 T_0 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 T_1 or T_2 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 notablely 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 botanic phenotypes associated with *TaWOX5* overexpression.

Methods

Plant materials and cultivation conditions. Common wheat cultivars/lines Zhengmai7698, Zhengmai1342, Zhengmai1860 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 (ICS-CAAS), Beijing, China; Luohanmai by M. Hao at Sichuan Agricultural University, Chengdu, China; and Cang6005 and Ji5265 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 Sichuan Agricultural University, Chengdu, China. Barley (HH, 2n = 14) cultivars Vlamingh, Supi3, Zhepi8, Baudin and Buloke were provided by Y. Xu at Yangtze University, Jingzhou, China. *T. monococcum* (AA, 2n = 14) accessions PI428182 and CItrl3961 and *Ae. speltoides* (SS, 2n = 14) accession PI554241 were provided by Y. Yan at Capital Normal University, Beijing, China, and triticale (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 (AJ012310) in NCBI was used as a query to search the homologous gene in common wheat (*T. aestivum*) via tblastn, resulting in only one sequence, FN564431.1. 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 CItrl3961 and *Ae. speltoide* accession PI554241.

Because *TaWOX5* is not an *AtWUS* orthologue, the protein sequence of AtWUS was used as a query for tblastn in IWGSC, with three contigs obtained. According to contig sequences, a pair of specific primers (5′-ATGGACAAGCAGAGCGTC-3′ and 5′-TAGGACAATGACGGGAGCACT-3′) was designed to amplify the sequence, designated as *TaWUS*.

Vector construction. The primers CB1SmaF: 5′-AAACCCGGGATGGAG GCGCTGAGCGG-3′ and CB1KpnR: 5′-AAAGGTACCTTAGACCAGATAC CGAT-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 *Kpn*I and *Sma*I to obtain a 773-base-pair (bp) product and a 4,535-bp vector backbone. Next, the target PCR product and vector backbone were ligated to generate intermediate expression vector *pWMB003-TaWOX5*. Vectors *pWMB003-TaWOX5* and *pWMB111* (containing a *Bar* expression cassette controlled by *ZmUbi* promoter MZ458107) were digested with *Hin*dIII 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. [20]), was used to detect transient transformation efficiency in different wheat genotypes. The *TaWOX5* expression cassette was inserted into the vector *pLC41* containing a *Bar* expression frame controlled by a *35S* 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 C58C1, and vector *pLC41-TaWOX5* was introduced into *Agrobacterium* strains EHA105 and LBA4404, for wheat and maize transformation, respectively. According to our previously published methods[23], the single-guide RNA of *TaQ* controlled by promoter *TaU3* was constructed into vector *pWMB110-SpCas9* containing *SpCas9* and *Bar* genes driven by promoters *ZmUbi* and *35S*, respectively, to generate 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 constructed 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 cocultivation WLS[1] liquid 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 acetosyringone (AS) 100 μM, pH 5.8) at room temperature, and cocultivated for 2 days on cocultivation medium (WLS liquid medium plus $AgNO_3$ 0.85 mg l^{-1}, $CuSO_4 \cdot 5H_2O$ 1.25 mg l^{-1} and agarose 8 g l^{-1}), with the scutellum facing upwards, at 25 °C under darkness. After cocultivation, embryonic axes were removed with a scalpel and remaining scutella were transferred onto plates containing callus induction medium (LS salts, MS vitamins, 2,4-D 0.5 mg l^{-1}, picloram 2.2 mg l^{-1}, $AgNO_3$ 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 phosphinothricin (PPT, Sigma, no. 45520) 5 mg l^{-1} without cefotaxime) for further callus induction. Two weeks later, calli 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 PPT 5 mg l^{-1} without zeatin (other than LSZ-P5 (ref. [1]) medium in PureWheat methods containing zeatin) at 25 °C under 100 μmol m^{-2} s^{-1} light. Regenerated shoots were transferred into cups filled with rooting medium plus PPT 5 mg l^{-1} for

elongation and root formation. Plantlets with well-developed root systems were transplanted into pots and cultivated in a growth chamber. Transformation of *T. monococcum*, rye and triticale was performed by the same methods used for wheat.

Barley. Barley transformation was performed following previously published protocols[24], with a slight modification. Immature embryos were isolated after sterilization of immature grains by the methods used for wheat, subsequently incubated (1/10 MS medium (Sigma, no. M5524) plus glucose 10 g l^{-1} and AS 100 µM) with *Agrobacterium* for 10 min, and cocultivated for 2 days on CM medium (1/10 MS medium plus glucose 10 g l^{-1}, AS 100 µM and agarose 8 g l^{-1}). Embryo axes were then removed and the remaining scutella cultured on the first selection medium (MS medium plus myoinositol 0.35 g l^{-1}, proline 0.69 g l^{-1}, thiamine HCl 1 mg l^{-1}, dicamba 2.5 mg l^{-1}, casein hydrolysate 1 g l^{-1}, PPT 5 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}). After 2 weeks, tissues were transferred to the second selection medium (first selection medium plus PPT 10 mg l^{-1} without cefotaxime). Three weeks later, the embryonic calli thus produced were cultured on DM medium (first selection medium without cefotaxime plus $CuSO_4 \cdot 5H_2O$ 2.5 mg l^{-1}, kinetin 1 mg l^{-1}, 6-benzylaminopurine 0.5 mg l^{-1} and 1-naphthaleneacetic acid 0.05 mg l^{-1}) at 25 °C under 100 µmol m^{-2} s^{-1} light for differentiation. Regenerated shoots were moved into a plastic box containing RT medium (first selection medium without cefotaxime plus indole butyric acid (IBA) 1 mg l^{-1}). Lastly, regenerated plants were transplanted into pots.

Maize. Maize transformation was performed following previously published protocols[25]. Between 8 and 15 DPA, maize spikes containing immature embryos were sampled. Immature embryos were isolated and infected by *Agrobacterium* for 5 min. Infected embryos were then transferred to LS-AS solid medium (1/10 LS medium plus 2,4-D 1.5 mg l^{-1}, sucrose 20 g l^{-1}, glucose 10 g l^{-1}, proline 0.7 g l^{-1}, MES 0.5 g l^{-1} and agarose 8 g l^{-1}) with scutella facing upwards, and incubated under darkness at 25 °C for 7 days. After cocultivation steps, embryos were transferred to the first selection medium (LS medium plus 2,4-D 1.5 mg l^{-1}, sucrose 20 g l^{-1}, glucose 10 g l^{-1}, proline 0.7 g l^{-1}, carbenicillin 250 mg l^{-1}, cefotaxime 250 mg l^{-1}, PPT 5 mg l^{-1}, MES 0.5 g l^{-1} and agar 8 g l^{-1}) for 10 days, and then moved to the second selection medium (first selection medium plus PPT 10 mg l^{-1}) for culture for 21 days. Calli were cut into pieces of diameter 3–5 mm and transferred onto a second, fresh selection medium. After culture for a further 21 days, embryonic calli were cut into pieces of diameter 2–3 mm and transferred to LSZ medium (LS medium plus zeatin 5 g l^{-1}, $CuSO_4 \cdot 5H_2O$ 2.5 mg l^{-1}, sucrose 20 g l^{-1}, carbenicillin 250 mg l^{-1}, cefotaxime 100 mg l^{-1}, PPT 5 mg l^{-1}, MES 0.5 g l^{-1} and agar 8 g l^{-1}) for shoot differentiation. Regenerated shoots were transferred to cups filled with LSF medium (LS medium plus IBA 0.2 mg l^{-1}, sucrose 15 g l^{-1}, MES 0.5 g l^{-1} and gellan gum 3 g l^{-1}). Finally, regenerated plants with developed roots were transplanted into pots.

Detection of transgenic plants. *QuickStix detection.* Transgenic plants were detected for the *Bar* gene using the QuickStix Kit (EnviroLogix, no. AS013LS) according to the manufacturer's instructions.

PCR analysis. Genomic DNA was extracted from the leaves of transgenic plants using a NuClean PlantGen DNA Kit (CWBIO, no. CW0531M). The primer pair 5′-ACCATCGTCAACCACTACATCG-3′ and 5′-GCTGCCAGAAACCACGTCATG-3′ was used to amplify the 429-bp fragment specific to the *Bar* gene in transgenic plant samples.

ddPCR. Total genomic DNA was extracted following a standard CTAB method[26]. A single-copy wheat *TaWaxy* gene on genome D was used as reference gene for ddPCR, and its specific primers (TaWaxy-DF: 5′-GCCACGTCGAAGAAGGAGATC-3′ and TaWaxy-DR: 5′-GACAGGTTCAGCCGGTATGTG-3′) and probe (TaWaxy-DProbe: VIC-CCTGCACTGTTGCTCGC CGCT-BHQ) were employed[27]. The primer pairs (BarF: 5′-TCGTCAACCACTACATCGAGACA-3′ and BarR: 5′-GTCCACTCCTGCGGTTCCT-3′) and probe (BarProbe: FAM-ACTTCCGTACCGAGCCG-MGB) for the *Bar* gene were designed to detect the transgene copy number in transgenic wheat plants. Duplexing a *TaWaxy*-D primer pair with a *Bar* primer pair increased the accuracy of ddPCR assay by enabling normalization and direct amplicon comparison within and across samples. Each reaction mixture (20 µl) consisted of 10 µl of ddPCR TM supermix (Bio-Rad, no. 1863010), 0.9 µM primers, 250 nM probe and 200 ng of DNA. The mixtures and droplet generation oil (Bio-Rad, no. 1863005) were added in cartridges and loaded into a QX200 droplet generator (Bio-Rad) for droplet generation. Droplet emulsions were transferred to a 96-well PCR plate and sealed with a foil heat seal at 180 °C for 10 s. PCR amplification was run at 95 °C for 10 min, 40 cycles of 94 °C for 30 s and 54 °C for 40 s, with a final cycle of 98 °C for 10 min. A QX200 Droplet Reader (Bio-Rad) was used for automatic measurement of the fluorescence signal of each droplet. Quantification of target 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 *pWMB111* 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.

Received: 20 May 2021; Accepted: 1 December 2021;
Published online: 13 January 2022

References

1. Ishida, Y. Tsunashima, M. Hiei, Y. & Komari, T. in *Agrobacterium Protocols* Vol. 1 (ed. Wang, K.) 189–198 (Springer, 2015).
2. Richardson, T., Thistleton, J., Higgins, T. J., Howitt, C. & Ayliffe, M. Efficient *Agrobacterium* transformation of elite wheat germplasm without selection. *Plant Cell Tissue Organ Cult.* **119**, 647–659 (2014).
3. Wang, K., Liu, H. Y., Du, L. P. & Ye, X. G. Generation of marker-free transgenic hexaploid wheat via an *Agrobacterium*-mediated co-transformation strategy in commercial Chinese wheat varieties. *Plant Biotechnol. J.* **15**, 614–623 (2017).
4. Zakizadeh, H., Stummann, B. M., Lutken, H. & Muller, R. Isolation and characterization of four somatic embryogenesis receptor-like kinase (RhSERK) genes from miniature potted rose (*Rosa hybrida* cv. Linda). *Plant Cell Tissue Organ Cult.* **101**, 331–338 (2010).
5. Lotan, T. et al. *Arabidopsis* LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. *Cell* **93**, 1195–1205 (1998).
6. Stone, S. L. et al. LEAFY COTYLEDON2 encodes a B3 domain transcription factor that induces embryo development. *Proc. Natl Acad. Sci. USA* **98**, 11806–11811 (2001).
7. Nishimura, A. et al. Isolation of a rice regeneration quantitative trait loci gene and its application to transformation systems. *Proc. Natl Acad. Sci. USA* **102**, 11940–11944 (2005).
8. Boutilier, K. et al. Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell* **14**, 1737–1749 (2002).
9. Bouchabke-Coussa, O. et al. Wuschel overexpression promotes somatic embryogenesis and induces organogenesis in cotton (*Gossypium hirsutum* L.) tissues cultured *in vitro*. *Plant Cell Rep.* **32**, 675–686 (2013).
10. Zuo, J. R., Niu, Q. W., Frugis, G. & Chua, N. H. The *WUSCHEL* gene promotes vegetative-to-embryonic transition in *Arabidopsis*. *Plant J.* **30**, 349–359 (2002).
11. Lowe, K. et al. Morphogenic regulators baby boom and wuschel improve monocot transformation. *Plant Cell* **28**, 1998–2015 (2016).
12. Lowe, K. et al. Rapid genotype 'independent' *Zea mays* L. (maize) transformation via direct somatic embryogenesis. *In Vitro Cell Dev. Biol. Plant* **54**, 240–252 (2018).
13. Gao, H. R. et al. Superior field performance of waxy corn engineered using CRISPR–Cas9. *Nat. Biotechnol.* **38**, 579–581 (2020).
14. Hoerster, G. et al. Use of non-integrating Zm-Wus2 vectors to enhance maize transformation. *In Vitro Cell Dev. Biol. Plant* **56**, 265–279 (2020).
15. Debernardi, J. M. et al. A GRF-GIF chimeric protein improves the regeneration efficiency of transgenic plants. *Nat. Biotechnol.* **38**, 1274–1279 (2020).
16. Su, Y. H. et al. Auxin-induced WUS expression is essential for embryonic stem cell renewal during somatic embryogenesis in *Arabidopsis*. *Plant J.* **59**, 448–460 (2009).
17. van der Graaff, E., Laux, T. & Rensing, S. A. The WUS homeobox-containing (WOX) protein family. *Genome Biol.* **10**, 248 (2009).
18. Sarkar, A. K. et al. Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* **446**, 811–814 (2007).
19. Zhang, W. et al. Regeneration capacity evaluation of some largely popularized wheat varieties in China. *Acta Agron. Sin.* **44**, 208–217 (2018).
20. Riaz, B. et al. Overexpression of maize *ZmC1* and *ZmR* transcription factors in wheat regulates anthocyanin biosynthesis in a tissue-specific manner. *Int. J. Mol. Sci.* **20**, 5806 (2019).
21. Wang, X. M. et al. Effects of environmental temperature on the regeneration frequency of the immature embryos of wheat (*Triticum aestivum* L.). *J. Integr. Agric.* **13**, 722–732 (2014).
22. Tao, L. L. et al. Improvement of plant regeneration from immature embryos of wheat infected by *Agrobacterium tumefaciens*. *Agric. Sci. China* **10**, 317–326 (2011).

23. Liu, H. Y. et al. Efficient induction of haploid plants in wheat by editing of *TaMTL* using an optimized *Agrobacterium*-mediated CRISPR system. *J. Exp. Bot.* **71**, 1337–1349 (2021).
24. Bartlett, J. G., Alves, S. C., Smedley, M., Snape, J. W. & Harwood, W. A. High-throughput *Agrobacterium*-mediated barley transformation. *Plant Methods* **4**, 22 (2008).
25. Ishida, Y., Hiei, Y. & Komari, T. *Agrobacterium*-mediated transformation of maize. *Nat. Protoc.* **2**, 1614–1621 (2007).
26. Sambrook, J. Russell, D. W. *Molecular Cloning, A Laboratory Manual* 3rd ed. Chapter 1, 34–36 (Cold Spring Harbor Laboratory Press, 2001).
27. Paternò, A. et al. In-house validation and comparison of two wheat (*Triticum aestivum*) taxon-specific real-time PCR methods for GMO quantification supported by droplet digital PCR. *Food Anal. Methods* **11**, 1281–1290 (2018).

Acknowledgements

We thank W. Xiao at Saint Louis University, USA and H. Li at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences for critical revisions of this manuscript. This research was financially supported by grants from the National Natural Science Foundation of China (no. 31971946) to K.W., the Science and Technology Department of Ningxia in China (no. 2019BBF02020) to X.Y. and the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (nos. S2021ZD03 and 2060302-2-19) to K.W. and X.Y..

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.

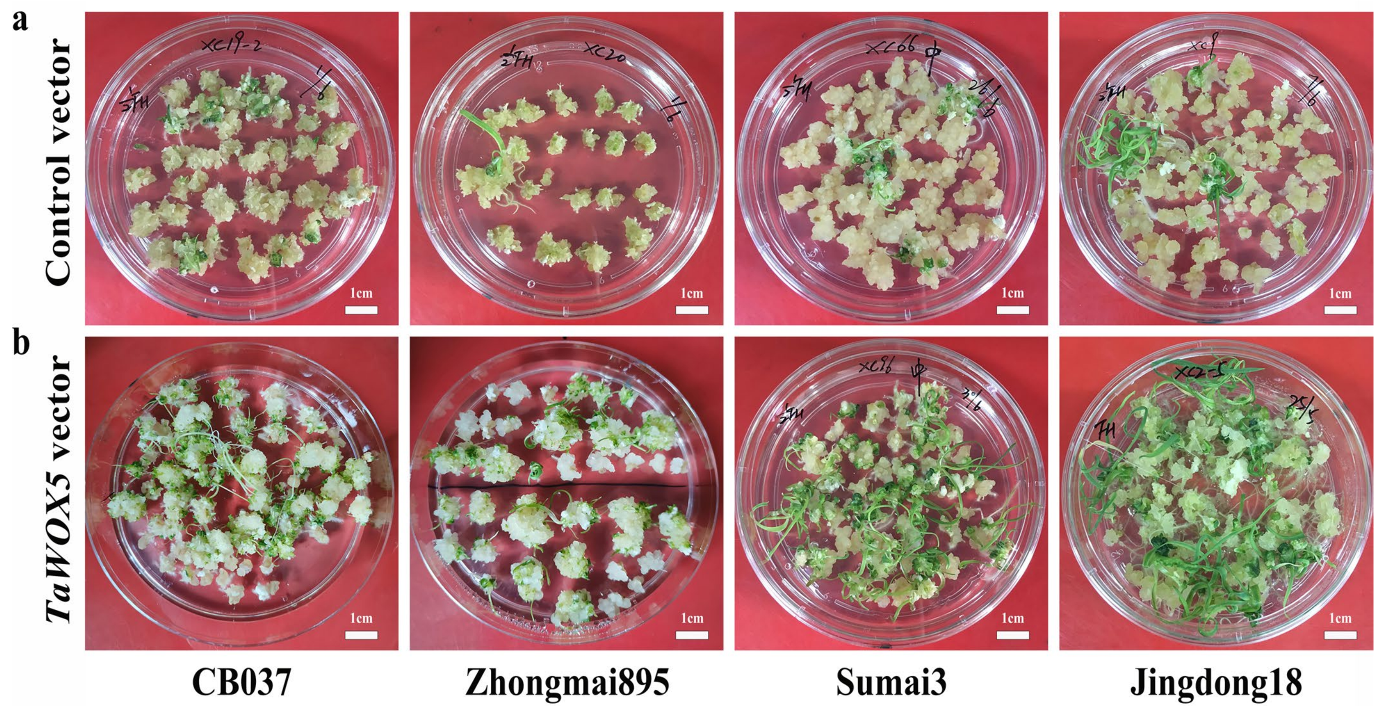
Peer review information *Nature Plants* thanks Sadiye Hayta and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

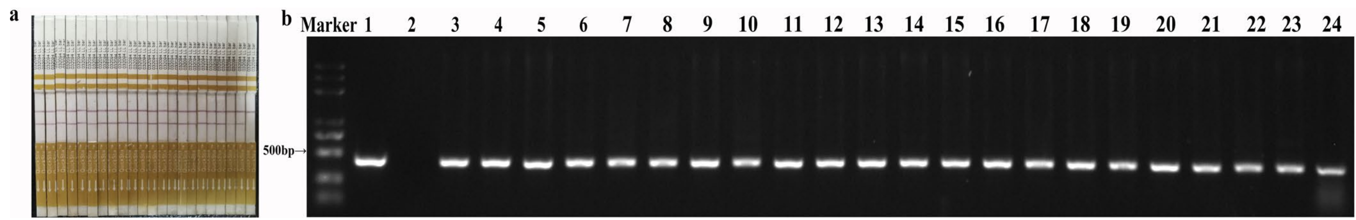
Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2022

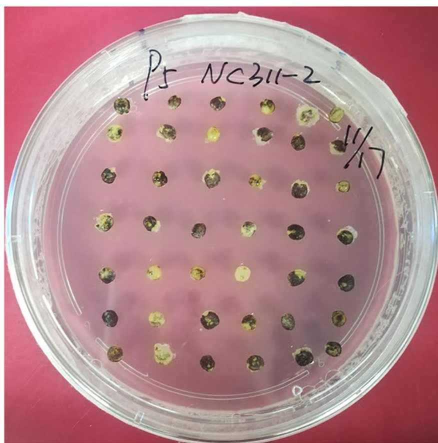
Extended Data Fig. 1 | Phylogenetic relationships among TaWOX5 and TaWUS proteins from wheat, and WOX proteins from *Arabidopsis*. The phylogenetic tree was constructed based on the sequences of TaWOX5 and TaWUS proteins in wheat and WOX proteins in *Arabidopsis* in MEGA X using the neighbor-joining 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.

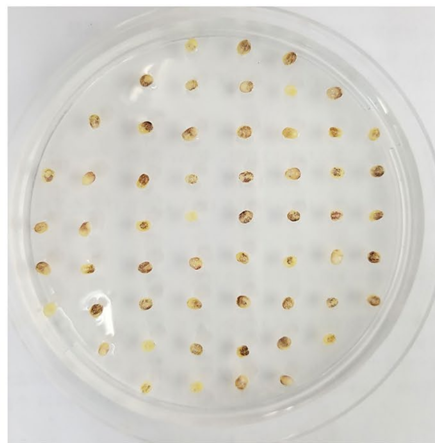


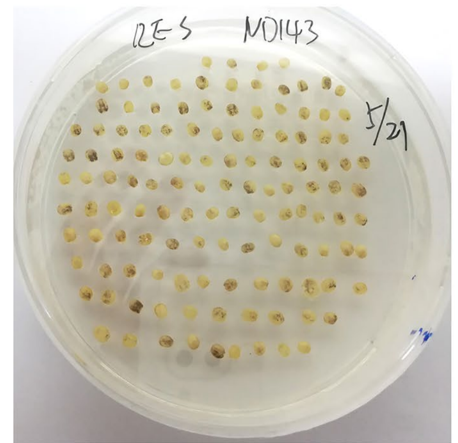
Extended Data Fig. 2 | Shoot regeneration of the immature embryos of different wheat genotypes promoted by the *TaWOX5* gene. a: Shoot regeneration of the wheat embryos transformed with control vectors. b: Shoot regeneration of the wheat embryos transformed with *TaWOX5* gene containing vector. The calli on plates were some overcrowding.

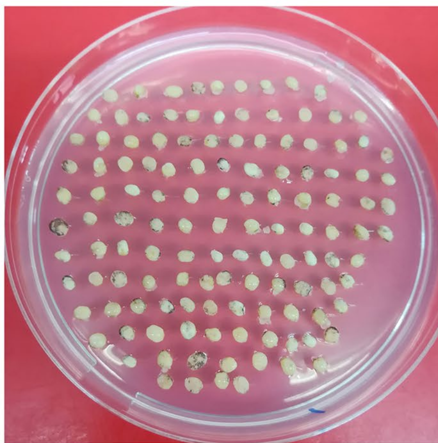


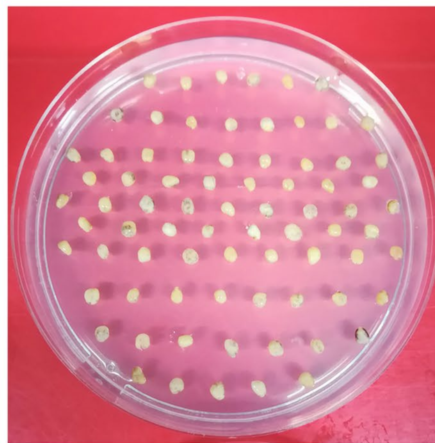
Extended Data Fig. 3 | Detection of transgenic wheat plants by QuickStix Kit and PCR. a: QuickStix Kit assay for the Bar protein; 1-21: transgenic plants; 22: wild-type Fielder. b: PCR detection for *Bar* gene, this testing experiment being repeated at least three times with similar results; 1: plasmid of *TaWOX5* vector; 2: wild-type Fielder; 3-24: transgenic plants.

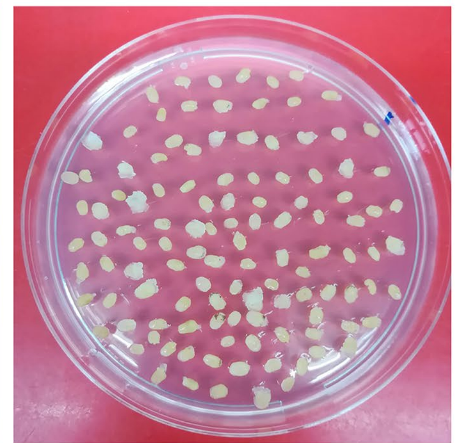




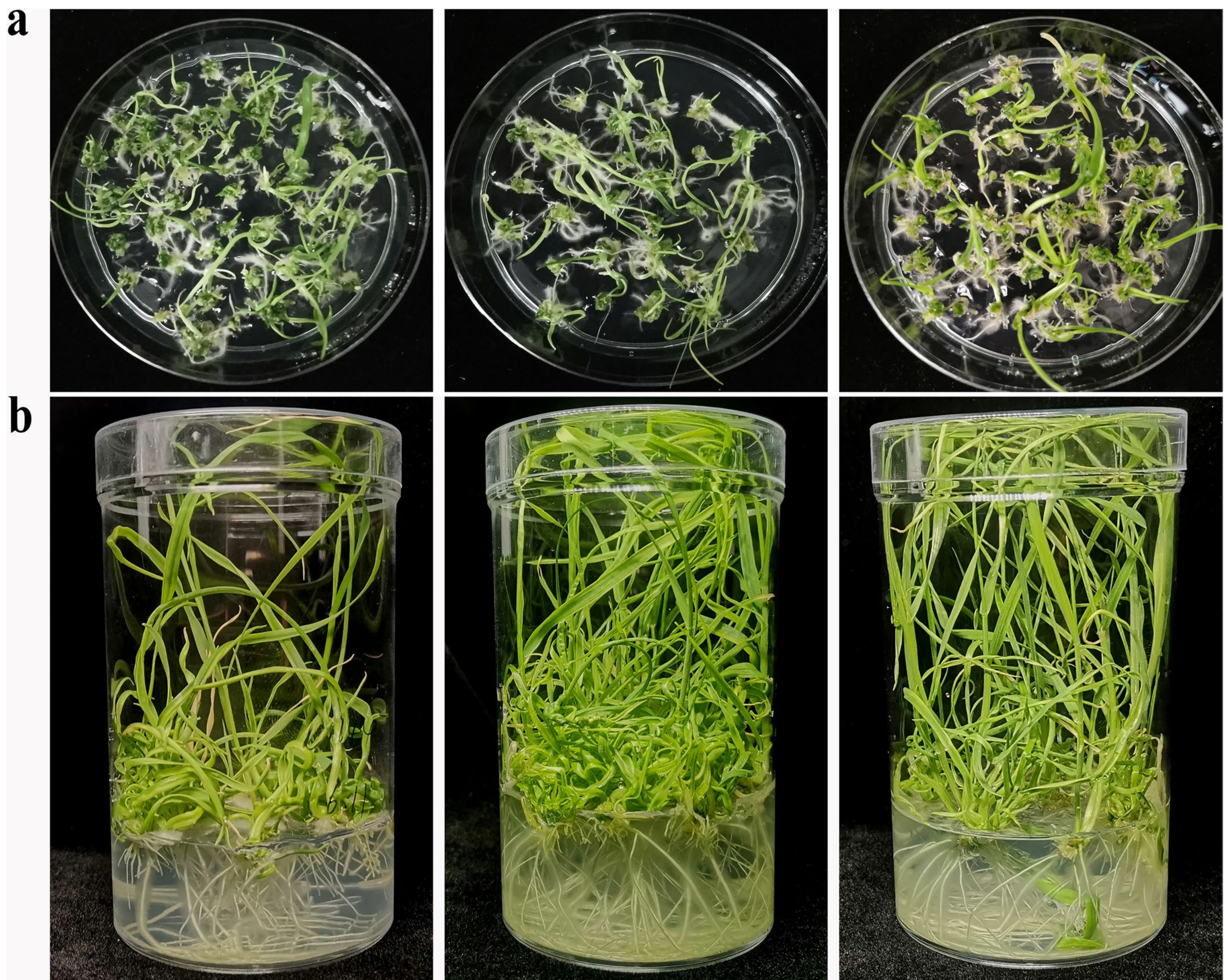




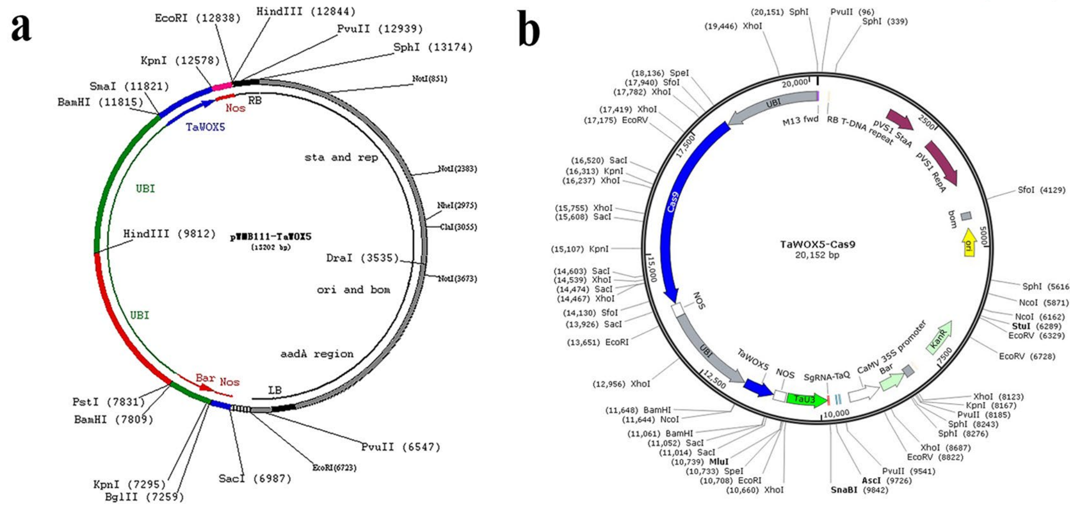




Extended Data Fig. 4 | Comparison of the transient infection efficiency of different wheat varieties by expressing anthocyanin biosynthesis genes *ZmR* and *ZmC1* as visible markers.



Extended Data Fig. 5 | Normal growth of the regeneration shoots and roots derived from a transformed immature embryo of Fielder using the *TaWOX5* 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-TaQ*.

nature research

Corresponding author(s): Xingguo Ye

Last updated by author(s): Nov 18, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

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.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The accession numbers and gene names are available in the phylogenetic tree in Extended data Fig. 1. The accession numbers of the genes identified in this study are available in Supplementary Table 1, and their sequences were also provided in Supplementary sequence file. Accession number of pWMB111 was MZ458107. The raw data for different experiments are available in Supplementary Tables 2 and 3. The transgenic lines and plasmids generated are available from the corresponding authors upon request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size: The number of experiments (1-68) is indicated for each graph and analysis and the total number of embryos (40-5459) is indicated below each box-plot and Supplementary Table 2 for each genotype.

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

Blinding: All the experiments were used the numbers instead of genes to achieve blind experiments

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description: *Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*

Research sample: *State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*

Sampling strategy: *Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*

Data collection: *Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*

Timing: *Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*

Data exclusions: *If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*

Non-participation: *State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*

Randomization: *If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description: *Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*

Research sample: *Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*

Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☒ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study	
☒	☐	Antibodies
☒	☐	Eukaryotic cell lines
☒	☐	Palaeontology and archaeology
☒	☐	Animals and other organisms
☒	☐	Human research participants
☒	☐	Clinical data
☒	☐	Dual use research of concern

Methods

n/a	Involved in the study	
☒	☐	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.*
Validation	*Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	*State the source of each cell line used.*
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*

Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	*For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.*
Wild animals	*Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation: *Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*

Instrument: *Identify the instrument used for data collection, specifying make and model number.*

Software: *Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*

Cell population abundance: *Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*

Gating strategy: *Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type: *Indicate task or resting state; event-related or block design.*

Design specifications: *Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*

Behavioral performance measures: *State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s): *Specify: functional, structural, diffusion, perfusion.*

Field strength: *Specify in Tesla*

Sequence & imaging parameters: *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*

Area of acquisition: *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software: *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization: *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*

Normalization template: *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal: *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

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

Statistical modeling & inference

Model type and settings | Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested | Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016) | Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction | Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity | Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis | Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis | Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.