The gene *TaCB1* overcomes genotype dependency in wheat genetic transformation

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Letter

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Abstract

Genotype dependency is the most important factor in wheat genetic transformation, which further limits 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. In the present study, a wheat gene *TaCB1* in the *WUSCHEL* family was identified to dramatically increase the transformation efficiencies of many wheat varieties without genotype dependency after its over-expression. The expression of *TaCB1* in wheat calli did not prohibit shoot differentiation and root development. The application of *TaCB1* can lighten the requirement to wheat immature embryo for plant regeneration. Transgenic wheat plants can be clearly recognized by the visible phenotype of wide flag leaves. The promise function of *TaCB1* on improving transformation efficiency was also tested in *T. monococcum*, triticale, rye, barley, and maize.

Main

The cereal crops, such as common wheat (*Triticum aestivum* L.) and its relative species, maize (*Zea mays* L.), barley (*Horderum vulgare* L.), and *Indica* rice (*Oryza sativa* L. ssp. *indica*), are not easy to be transformed and their transformation efficiencies are usually low. Recently, a big progress on wheat transformation mediated by *Agrobacterium tumefaciens* called as PureWheat technique has been made by Japan Tobacco Inc using modified tissue culture media and transformant selection regime, by which the transformation efficiency for a model wheat genotype Fielder is up to 50%[1]. Richardson et al.[2] employed this technique and obtained a transformation efficiency around 45% for a few Australian wheat cultivars. Moreover, 15 commercial Chinese hexaploid wheat cultivars were transformed with the efficiency from 2.9% to 22.7% using of the PureWheat technique[3]. However, PureWheat still has limitation in transforming many commercialized wheat cultivars which show strong genotype-dependency, being low efficiencies or recalcitrant to transformation even using PureWheat[2,3]. Therefore, overcoming genotype-dependence in transformation is still needed for wheat improvement.

Although different tissue culture media and physical treatment displayed some effects on the improvement of the genetic transformation efficiency of cereal crops, it is well known that the genotype restriction is still the key limited factor in the transformation manipulation. Many researches have been conducted on the identification of plant genes related to *in vitro* regeneration, and a few candidate genes affecting somatic embryogenesis have been characterized such as *somatic embryogenesis receptor kinase* (SERK[4]), *LEAFY COTYLEDON1(LEC1) (LEC1*[5]), *LEAFY COTYLEDON2(LEC2*[6]), *NiR*[7], *BABY BOOM* (*BBM*[8]), *WUSCHEL* (*WUS*[9,10]). Especially, co-overexpression of *BBM* and *WUS2* genes led to high transformation frequencies in several previously transformation-recalcitrant maize inbred lines[11]. Moreover, maize mature embryos and leaf tissues were all used to generate transgenic plants with available efficiencies assisting by the two morphogenic regulators of BBM and WUS2. In addition, the co-expression of *BBM* and *WUS2* obviously enhanced the transformation efficiencies of some recalcitrant genotypes of sorghum [*Sorghum bicolor* (L.) Moench], *Indica* rice (*Oryza sativa* L. ssp. *Indica*), and sugarcane (*Saccharum officinarum* L.). However, over-expression of *WUS2* and *BBM* in the cereal crops led to many negative effects such as callus necrosis, difficult differentiation of shoots and roots, decreased fertility of transgenic plants, aberrant, stunted, and twisted phenotypes[11]. Most recently, the expression of *GRF4-GIF1* chimera was found to achieve a transformation efficiency of 60% in teraploid wheat (Desert King and Kronos) and hexaploid wheat (Fielder), even 9-19% in two non-transformable hexaploid wheat (Hahn and Cadenza), using the PureWheat protocol[12]. In this paper we report that overexpression of a wheat gene *TaCB1* related to efficient plant regeneration in the *WUS*

family dramatically improve the transformation frequency of wheat as well as a few other cereal plants with less genotype-dependence and no obvious negative effects on callus differentiation and plant phenotype.

The *WUS* gene is an important regulator for somatic embryogenesis in *Arabidopsis*[13]. Based on the *Arabidopsis WUS* sequence, two wheat homologous genes (*TaCB1* and *TaCB2*) were obtained. Gene *TaCB1* was more closely related to *Arabidopsis WOX5* and *WOX7* genes containing WUS related homeobox domain (Extended data Fig. 1) according to the description on the classification of AtWUS protein structure by Graaff et al.[14], which belongs to WOX5 type in the *WUS* gene family and specifically is expressed in roots tips[15]. While, *TaCB2* gene was more closely related to *AtWUS* gene (Extended data Fig. 1) and can be known as *TaWUS* exactly. Further, six homologous *TaCB1* genes were obtained from a common wheat line CB037 (AABBDD, 2n=42), and three from a *T. monococcum* accession CItrl3961 (AA, 2n=14), and an *Ae. speltoides* (SS, 2n=14) accession PI554241 (Supplementary Table 1), respectively. Thus, we speculated that *TaCB1* has at least nine copies in common wheat. Three homologous *TaCB2* genes (*TaWUS-A*, *TaWUS-B* and *TaWUS-D*) were also obtained from CB037 (Supplementary Table 1).

The genomic sequences of *TaCB1*, *TaCB2-A* (*TaWUS-A), TaCB2-B* (*TaWUS-B)* and *TaWUS-D* isolated from CB037 were manipulated under the control of maize *ZmUbi* promoter and *Nos* terminator and then linked onto an expression vector *pWMB111*, respectively, which only contains the *Bar* gene in the T-DNA region (Fig. 1a). When the construct *pWMB111-TaCB1* was introduced into two wheat genotypes (Fielder and CB037) by *Agrobacterium*-mediated approach, the transformation efficiencies were nearly 100%, but the transformation efficiencies using the control vector *pWMB111* were around 50% (Fig. 1a). While the transformation efficiencies using the constructs *TaWUS-A, TaWUS-B* and *TaWUS-D* were lower than that using pWMB111 because many regeneration shoots in the transformation experiments did not produce roots and were excluded in the transgenic plants. We observed that *TaCB2* greatly promoted shoot production among which *TaWUS-D* was most effective. Therefore, to further verify the function of *TaCB1* and *TaCB2* in regeneration, vectors *pWMB111*, *pWMB111-TaCB1* and *pWMB111-TaWUS-D* were transformed into several widely commercialized wheat cultivars, such as Zhongmai895, Jimai22, Jing411, Lunxuan987 and Zhengmai9023. Results showed that *TaWUS-D* could improve the transformation efficiencies of the cultivars except Zhengmai9023. While the efficiencies of all the varieties were greatly improved by *TaCB1*, nearly reaching 100% in Fielder, CB037 and Zhongmai 895 (Fig. 1b, 1d). These results indicate that *TaCB1* is the best choice in the transformation efficiency improvement.

In the past five years, 29 hexaploid wheat varieties in total including some special recalcitrant varieties were successfully transformed with various target genes by application of *TaCB1*. Without using *TaCB1* in the transformation, Ningchun4 didn't regenerate green shoots, Jimai22 and Kenong199 showed only a few green shoots, and Fielder showed less than 10 green shoots on each immature embryo. Using *TaCB1*, all varieties displayed dozens of green shoots on each immature embryo (Fig. 2). Jimai22 is the most widely cultivated variety in China with 2 million hectares each year, and its immature embryos derived calli are very poor in quality. However, the quality of the Jimai22 calli overexpressing *TaCB1* was dramatically improved (Fig. 2), and the transformation efficiency of this cultivar was increased from 5.8% to 55.4% (Fig. 1c and Supplementary Table 2). Similar improved results were also achieved in other wheat cultivars with the assistance of *TaCB1* for obtaining transgenic plants (Extended data Fig. 2 and Supplementary Table 2). As shown in Fig.1d and Supplementary Table 2, the application of *TaCB1* increased the transformation efficiencies of CB037, Fielder and Kenong199, which are regarded as easily transformed wheat varieties with a transformation efficiency of 20-45% under the using of a common vector, to 96.2%, 94.5% and 75.7% from 39.9%, 45% and 17.7%, respectively. With the assistance of *TaCB1*, the

transformation efficiencies of poorly transformed varieties Zhongmai895, Sumai3 and Jing411 (transformation efficiency is less than 8% using a common vector) were 82.7%, 57.4% and 17.5%, respectively. The transformation efficiencies of non-transformable varieties Bs366, Ningchun4, Aikang58, Xinong979, and Sunstate were 83.5%, 29.3%, 21.8%, 16.7%, and 9.1% using *TaCB1*, respectively. Moreover, transgenic plants were successfully generated from Zhengmai6694, Zhengmai9170, Zhongmai175, Luohanmai (a special landrace genotype) and Cang6005 using *TaCB1* with an efficiency of 6.1-97.8%. During the past a few years, 2322 immature embryos of Fielder were transformed with a common vector, and 3290 immature embryos of Fielder and 5459 immature embryos of Jimai22 were transformed with a *TaCB1* containing vector. The immature embryos used for transformation experiment from most varieties exceeded 1,000 in amount (Supplementary Table 2). Parts of the transgenic plants were confirmed by the Southern blotting, PCR and QuickStix Kit (Extended data Fig. 3). The above data demonstrated that the application of *TaCB1* greatly improves the transformation efficiency with less genotype-dependency in wheat.

Theoretically, *Agrobacterium*-mediated plant transformation depends on infection efficiency of bacteria and the ability of host cell regeneration. Obviously, the application of *TaCB1* can enhance the ability of the host tissues for callus induction and regeneration. In this case, whether a target gene could transfer into wheat cells will limit the final transformation efficiency, and high transformation efficiency could be achieved if the expression cassette can be well delivered into the target cells by the bacteria with a high efficiency. The sensitivity of different wheat genotypes to *Agrobacterium* varied in a large extent. Interestingly, despite genotypes differ in their transformation efficiency, the efficiencies were less related to their regeneration abilities even if *TaCB1* was applied. According to our evaluation in a previous study[16], the order of regeneration capacity for the widely cultivated Chinese commercial wheat cultivars was Zhongmai895, Ningchun4, Aikang58, Xinong979, and Jimai22. While the transformation efficiency of Jimai22 was 55.4% by virtue of *TaCB1*, being higher than Aikang58, Xinong979 and Ningchun4 in this study. To explain this phenomenon, two maize genes associated with anthocyanin biosysthesis *ZmR* and *ZmC1*[17] were transformed into three types of wheat genotypes with different transformation efficiency: high group (Fielder and Zhongmai895), medium group (Jimai22), and low group (Ningchun4, Aikang58, and Sunstate). The order of transient expression efficiency from high to low was Fielder, Zhongmai895, Jimai22, Ningchun4, Aikang58, and Sunstate (Extended data Fig. 4), which was consistent with the order of transformation efficiency using *TaCB1*. Therefore, we speculated that the transient transformation efficiencies might be more important than the regeneration ability on the premise of using *TaCB1*. In short, the application of *TaCB1* basically could overcome genotype limitation in wheat genetic transformation when *TaCB1* can be delivered into the immature embryos of the wheat genotypes by *Agrobacterium* infection.

The *TaCB1* gene can make wheat transformation more efficient and stable. High temperature during growth period of mother plants, especially at the grain filling stage, negatively affected production and differentiation of embryonic calluses derived from the immature embryos, and finally led to transformation failure[18,19]. In the PureWheat standard methods, the physiological status of wheat immature embryos is also addressed to determine plant regeneration ability and transformation efficiency. When wheat mother plants are subjected to biotic or abiotic stress, the physiological state of the immature embryos will be deteriorated. Usually, these wheat immature embryos cannot generate embryonic callus. There was no good quality callus produced from the immature embryos of CB037 and Kenong199 plants with poor growing status in normal transformation experiments (Fig. 3a); while in the transformation with the assistance of *TaCB1*, the immature embryos from CB037 plants produced brown and limited grown calluses, from which some green shoots were still generated (Fig. 3b, c). Although calli of Kenong199 produced many roots (generally in our experiences, the quality of the calluses producing roots is very

poor, which is normally difficult to generate shoots), but they also can generate many shoots by virtue of using *TaCB1*. Therefore, the application of *TaCB1* can lighten the requirements to wheat immature embryo status in *Agrobacterium*-mediated genetic transformation. Moreover, under the presence of *TaCB1* transgenic wheat plants were also obtained with an efficiency of 54.0% using the previously published media[18]

Gene *TaCB1* driven by the maize ubiquitin promoter was also tested in species in the tribe Triticeae. A *T. monococcum* line PI428182 and a tetraploid wheat line Dwarfing Polish wheat were transformed for generating transgenic plants under the assistance of *TaCB1*, and their transformation efficiencies were 94.5 and 15.1% (Table1), respectively. The transformation frequency of a barley cultivar Vlamingh was increased to 78.6% under the premise of using a vector containing *TaCB1* from 10.1% employing a common vector. A few commercial barley genotypes including Supi3, Zhepi8, Baudin and Buloke, which were all recalcitrant to transformation, gave transformation frequencies of 24.7%, 20.3%, 17.6%, and 12.2%, respectively, on the premise of using *TaCB1* (Table1). Additionally, *TaCB1* also played a positive function in the transformation of *Secale cereale* L. and triticale (× *Triticosecale* Wittmack) with the transformation frequencies of 7.8% for Lanzhou Heimai and 53.3% for Linfen45, respectively (Table1).

To test the function of *TaCB1* in more genotypes, *TaCB1* was transferred into Japan Tobacco Inc via patent collaboration treaty (PCT). The transformation efficiency of Fielder was increased to 100% from 60% in control; the transformation efficiency of another wheat line Norin61 was increased to 24.3% from 0 in control; while the transformation efficiency of Chinese Spring wheat also enhanced to 29.8% was tested in maize, and the transformation efficiencies of two inbred lines B73 and A188 were from 16.4% in the control (Table2). The function of *TaCB1* e increased to 23.2% and 38.1% from 0 and 8.1% in the control, respectively (Table2).

Compared to other reported regeneration related genes *BBM*, *WUS2*, and *GRF4-GIF1*, *TaCB1* has certain advantages in the genetic transformation of wheat. Firstly, application of *TaCB1* can significantly increase the transformation efficiency to high level. The transformation efficiency of some wheat varieties like Fielder, CB037, Zhoumai18, and Zhengmai6694 could be as high as 100% in a small scale and 90% in a large scale in many repeated experiments during last five years (Supplementary Table 2). Moreover, one callus could generate dozens of green shoots with three replications (Fig. 2d), and most shoots produced could develop into normal plants with healthy roots (Extended data Fig. 4). Therefore, the transformation efficiency is calculated based on the immature embryos used for transformation, and only one shoot is considered for each immature embryo even though more shoots might be generated from a single embryo. If all the generated shoots are considered, the transformation efficiency will be far more than 100%. We found that wheat *TaCB2*, which is the homologous gene of maize *WUS2* (Extended data Fig. 1), showed a much lower function on the promotion of transformation efficiency than wheat *TaCB1* (Fig. 1a and 1b). Secondly, *TaCB1* can be singly used to increase transformation efficiency while the other reported genes needed to be co-expressed together in maize, sorghum and wheat transformation[11,12,20]. Moreover, the two genes of *WUS2* and *BBM* needs to be eliminated from the transformed calli during the transformation process using *Cre/LoxP* system driven by dehydration inducible promoter because of their negative effects on callus differentiation and root development[11,20]. The *TaCB1* gene is not necessary to be removed during the transformation as it has no negative effects. Lastly, *TaCB1* can be used as a marker to distinguish transgenic plants. Although overexpression of *TaCB1* had no negative effect on shoot differentiation and root development, it has some phenotypes in transgenic wheat plants including wide, short flag leaves and thick stems (Fig. 3d and Supplementary Table2). Therefore, the *TaCB1* transgenic wheat plants can be easily recognized by its phenotype.

The newly identified *TaCB1* in this study as a promising regenerative gene has a great potential application in genetic transformation and genome editing for cereal crops. We have incorporated *TaCB1* onto the vectors containing double T-DNA region and *Cas9* expression cassette, on which *TaCB1* was linked with the *Bar* selection marker and *Cas9*, and *TaCB1* can be removed together with *Bar* and *Cas9* in the progenies of transgenic or edited plants. Although the application of *TaCB1* cannot directly contribute to the frequency improvement of generating marker-free or Cas9 cleavage plants, it can greatly help to obtain more candidate plants for identifying marker-free or mutants. Similarly, the application of *TaCB1* can help to reduce workload for identifying marker-free transgenic and Cas9-free edited plants by selecting the *TaCB1* phenotype in the segregation population.

In summary, a total of 31 hexaploid wheat cultivars were transformed by using *TaCB1*, and the genotype dependency in transformation was overcome. In addition, overexpression of *TaCB1* also significantly increased the transformation efficiencies of *T. monococcum*, triticale, rye (*Secale cereale*, RR, 2n=14), barley, and maize. The application of *TaCB1* can make the genetic transformation and genome editing of wheat and other crops more efficient and economical by improving plant regeneration, reducing the requirement for embryo quality, and identifying transgenic or edited plants by visible phenotypes.

Methods

Plant materials and cultivation conditions

Wheat cultivars Zhengmai7698, Zhengmai1342, Zhengmai1860, and Zhengmai6694 were kindly provided by Prof. Weigang Xu at Wheat Research Institute, Henan Academy of Agricultural Sciences, Zhengzhou, Henan province; Zhongmai895 and Zhongmai175 by Prof. Yong Zhang at Institute of Crop Sciences, Chinese Academy of Agricultural Science (ICS-CAAS), Beijing; Luohanmai by Prof. Ming Hao at Sichuan Agricultural University, Chengdu, Sichuan province; Cang6005 and Ji5265 provided by Prof. Xiulin Guo and Hui Li at Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang, Hebei province, respectively. Fielder and Chinese Spring were obtained from Yokohama City University. Norin61 was obtained from Kyoto University. Wheat cultivars CB037, Ningchun4 and Kenong199 and a rye cultivar Lanzhou Heimai were maintained in our laboratory. A tetraploid (*Triticum polonicum*, AABB, 2n=28) line Dwarfing Polish wheat was provided by Prof. Minyi Wang at Sichuan Agricultural University, Chengdu, Sichuan province. Other wheat genotypes, including winter wheat cultivars Jimai22, Aikang58, Xinong979, and Jing411, were requested from the National Germplasm Bank at ICS-CAAS. All the barley cultivars Vlamingh, Supi3, Zhepi8, Baudin, and Buloke were kindly provided by Prof. Yanhao Xu at Yangtze University, Jingzhou, Hubei province. A *T. monococcum* (AA, 2n=14) accession PI428182 was kindly provided by Prof. Yueming Yan at Capital Normal University, Beijing; and triticale (AABBRR, 2n=42) cultivars ZS3297, ZS1257, ZS3224 and Linfen45 by Prof. Zengyuan Wang at ICS-CAAS and Prof. Fangpu Han at the Institute of Genetics and Developmental Biology of Chinese Academy of Sciences, Beijing, respectively. Maize inbred lines A188 and B73 were obtained from National Agriculture and Food Research Organization in Japan.

Cloning of *TaCB1*

The amino acid coding sequence of *Arabidopsis* WUS (AJ012310) registered in NCBI was used as a query for searching the homologous gene of wheat via tblastn, in which the organism was set as *Triticum aestivum*, resulting in only one sequence FN564431.1. Based on this sequence, a pair of primers of *TaCB1F* (GTGTCAATGGAGGCGCTGAGCG) and *TaCB1R* (ATGCGTGCGTGCGACGTTGATT) were designed to amplify *TaCB1* from the genomic DNA of wheat line CB037.

It was found that TaCB1 is not the original WUS, so the protein sequence of AtWUS was used as a query for tblastn in IWGSC, and three contigs were obtained. According the contig sequences, a specific primer pairs was designed to amplify the sequence, designated *TaCB2*. The identification and application of *TaCB1* has been granted for Chinese invention patent (ZL**201710422896.6)** and applied for PCT with Japan Tobacco Inc (PCT/CN2018/090239).

Vector construction

The primers CB1SmaF: AAACCCGGGATGGAGGCGCTGAGCGG and CB1KpnR: AAAGGTACCTTAGACCAGATACCGAT were used to perform PCR amplification using *pMD-18T-TaCB1* as a template with high fidelity enzyme KOD. Then, the PCR product and the *pWMB003* vector (containing the *ZmUbi* promoter and the *Nos* terminator) were digested with *Kpn*I and *Sma*I to obtain a 773 bp product and a 4535 bp vector backbone. Next, the target PCR product and the vector backbone were ligated to generate an intermediate expression vector *pWMB003-TaCB1*. The vectors *pWMB003-TaCB1* and *pWMB111* (containing a *Bar* expression cassette controlled by the *Ubi* promoter) were digested with *Hin*dIII to produce a 3033 bp *TaCB1* expression frame and a 10,170 bp vector backbone, respectively. Finally, the two enzyme-digested products were ligated to generate the target expression vector *pWMB111-TaCB1* for the transformation of wheat, *T. monococcum*, rye, triticale, and barley. The *TaCB1* expression cassette was inserted into the vector pLC41 containing a *Bar* expression frame controlled by the *35S* promoter and the *Nos* terminator for wheat varieties Chinese Spring and Norin61, and maize transformation. The expression vector containing *TaCB2* was also constructed by the same method. The *pWMB202* vector containing two anthocyanin biosynthesis related genes *ZmR* and *ZmC1*[17] were used to detect the transient transformation efficiency in different wheat genotypes.

Plant transformation

Wheat transformation. Spikes of wheat were harvested at 14 d post anthesis (DPA). Immature wheat grains were carefully collected. In the aseptic conditions, grains were surface-sterilized with 70% ethanol for 1 min, 5% sodium hypochlorite (NaClO) for 15 min, and rinsed 5 times with sterile water. Fresh immature embryos of wheat were isolated and transformed by *Agrobacterium*-mediated transformation to obtain transgenic plants following the protocol described by Ishida et al.[1] with slight modifications. In brief, immature wheat embryos were incubated with *Agrobacterium* strain C58C1 harbouring vectors for 5 min in WLS-inf medium at room temperature, and co-cultivated for 2 d on WLS-AS medium with scutellum facing upwards at 25 °C in darkness. After co-cultivation, embryonic axes were removed with a scalpel and the scutella were transferred onto plates containing WLS-Res medium for delay culture for 5 d under the same condition. Afterwards, tissues were cultured on WLS-P5 medium for callus induction. Two weeks later, calli were placed on WLS-P10 medium for 3 weeks in darkness. Embryogenic calli were then differentiated on LSZ-P5 medium at 25°C with 100 μmol/m^2/s light. Regenerated shoots were transferred into cups filled with LSF-P5 medium for elongation and root formation. Plantlets with well-developed root systems were transplanted into pots and cultivated in growth chambers. The transformation of *T. monococcum*, rye, and triticale were performed by the same methods aforementioned for wheat.

Barley transformation. Barley transformation was performed following the previously published protocols[21] with a slight modification. Immature embryos of barley were isolated after sterilization of the immature grains by the same methods as wheat, subsequently incubated with *Agrobacterium* for 10 min, and co-cultivated for 2 d on CM medium. Then, embryo axes were removed and the scutella were cultured on the first selection medium. After 2 weeks, tissues were transferred onto the second selection medium. Three 3 weeks later, embryonic calli were

cultured on DM medium at 25 °C with 100 μmol/m^2/s light for differentiation. Shoots were timely moved into a plastic box containing RT medium. Lastly, plants were transplanted into pots filled with soil.

Maize transformation. Maize transformation was performed following the previously published protocols[22]. Between 8 and 15 DPA, spikes containing immature embryos were harvested. Immature embryos were isolated and infected by *Agrobacterium* for 5 min. Then, embryos were transferred onto LS-AS solid medium with the scutellum face up, and incubated in darkness at 25°C for 7 d. After the co-cultivation steps, embryos were transferred onto the first selection medium for 10 d, and then moved to the second selection medium and the third selection medium for 21 d, respectively. Next, embryonic calli were transferred onto LSZ medium for shoot differentiation. The regenerated shoots were transferred into cups filled with LSF medium. Finally, plants with developed roots were transplanted into pots containing appropriate soil.

Detection of transgenic plants

Quickstix detection. Transgenic plants were detected for the *Bar* gene by the QuickStix Kit (EnviroLogix) according to the manufacturer's instructions, which is used to detect the protein encoded by the *Bar* gene.

PCR analysis. Genomic DNA was extracted from the leaves of transgenic plants using a NuClean PlantGen DNA kit (CWBIO, Taizhou, China). The primer pair 5'-ACCATCGTCAACCACTACATCG-3' and 5'-GCTGCCAGAAACCACGTCATG-3' was used to detect the *Bar* gene in the transgenic plant samples by PCR amplification with a 429-bp fragment.

Southern blotting analysis. Total genomic DNA was extracted followed a standard CTAB method[23], and 10 μg DNA from each sample was digested with *Hin*dШ. Then, the digested DNA samples were fractionated on a 0.8% agarose gel and transferred onto a nylon Hybond-N^+ membrane (Roche, Mannheim, Germany) with a membrane transfer instrument (Model 785, Hercules, California, Bio-Rad). The PCR products of *Bar* gene (429-bp) were labeled with Digoxigenin and used as probes to hybridize with the digested DNA on the membrane. The hybridization and detection steps were performed according to the instructions for the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche, Mannheim, Germany).

Declarations

Data availability

The transgenic lines and plasmids generated in this study are available from the corresponding authors upon request. Source data are provided with this paper.

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Author contributions

K.W. contributed to funding acquisition, experiment designing, vector construction, wheat and barley transformation, data analysis, and manuscript writing. S.L. contributed to gene identification, vector construction, and transgenic detection. X.L performed media 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 *T. monococcum* and rye transformation. Y.C. performed triticale transformation. Y.H. performed maize transformation. C.Y. contributed to vector construction. L.D. contributed material management and media making. Y.I. contributed to experiment designing, wheat transformation and manuscript editing. XY. contributed to study conceiving, experiment supervision, formal analysis, project administration, funding acquisition and manuscript editing.

Competing interests

The authors declare that there are no competing interests on this study.

References

1. Ishida, Y. Tsunashima, M. Hiei, Y. & Komari, T. Methods in molecular biology, *Agrobacterium* protocols, 3rd edn. Wheat (*Triticum aestivum* L.) transformation using immature embryos. In *Agrobacterium* Protocols, Vol. 1 (ed. Wang, K.) **1223**, 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 Tiss. Org.* **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 Tiss. Org.* **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. Debernardi J. M. et al. A GRF-GIF chimeric protein improves the regeneration efficiency of transgenic plants. *Nat. Biotechnol*. **38**, 1274–1279 (2020).
13. Su, Y. H. Zhao, X. Y. Liu, Y. B. Zhang, C. L. O'Neill, S. D. & Zhang, X. S. Auxin-induced WUS expression is essential for embryonic stem cell renewal during somatic embryogenesis in *Arabidopsis*. *Plant J.* **59**, 448–460 (2009).
14. van der Graaff, E. Laux, T. & Rensing, S. A. The WUS homeobox-containing (WOX) protein family. *Genome Biol.* **10**, 248 (2009).
15. Sarkar, A. K. et al. Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* **446**, 811–814 (2007).
16. Zhang, W. Yin, M. Q. Zhao, P. Wang, K. Du, L. P. & Ye, X. G. Regeneration capacity evaluation of some largely popularized wheat varieties in China. *Acta Agro. Sin.* **44**, 208–217 (2018).
17. Riaz, B. Chen, H. Q. Wang, J. Du, L. Wang, K. & Ye, X. G. 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).
18. 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).
19. 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).
20. Mookkan, M. Nelson-Vasilchik, K. Hague, J. Zhang, Z. Y. J. & Kausch, A. P. Selectable marker independent transformation of recalcitrant maize inbred B73 and sorghum P898012 mediated by morphogenic regulators BABY BOOM and WUSCHEL2. *Plant Cell Rep.* **36**, 1477–1491 (2017).
21. 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).
22. Ishida, Y. Hiei, Y. & Komari, T. *Agrobacterium*-mediated transformation of maize. *Nat. Protoc.* **2**, 1614–1621 (2007).
23. Sambrook, J. Fritsch, E. F. & Maniatis, T. Molecular Cloning, A Laboratory Manual, 2nd edn. (Cold Spring Harbor Laboratory Press, 1989).

Tables

Table 1 Overexpression of *TaCB1* increased the transformation efficiency in *T. monococcum,* barley, *Secale cereale* and triticale.

Genotype	Species	Vector	No. explants transformed	Positive plants	Transformation efficiency (%)
PI428182	*Triticum monococcum*	TaCB1	273	258	94.5
Dwarf Polish wheat	*T. polonicum*	TaCB1	166	25	15.1
Vlamingh	*Hordeum vulgare*	Common	138	14	10.1
Vlamingh	*H. vulgare*	TaCB1	159	125	78.6
Buloke	*H. vulgare*	TaCB1	82	10	12.2
Baudin	*H. vulgare*	TaCB1	159	28	17.6
Zhepi8	*H. vulgare*	TaCB1	128	26	20.3
Supi3	*H. vulgare*	TaCB1	85	21	24.7
Linfen45	Triticale	TaCB1	75	40	53.3
ZS3297	Triticale	TaCB1	51	21	41.2
ZS1257	Triticale	TaCB1	56	11	19.6
ZS3224	Triticale	TaCB1	67	11	16.4
Lanzhou Heimai	*Secale cereale*	TaCB1	4	51	7.8

Table 2 Overexpression of *TaCB1* increased the transformation efficiency in wheat and maize in Japan Tobacco.

Varieties	Species	gus-bar vector			*TaCB1* vector		
		No. of explants transformed	No. of positive plants	Transformation efficiency (%)	No. of explants transformed	No. positive plants	Transformation efficiency (%)
Fielder	*Triticum. aestivum*	10	6	60	11	11	100
Norin61	*T. aestivum*	44	0	0	37	9	24.3
Chinese Spring	*T. aestivum*	55	9	16.4	57	17	29.8
B73	*Zea mays*	203	0	0	578	134	23.2
A188	*Z. mays*	62	5	8.1	42	16	38.1

Figures

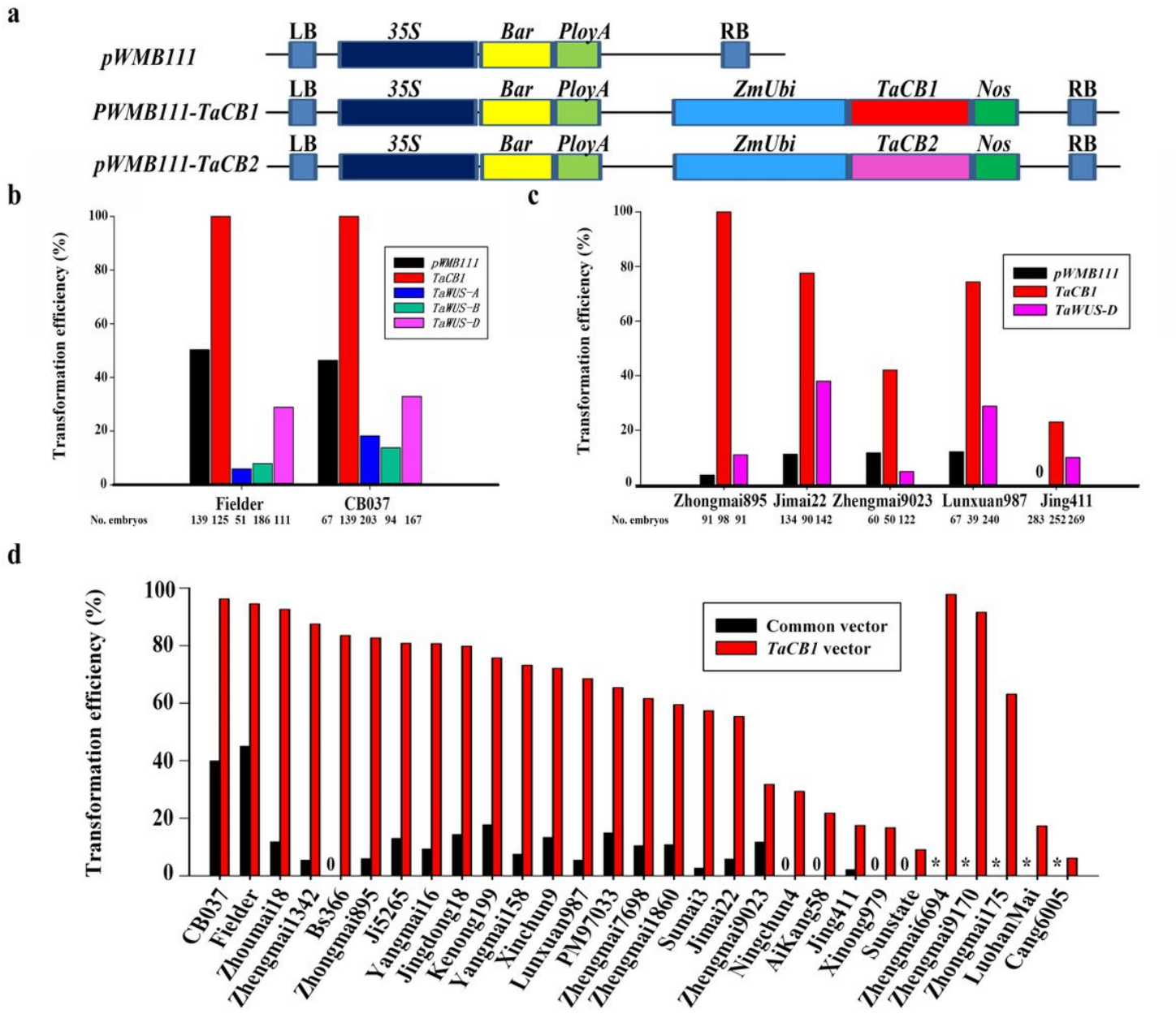

Figure 1

Effects of application of genes TaCB1 and TaCB2 on the transformation efficiencies of different wheat genotypes. a: The structure of transfer DNA (T-DNA) region of plasmids pWMB111, pWMB111-TaCB1 and pWMB111-TaCB2. b: The transformation efficiencies of Fielder and CB037 using the vectors containing TaCB1 and TaCB2 genes as well as the common vector pWMB111. c: The transformation efficiencies of Zhongmai895, Jimai22, Zhengmai9023, Lunxuan987 and Jing411 on the premise of using the vectors containing TaCB1 and TaWUS-D genes as well as the common vector pWMB111. d: The transformation efficiencies of 29 wheat varieties using the TaCB1 containing vector and the common vector. 0: the transformation efficiency is 0%; and *: no data.

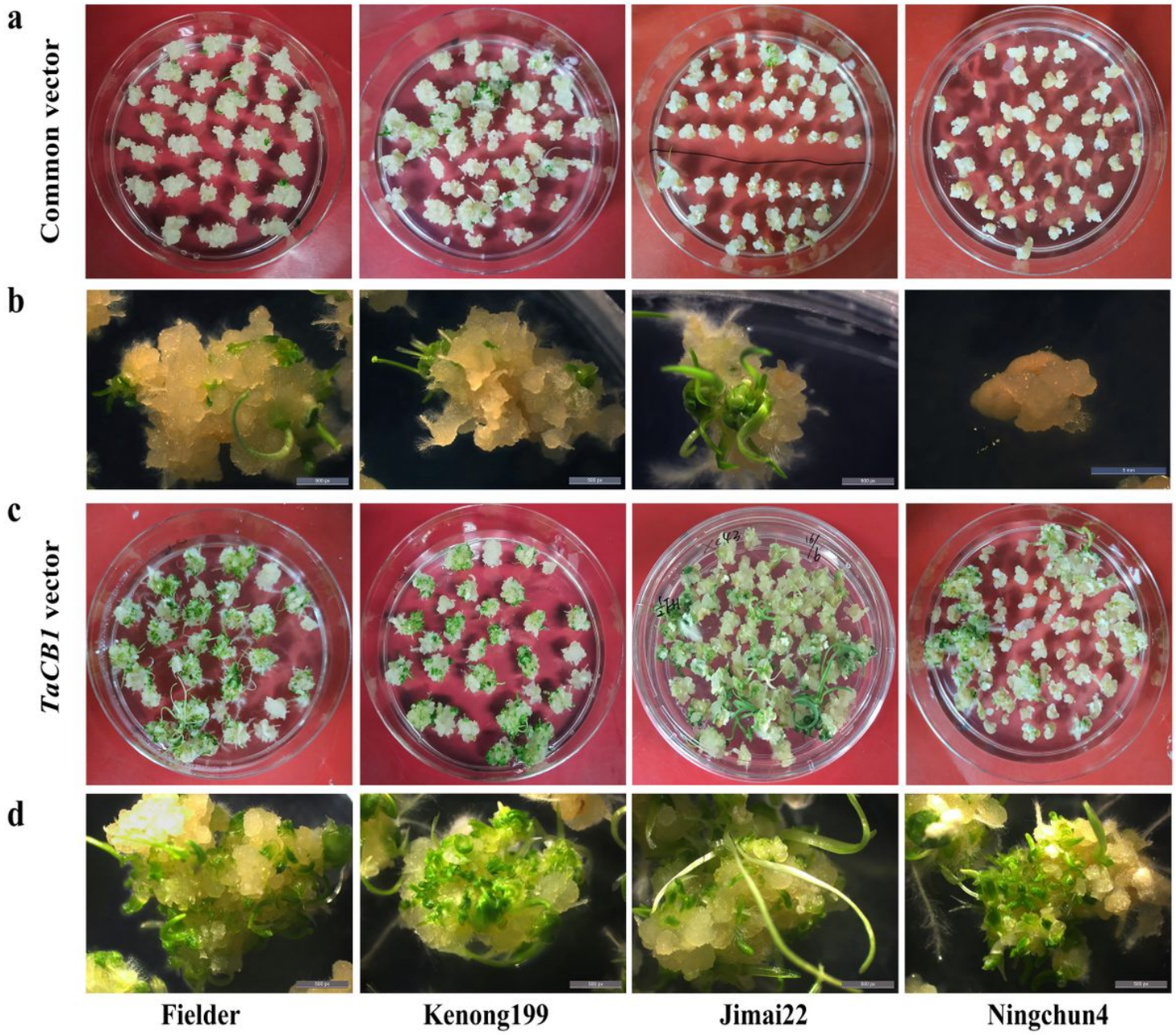

Figure 2

Shoot regeneration of different wheat genotypes with an increased frequency using TaCB1. a, b: Shoot regeneration of the wheat immature embryos transformed with the common vector. c, d: Shoot regeneration of the wheat immature embryos transformed with TaCB1 gene-containing vector.

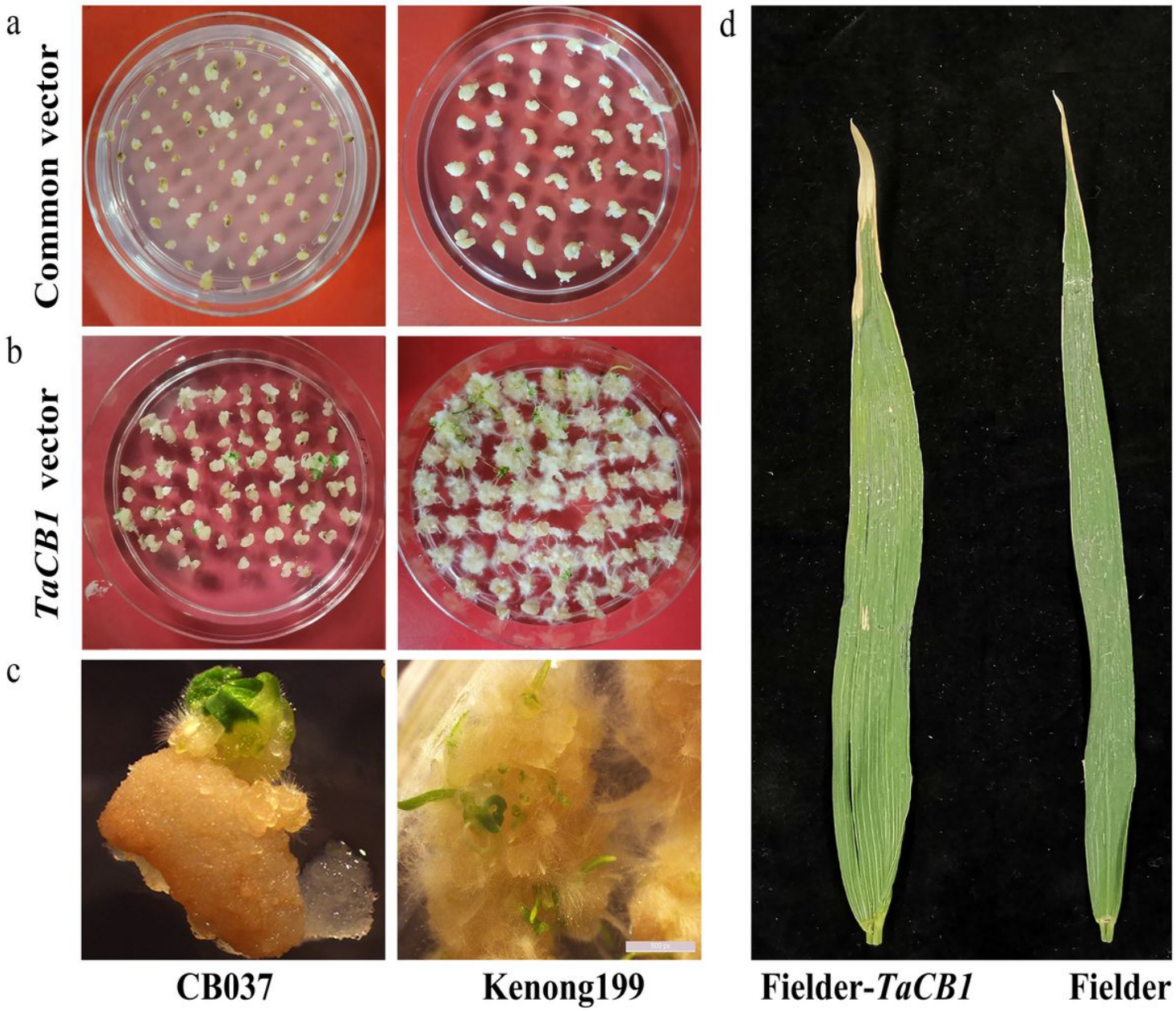

Figure 3

Shoot regeneration of different wheat genotypes with an increased frequency using TaCB1. a, b: Shoot regeneration of the wheat immature embryos transformed with the common vector. c, d: Shoot regeneration of the wheat immature embryos transformed with TaCB1 gene-containing vector.

Supplementary Files

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