

Comparison of three wheat near isogenic lines originated from CB037 on tissue culture and transformation capacities

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Research Article

Keywords: Wheat, Near isogenic lines, Disease resistance, Tissue culture, Genetic transformation

Posted Date: July 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1787660/v1>

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Abstract

Wheat is an important worldwide food crop. To meet the increasing demand for wheat grains, it is essential to continuously improve wheat by commercial and advanced technologies. Wheat line CB037 has ideal agronomic traits, good bread quality, strong resistance to powdery mildew (PM), and high regeneration ability, and therefore it has been widely used in hybridization breeding and genetic transformation. Three near isogenic lines (NILs) from CB037 were identified in a previous study based on glutenin composition. In this study, the three NILs of CB037A, CB037B, and CB037C were investigated comparatively for agronomic traits, disease resistance for PM and stripe rust, and tissue culture and transformation abilities. Results indicated that the three NILs were high resistant to PM, and CB037B was not only high resistant to stripe rust but also had significantly higher regeneration ability than CB037A and CB037C in anther culture, immature and mature embryo culture. Particularly, positive transgenic wheat plants were obtained only from CB037B. Thereby CB037B was identified to be a desirable material for wheat genetic transformation, which was of great significance for improving the transformation efficiency and accelerating the application of genetic engineering breeding technology in wheat.

Key Message

CB037B was high resistant to PM and stripe rust. It had high regeneration ability in anther, immature, and mature embryo culture, and could be used in wheat genetic transformation for generating transgenic plants.

Introduction

As one of the most important food and forage crops, wheat (*Triticum aestivum* L., $2n = 6X = 42$, AABBDD) is globally cultivated in the long history. With the increasing demand of human beings and animals for wheat grains, wheat production is facing a great challenge. Therefore, it is necessary to improve various economically important traits of wheat by traditional hybridization or mutation and genetic engineering breeding approaches for high and stable yield (Tester and Langridge 2010; He et al. 2011; Ishida et al. 2015). Either by traditional technique or genetic engineering strategy, the selection of parents or receiving genotypes is an essential step for developing new wheat varieties with desirable and targeted traits.

In wheat hybridization breeding program, parents are required to have good comprehensive agronomic traits and high general combining ability. In wheat cell and genetic engineering breeding program, the receiving genotypes are required to have high tissue culture or transformation response except good agronomic traits. As a model wheat genotype, Bobwhite was widely used in wheat transformation during 1995 to 2014 (Zhou et al. 1995; Zhang et al. 2000; Abebe et al. 2003; Han et al. 2012; Wang et al. 2014a). According to previous reports, Bobwhite and its sister lines were used in more than 40% of studies on wheat genetic transformation as acceptor materials (Cheng et al. 1997; Pellegrineschi et al. 2002; Ye et al. 2014). Additionally, several wheat varieties such as Verry5, Cadenza, and Florida were also normally used in wheat genetic transformation (Hu et al. 2003; Ye et al. 2005; Ogawa et al. 2008; Wu et al. 2008). Although Bobwhite and other model wheat lines have good regeneration capability, their agronomic characters are not desirable. At the same time, wheat haploid breeding *via* anther culture has been conducted with less efficiency due to the lack of ideal wheat parents having good agronomic traits and high anther culture response (Orshinsky et al. 1997; Zhao et al. 2015). Therefore, it is necessary to evaluate more available wheat genotypes with both of high regeneration ability and ideal agronomic traits for transgenic, double haploid, and genome editing breeding.

In the latest eight years, another spring wheat variety Fielder was collectively employed in wheat genetic transformation (Ishida et al. 2015; Hayta et al. 2021). Even though transgenic wheat plants were also generated from more than twenty Australian, American, and Chinese varieties or lines using the PureWheat technique, Fielder showed the highest transformation efficiency followed by a few other genotypes such as CB037 and Westonia (Richardson et al. 2014; Wang et al. 2017; Liu et al. 2020a, 2020b). Among the newly identified wheat genotypes for wheat tissue culture and genetic transformation, CB037 showed the special features of a model genotype including short plant height, early maturity, high tissue culture response from three explants (anthers, immature embryos, and mature embryos), and strong resistance to powdery mildew (PM) (Tao et al. 2011; Yin et al. 2011; Zhang et al. 2012, 2017, 2018; She et al. 2013; Wang et al. 2014b; Bie et al. 2017, 2020). Additionally, CB037 displayed good agronomic and bread-making quality traits (Zhang et al. 2012, 2017; Wang et al. 2016; Dong et al. 2017).

However, the original CB037 is a mixed population. Three near isogenic lines (NILs) of CB037A, CB037B, and CB037C were identified in the original CB037 according to the glutenin composition (Wang et al. 2016; Wu et al. 2017). Namely, comparing with the composition of high molecular weight glutenin subunits (HMW-GS) in CB037B at the *Glu-1* locus (1Ax1, 1Bx17 + 1By18, 1Dx5 + 1Dy10), CB037A had the composition of 1Ax1, 1Bx17 + 1By18, 1Dx2 + 1Dy10 at this locus; CB037C had the same HMW-GS composition at *Glu-1* to CB037B. For the composition of low molecular weight glutenin subunits (LMW-GS) at the *Glu-3* locus, CB037B and CB037A had *Glu-A3c*, *Glu-B3h*, and *Glu-D3a* subunits, whereas CB037C only had the *Glu-A3c* and *Glu-D3a* subunits and missed *Glu-B3h* subunit (Wang et al. 2016; Wu et al. 2017). Recently, the original CB037 was identified to be genetically heterogeneous for resistance to wheat stripe rust, and the *Pst*-resistant line CB037-PstR and *Pst*-susceptible line CB037-PstS were isolated; the CB037-PstR carried a single dominant resistance gene, which was a 1BL/1RS translocation line and likely carried *Yr9* (Zheng et al. 2020). Update, it is not clear that which one in the three NILs has good regeneration and transformation abilities and stripe rust resistance.

The purpose of this investigation is to compare the three NILs of CB037 in multiple traits including tissue culture response and transformation efficiency using immature and mature embryos, and PM and stripe rust resistances. An ideal NIL will be evaluated for the further application in wheat cell engineering and genetic engineering breeding. Our output will facilitate the trait-oriented use of CB037 in wheat improvement by cell and genetic engineering technologies.

Materials And Methods

Materials

Three wheat NILs of CB037A, CB037B, and CB037C were identified from the original CB037 and provided by Prof. Yueming Yan at Capital Normal University, which were found to be different in glutenin composition (Wang et al. 2016). Two wheat NILs of *Pst*-resistant line CB037-PstR and *Pst*-susceptible line CB037-PstS were identified from the original CB037 and provided by Prof. Jiajie Wu at Shandong Agricultural University (Zheng et al. 2020), which were found to be different in stripe rust resistance. Spring wheat line Chinese Spring (CS) which was preserved in our laboratory. The wheat materials were planted by three replications in the February of 2018 at the experimental station of Institute of Crop Sciences (ICS), Chinese Academy of Agricultural Sciences (CAAS), Beijing, for anther, immature embryo, and mature embryo culture, and PM and stripe rust resistance identification.

Expression vector pWMB110-*GUS* was constructed by our laboratory (Figure S1). *Agrobacterium* strain C58C1 was kindly provided by Prof. Tom Clemente at University of Nebraska-Lincoln, USA.

Genomic in situ hybridization

Wheat roots were collected from the germinating seeds on an absorbent filter paper for 2–3 d at 25 °C in darkness. The root tips were treated with nitrous oxide for 2 h on 0.1 Mpa, and then fixed for 5 min with 90% glacial acetic acid. Chromosomes were prepared according to a previously described method (Yuan et al. 2014). The total genomic DNA of *D. villosum* labeled with fluorescein-12-dUTP using the nick translation method and using genomic DNA of CS as a block was employed as a probe for genomic *in situ* hybridization (GISH) to identify the chromosome constitution of the three NILs of CB037 following the methods described previously (Yuan et al. 2014; Guo et al. 2016). Slides were visualized using an Olympus BX-51 fluorescence microscope (Olympus Corporation, Japan) and photographed using a charge-coupled device system.

Powdery Mildew And Stripe Rust Resistance Test

For PM resistance test, the wheat plants at the tillering stage were inoculated with *Blumeria graminis* (*Bgt*) mixture collected from the infected wheat seedlings grown in artificial conditions. Two weeks after inoculation, disease symptoms of the wheat plants were investigated. In immune type there were no lesions and *Bgt* pathogens on the leaves, and in susceptibility type there were continuous lesions and a serious covering of *Bgt* pathogens on the leaves (Xiao et al. 2013).

For stripe rust resistance test, the wheat plants at the seedling stage were inoculated with *Puccinia striiformis* f. sp. *tritici* (*Pst*) and talc powder which were mixed in 1:10 ratio using a small brush to gently smear on the area of one leaf. Two weeks after inoculation,

a large number of spores were found in the inoculated leaf area of the susceptible plants, while no lesions and *Pst* spores were observed in the inoculated leaf area of the resistant plants (Zheng et al. 2020).

Anther culture

The wheat spikes with two stem nodes and leaves in which the development of the pollen cells was at uni-nucleus middle-late stage after mitosis were collected in a plastic bag and pretreated under 4 °C for 3 d in a refrigerator. Then, the leaves and second stem nodes were removed; the remaining tissues consisting of spike, sheath and the first stem node were carefully wiped with 70% alcohol for surface disinfection; the sheath and glumes were peeled off by tweezers; the anthers were inoculated on W14 medium (pH 6.0) containing 2.0 mg/l 2,4-D, 0.5 mg/l KT, 100 g/l sucrose, and 2.4 g/l gelrite (Zhang et al. 2018). The isolated tissues were firstly cultured at 30 °C for 3 d, and then at 28 °C for 30–45 d in darkness for callus induction. The calluses were moved onto FHCK medium (basic MS medium containing 30 g/l sucrose and 2.4 g/l gelrite, pH 6.0) for 20–25 d at 25 °C for shoot regeneration under a 16 h-light/8 h-dark photoperiod with a 100 $\mu\text{mol}/\text{m}^{-2}/\text{s}^{-1}$ photosynthetic photon flux density (She et al. 2013; Zhang et al. 2018). All media were autoclaved at 121°C for 15 min. Each experiment was duplicated at least three times.

Immature embryos culture

Wheat seeds were planted in a growth chamber with a temperature regime of 25 °C-day/20 °C-night, a photoperiod of 16 h-light/8 h-dark, a photosynthetic photon flux density of 300 $\mu\text{mol}/\text{m}^{-2}/\text{s}^{-1}$, and a relative humidity of 45%. Wheat immature grains at 13–14 d post anthesis (DPA) were collected, surface sterilized with 70% ethanol for 1 min and 15% sodium hypochlorite for 10 min in order, and rinsed for four times with sterile water. Wheat immature embryos of 1.2–1.5 mm in size were aseptically excised from the sterilized grains after the embryogenic axes were carefully removed, and then incubated with scutellum upwards on callus induction medium (CIM containing MS inorganic, 10.0 mg/l vitamin B₁, 150 mg/l asparagine, 2.0 mg/l 2,4-D, 30 g/l sucrose, and 2.4 g/l gelrite, pH 6.0) in 90-mm of diameter petri dishes in darkness at 25 °C for 14–21 d for callus induction (She et al. 2013; Zhang et al. 2018). In total, 150 immature embryos approximately from each wheat line were incubated on three petri dishes for duplication. The embryonic calluses were moved onto FHCK medium for another 14–21 d at 25 °C in light conditions as aforementioned description for shoot differentiation. Three duplicates at least were set up for this experiment.

Mature embryos culture

Mature wheat seeds were sterilized with 70% ethanol for 10 min and 25% sodium hypochlorite for 25 min, and then soaked in sterile water overnight at 25°C in darkness after being rinsed with sterile water for four times. The slightly germinated seeds were sterilized again with 25% sodium hypochlorite for 15 min and then rinsed with sterile water for four times (Yin et al. 2011; Zhang et al. 2018). The mature embryos were scraped into small pieces on seeds with a sharp knife and cultured on Adi medium (4.3 g/l MS inorganic, 30 g/l maltose, 1.0 g/l casein hydrolysate, 0.35 g/l myoinositol, 0.69 g/l proline, 1.0 mg/l VB₁, 2.5 mg/l dicamba, 0.5 mg/l 2,4-D, 3.5 g/l gelrite, pH 6.0) at 25 °C in darkness for 7 d for initial callus induction, in which every two embryos were scraped and cultured together. Then, the initiative calluses were moved onto fresh Adi medium for 3 weeks at 25 °C in darkness. Finally, the embryonic calluses were cultured on FHCK medium for 2 weeks at 25 °C in light conditions as aforementioned described for shoot regeneration. Each experiment was duplicated at least three times.

Agrobacterium-mediated transformation of wheat immature embryos

Wheat heads grown in the growth chamber were sampled at 14–15 DPA. The immature grains were carefully collected, then surface sterilized with ethanol and sodium hypochlorite in order, and finally rinsed with sterile water in aseptic conditions by the aforementioned procedure. A proprietary *Agrobacterium* mediated transformation system of wheat developed by Japan Tobacco Company (Ishida et al. 2015) was applied with a slight modification. In brief, immature wheat embryos of 2.0–2.5 mm in size were cautiously isolated from the sterilized grains aseptically under a stereoscopic microscope, incubated with the *Agrobacterium* strain C58C1 harboring pWMB110-*GUS* expression vector for 5 min on WLS-inf medium at room temperature, and co-cultivated with the scutellum facing upwards for 2 d on WLS-AS medium at 23°C in darkness. After co-cultivation, embryonic axes were removed and the remaining scutella were transferred onto WLS-Res medium. After a delay culture for 5 d, the tissues were transferred onto callus induction (WLS-P5) medium. Two weeks later, the survived calli were sliced vertically into halves and evenly cultured on WLS-P10 medium for 3 weeks in darkness. Embryogenic calli were then moved onto LSZ-P5 medium at 25 °C in light conditions as aforementioned description. Regenerated shoots were transferred into cups filled with MSF-P5 medium for shoot elongation and

root formation. Plantlets with well-developed root systems were transplanted into pots and cultivated in the growth chamber as aforementioned described.

Agrobacterium-mediated transformation of wheat mature embryos

Wheat mature embryo tissues were prepared by the description mentioned above. The initiative calli produced from the fine wheat mature embryo pieces which were cultured on Adi medium for 7 d were incubated with the *Agrobacterium* harboring the pWMB110-*GUS* vector for 30 min in WLS-inf medium at room temperature, and co-cultivated for 3 d on a sterile filter paper at 23°C in darkness. Then, the infected calli were transferred onto Adi-Res medium (basic Adi medium containing 0.25 g/l carbenicillin and 1.0 g/l vitamin C, 0.85 mg/l AgNO₃, pH 6.0). After 5 d at 25 °C under darkness, the tissues were transferred onto callus selection medium (Adi-P5: basic Adi medium containing 0.25 g/l carbenicillin, 1.0 g/l vitamin C, 0.85 mg/l AgNO₃, and 5 mg/l phosphinothricin (PPT), pH 6.0). After two weeks, callus cultures were placed on Adi-P10 medium (basic Adi medium containing 0.25 g/l carbenicillin, 1.0 g/l vitamin C, 0.85 mg/l AgNO₃ and 10 mg/l PPT, pH 6.0) for three weeks at 25 °C in darkness. Embryogenic calli were then cultured for differentiation on FHP5 medium (basic FHCK medium containing 0.25 g/l carbenicillin and 5 mg/l PPT, pH 6.0) at 25 °C in aforementioned light conditions. Regenerated shoots were transferred into cups filled with FHP5 medium for rooting. Plantlets with well-grown shoots and roots were transplanted into pots and cultivated in the growth chamber as aforementioned described.

Detection of transgenic wheat plants by PCR

Genomic DNA was extracted from the leaves of the putative transgenic wheat plants using a NuClean PlantGen DNA kit (CW BIO, China). The presence of the *GUS* gene in the transgenic plants was tested by an amplification fragment of 898 bp using the specific PCR primer pair 5'-GACCACCAGTGCAAGAACCCTC-3' and 5'-ATCCACGACCGACACTTTCACG-3'. The presence of the *bar* gene in the transgenic plants was examined by an amplification fragment of 429 bp using the specific PCR primer pair 5'-ACCATCGTCAACCACTACATCG-3' and 5'-GCTGCCAGAAACCACGTCATG-3'.

Histochemical staining and Quickstix stripe detection of transgenic wheat plants

Histochemical staining analysis for *GUS* expression was conducted by a described method (Jefferson et al. 1987). The young leaves collected from the putative transgenic wheat plants were immersed directly in 0.1 M NaPO₄ buffer (pH 7.0) containing Na₂-EDTA 10 mM, ferricyanide 0.2 mM, ferrocyanide 0.2 mM, X-gluc 0.8 g/l, methanol 20%, and Triton-100 0.5%, and incubated overnight at 37°C. Additionally, a QuickStix Kit (EnviroLogix, LibertyLink) was applied to detect the Bar protein in putative transgenic wheat plants by the manufacturer's instructions.

Data statistical analysis

For tissue culture, the numbers of primary calli, embryonic calli, and green plantlets were counted in each experiment, and then the data was sorted by Microsoft Excel. The regeneration efficiencies of wheat immature embryos and mature embryos for the three near-isogenic lines were compared by calculating embryogenic callus induction rate (number of embryogenic calli/number of incubated immature or mature embryos × 100%), differentiation callus rate (number of the calli showing green shoots/number of incubated immature or mature embryos × 100%), shoot production rate (number of regeneration shoots/number of incubated immature or mature embryos × 100%).

Results

GISH identification of the three NILs CB037A, CB037B, and CB037C

Firstly, we identified the three NILs by GISH using the total genomic DNA of *D. villosum* as a probe. A pair of translocated chromosomes displaying the green signal of *D. villosum* were clearly observed in the three NILs (Fig. 1). This result indicated that the three NILs of CB037A, CB037B, and CB037C were genetically stable and harbored a pair of wheat-*D. villosum* translocated chromosomes 6DL·6V#2S.

Powdery mildew and stripe rust resistance identification of the three wheat lines CB037A, CB037B, and CB037C

The three NILs were inoculated with *Bgt* mixture for PM test, and *Pst* mixture and talc powder for tripe rust test, respectively, at the seedling stage under greenhouse conditions, and their resistance to the two diseases were examined at the jointing stage, booting stage, and grain filling period. During the whole growth period, the three near isogenic wheat lines all showed strong PM resistance, and no disease lesion or pathogen were observed on their leaves (Fig. 2A1-A3), while the control cultivar of Fielder was seriously susceptible to the disease (Fig. 2A4). However, compared with the susceptible and resistant lines CB037-PstS and CB037-PstR to stripe rust (Fig. 2B4-B5), CB037A and CB037C were susceptible to the disease and a large number of spores parasitized on the leaves (Fig. 2B1 and B3), while CB037B was resistant to the disease, and there wasn't any lesions and *Pst* spores on the leaves (Fig. 2B2). Results indicated that the three NILs were high resistant to PM which all contained *Pm21* gene from *D. villosum* 6VS and only CB037B was high resistant to stripe rust.

Regeneration ability comparison of the three NILs in tissue culture

For the anther culture of the three near isogenic wheat lines, 899, 857, and 764 anthers from CB037A, CB037B, and CB037C, respectively, were cultured. One more months later, 13, 183, and 16 embryonic calli were produced from the inoculated anthers of the three lines, respectively. On the regeneration medium, 11, 324, and 15 green shoots were differentiated from the induced calli, respectively. Callus induction rate were 3.6%, 22.6%, and 4.6%, and shoot induction rate were 1.2%, 37.8%, and 2.0% for the three lines, respectively (Fig. 3; Table 1). Results indicated that CB037B had significantly higher plant regeneration ability than CB037A and CB037C in anther culture.

Table 1
Regeneration statistical analysis of the three near isogenic lines of CB037 in anther culture

Lines	No. of experiments	No. of anthers	Produced calli	Embryogenic calli	Differentiated calli	Regenerated shoots	CIR (%)	CDR(%)	SIR(%)
CB037A	3	899	32	13	10	11	3.6	1.1	1.2
CB037B	4	857	194	183	142	324	22.6	16.6	37.8
CB037C	3	764	35	16	12	15	4.6	1.6	2.0
CIR, callus induction rate; CDR, callus differentiated rate; SIR, shoot induction rate									

For the immature embryo culture of the three wheat lines, totally, 158, 149, and 160 immature embryos were cultured, and 134, 134, and 141 embryogenic calli were produced from CB037A, CB037B, and CB037C, respectively, among which 92, 128, and 95 embryogenic calli generated green shoots. The plant regeneration efficiencies of the three lines were 58.2%, 85.9%, and 59.4% in order (Fig. 4A-4C; Table 2). Results showed that CB037B had higher plant regeneration ability than CB037A and CB037C in immature embryo culture.

Table 2
Regeneration statistical analysis of the three near isogenic lines of CB037 in immature and mature embryo culture

Lines	No. of experiments	No. of embryos	Produced calli	Embryogenic calli	Differentiated calli	Regenerated shoots	CIR (%)	CDR(%)	SIR(%)
Immature									
CB037A	3	158	147	134	92	198	93.0	58.2	125.3
CB037B	3	149	140	134	128	468	94.0	85.9	314.1
CB037C	3	160	150	141	95	236	93.7	59.4	147.5
Mature									
CB037A	3	147	95	75	45	45	64.6	30.6	30.6
CB037B	3	175	125	113	82	89	71.4	46.9	50.9
CB037C	3	183	115	89	58	58	62.8	31.7	31.7
CIR, callus induction rate; CDR, callus differentiated rate; SIR, shoot induction rate									

For the mature embryo culture of the three wheat lines, 147, 175, and 183 mature embryos were cultured on callus induction medium, and 75, 113, and 89 embryogenic calli were induced from CB037A, CB037B, and CB037C, respectively, among which 45, 82, and 58 embryogenic calli generated green shoots. The plant regeneration efficiencies of the three lines were 30.6%, 46.9%, and 31.7% in order (Fig. 4D-4F; Table 2). Results suggested that CB037B had higher plant regeneration ability than CB037A and CB037C in mature embryo culture.

Transformation ability comparison of the three NILs using immature and mature embryos

In total, 498, 521, and 532 immature embryos of the three wheat lines CB037A, CB037B, and CB037C were infected by *Agrobacterium*-mediated for stable genetic transformation, respectively. After callus induction, selection, and differentiation, 160 transgenic plants were obtained from CB037B which were tested by PCR, *GUS* staining, and Quickstix stripe (Fig. 5B, Fig. 6), and the transformation efficiency was 29.2% (Table 3). No transgenic plants were obtained from the transformed immature embryos of CB037A and CB037C (Table 3, Figs. 5A and 5C).

Table 3
Agrobacterium-mediated transformation efficiencies of the three near isogenic lines of CB037 using immature embryos and mature embryo derived calli

Lines	No. of experiments	No. of embryos transformed	No. of regeneration plants	No. of transgenic plants	Transformation efficiency (%)
Immature					
CB037A	5	498	0	0	0
CB037B	5	521	160	152	29.2
CB037C	5	532	0	0	0
Mature					
CB037A	10	1210	0	0	0
CB037B	10	1311	8	8	0.61
CB037C	10	1259	0	0	0

In another transformation experiment, 1210, 1311, and 1259 calli derived from the mature embryos of CB037A, CB037B, and CB037C were infected by *Agrobacterium*, respectively. After callus selection and differentiation, 8 transgenic plants were obtained from CB037B which were tested by PCR, *GUS* staining, and Quickstix stripe (Fig. 5E, Fig. 6), and the transformation efficiency was

0.61% (Table 3). No transgenic plants were obtained from the transformed mature embryo derived calli of CB037A and CB037C (Table 3, Figs. 5D and 5F).

Discussion

Genetic engineering and haploid technologies can accelerate the breeding progress of new plant varieties, which is normally limited by plant regeneration efficiency (Ye et al. 1998). Wheat regeneration and transformation rates are affected by many factors such as genotype, explant, medium composition, hormone, culture conditions, and physiological status of mother plants (Ye et al. 1997; Mendoza et al. 2002; Sharma et al. 2005). Among them, genotype is one of the most important factors (Ye et al. 1997; Shah et al. 2009; Yin et al. 2011). Although wheat immature embryos, inflorescences, mature embryos, anthers, and microspores all can be used in tissue culture for plant regeneration, immature embryos are mostly often employed in transgenic study (Ishida et al. 2015; Wang et al. 2017). Currently, a model spring wheat cultivar Fielder was widely used in the transformation, and its transformation efficiency using immature embryos can be up to 45.3% (Wang et al. 2017). However, Fielder was susceptible to PM and stripe rust, which makes cultivation management some difficulties and low physiological status of the explants for plant regeneration and genetic transformation. Thereby, it is necessary to evaluate desirable wheat genotypes with good disease resistance and high regeneration and transformation efficiencies.

Except Fielder, another spring wheat line CB037 was found to have excellent agronomic traits, short plant height, early maturity, good bread-making quality, and immunity to PM (Zhang et al. 2012; Chen et al. 2013; Wang et al. 2016; Dong et al. 2017; Liu et al. 2017). Particularly, CB037 was proved to be high in regeneration ability in anther culture, immature embryo culture, and mature embryo culture (Tao et al. 2011; Yin et al. 2011; She et al. 2013; Wang et al. 2014b; Bie et al. 2017, 2020; Zhang et al. 2018). Therefore, this wheat line has been also commonly applied in the study on gene functional characterization via transgenic and genome editing strategies (Wang et al. 2017; Chen et al. 2022). However, the normally used CB037 was not pure in genetics. In an experiment on stripe rust resistance test, two different responses of high sensitivity line CB037-PstS and high resistance line CB037-PstR were confirmed in the original CB037 (Zheng et al. 2020). Previous to the aforementioned study, three near isogenic lines of CB037A, CB037B, and CB037C were identified in the original CB037 according to glutenin composition (Wu et al. 2011; Wang et al. 2016).

In present study, we proved that CB037A, CB037B, and CB037C were all wheat-*D. villosum* 6AL·6V#2S translocation lines by GISH (Fig. 1), and showed strong PM resistance (Fig. 2A). In the resistance test to stripe rust, only CB037B showed good resistance to *Pst*, and CB037A and CB037C were seriously susceptible to the pathogen infection (Fig. 2B). Combining a previously published finding that CB037-PstR contained a pair of wheat-rye translocated chromosomes of 1BL·1RS identified by molecular marker and GISH (Zheng et al. 2020), we concluded that CB037B is right the CB037-PstR (Fig. 2B), and carries the 1BL·1RS translocated chromosomes, on which a resistance gene *Yr9* to stripe rust might be contained. In fact, the original CB037 was developed from a complicated cross combination in which five varieties or lines were used as parents including CA9211, 93N40, Liaochun10, 93R137, and Jing711 (Zhang et al. 2012; Liu et al. 2017). Among the three parents, 93N40 showing PM resistance was a 6AL·6V#2S translocation line carrying a resistance gene *Pm21*, 93R137 showing PM resistance was a 6DL·6V#4S translocation line carrying a resistance gene *PmV*, and CA9211 was a 1BL·1RS translocation line showing resistance to stripe rust (Zhang et al. 2012; Liu et al. 2017; Li et al. 2020). Therefore, we concluded that the stripe rust resistance in CB037B contributed by 1BL·1RS translocation should be derived from CA9211; the 6DL·6V#4S translocated chromosome was missed in the original CB037, in which the PM resistance was contributed by 6AL·6V#2S translocation.

Particularly, we first compared the regeneration and transformation efficiencies of immature embryos of the three wheat lines CB037A, CB037B, and CB037C in the current study, and found that CB037B had higher regeneration and transformation efficiencies than CB037A and CB037C (Table 2–3, Fig. 4–5). In other hands, CB037B also showed higher regeneration efficiency than CB037A and CB037C in anther culture and mature embryo culture (Table 1–2, Fig. 3–4). Additionally, CB037B showed strong resistance to PM and good resistance to stripe rust (Fig. 2A2, B2), which can allow the mother plants growing healthy for available physiological status for tissue culture and transformation. Combining its other desirable agronomic and quality traits (Zhang et al. 2012; Chen et al. 2013; Dong et al. 2017; Liu et al. 2017), CB037B is an ideal genotype for wheat genetic transformation and haploid breeding.

Conclusions

Wheat line CB037 showing ideal agronomic traits, good bread quality, strong resistance to powdery mildew (PM) and high regeneration ability is widely applied in wheat genetic transformation. However, the original CB037 is a mixture of three genotypes. In the present study, three near isogenic lines (NILs) of CB037A, CB037B, and CB037C originated from CB037 were proved to be high resistant to PM, and CB037B was not only high resistant to stripe rust but also had high regeneration ability in anther culture, immature, and mature embryo culture. Positive transgenic wheat plants were finally obtained only from CB037B. Therefore, CB037B was an available wheat material for tissue culture and genetic transformation.

Declarations

Acknowledgements

The authors thank Dr. Fangpu Han, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, for his guidance on genomic *in situ* hybridization of wheat chromosomes. This study was financially supported by the National Natural Science Foundation of China (31971945) and the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (2060299-2-22).

Author contributions

XY and ZL conceived the research and designed the experiments. HL conducted most of the experiments. HM and ZL performed cytogenetics observation. WZ and WW participated in tissue culture. HM, ZL and JW identified PM and stripe rust resistance. KW performed vector construction and participated in genetic transformation. HL and XY analyzed the data, and drafted and revised the manuscript.

Availability of data and materials

There are supplementary data in this manuscript and all data are included in the main text. The materials used in this study will be available upon request.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Abebe T, Guenzi AC, Martin B, Cushman JC (2003) Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. *Plant Physiol* 131:1748–1755
2. Bie XM, Dong L, Li XH, Wang H, Gao XQ, Li XG (2020) Trichostatin A and sodium butyrate promotes plant regeneration in common wheat. *Plant Signal Behav* 15:1820681
3. Bie XM, Wang K, Liu C, Liu YW, Du LP, Mao XG, Ye XG (2017) Effects of soil drought stress on plant regeneration efficiency and endogenous hormone levels of immature embryos in wheat (& *Triticum aestivum* L.). *Pak J Bot* 49:1673–1679

4. Chen LF, Yan YP, Wang XD, Liu Y, Zhang HY, Zhang ZY (2013) Development and detection of wheat breeding materials resistant to both powdery mildew and barley yellow dwarf virus. *J Plant Genetic Resour* 14:925–929 (in Chinese with English abstract)
5. Dong LW, Li N, Lu XB, Prodanovic S, Xu YH, Zhang WY, Yan YM (2016) Quality properties and expression profiling of protein disulfide isomerase genes during grain development of three spring wheat near isogenic lines. *Genetika* 48:249–269
6. Guo X, Su HD, Shi QH, Fu SL, Wang J, Zhang XQ, Hu ZM, Han FP (2016) De novo centromere formation and centromeric sequence expansion in wheat and its wide hybrids. *PLOS Genetic* 12:e1005997
7. Han J, Lakshman DK, Galvez LC, Mitra S, Baenziger PS, Mitra A (2012) Transgenic expression of lactoferrin imparts enhanced resistance to head blight of wheat caused by *Fusarium graminearum*. *BMC Plant Biol* 12:33–41
8. He ZH, Xia XC, Chen XM, Zhuang QS (2011) Progress of wheat breeding in China and the future perspective. *Acta Agron Sinica* 37:202–215 (in Chinese with English abstract)
9. Ishida Y, Tsunashima M, Hiei Y, Komari Y (2015) Wheat (*Triticum aestivum* L.) transformation using immature embryos. In: Wang K (ed) *Agrobacterium* Protocols: Volume 1. *Methods in Molecular Biology*, vol 1223. Springer Science + Business Media, New York, pp 189–198
10. Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: betaglucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* 6:3901–3907
11. Khurana J, Chugh A, khurana P (2002) Regeneration from mature and immature embryos and transient gene expression via *Agrobacterium* mediated transformation in emmer wheat (*Triticum diccicum* Schuble). *Indian J Exp Biol* 40:1295–1303
12. Li SJ, Jia ZM, Wang K, Du LP, Li HJ, Lin ZS, Ye XG (2020) Screening and functional characterization of candidate resistance genes to powdery mildew from *Dasyphyrum villosum*#4 in a wheat line Pm97033. *Theor Appl Genet* 133:3067–3083
13. Liu C, Li SJ, Wang K, Ye XG, Lin ZS (2017) Developing of Specific Transcription Sequences *P21461* and *P33259* on *Dasyphyrum villosum* 6VS and Application of Molecular Markers in Identifying Wheat-*D. villosum* Breeding Materials with Powdery Mildew Resistance. *Acta Agron Sinica* 43:983–992 (in Chinese with English abstract)
14. Mendoza MG, Kaeppler HF (2002) Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of wheat (*Triticum aestivum* L.). *Vitro Cell Dev Biology Plant* 38:39–45
15. Orshinsky BR, Sadasivaiah RS (1997) Effect of plant growth conditions, plating density, and genotype on the anther culture response of soft white spring wheat hybrids. *Plant Cell Report* 16:758–762
16. Patnaik D, Khurana P (2003) Genetic transformation of Indian bread (*T. aestivum*) and pasta (*T. durum*) wheat by particle bombardment of mature embryo-derived calli. *BMC Plant Biol* 3:5–15
17. Shah M, Khalid Q, Khan U, Shah S, Shah S, Hassan A, Pervez A, Oliveira V, Caxito F, Gomes K (2009) Variation in genotypic responses and biochemical analysis of callus induction in cultivated wheat. *Genet Mol Res* 8:783–793
18. Sharma VK, Hnsch R, Mendel RR, Schulze J (2005) Influence of picloram and thidiazuron on high frequency plant regeneration in elite cultivars of wheat with long-term retention of morphogenecity using meristematic shoot segments. *Plant Breeding* 124:242–246
19. She MY, Yin GX, Li JR, Li X, Du LP, Ma WJ, Ye XG (2013) Efficient Regeneration potential is closely related to auxin exposure time and catalase metabolism during the somatic embryogenesis of immature embryos in *Triticum aestivum* L. *Mol Biotechnol* 54:451–460
20. Tao LL, Yin GX, Du LP, Shi ZY, She MY, Xu HJ, Ye XG (2011) Improvement of plant regeneration from immature embryos of wheat Infected by *Agrobacterium tumefaciens*. *Agricultural Sci China* 10:317–326
21. Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. *Science* 327:818–822
22. Wang C, Zeng J, Li Y, Hu W, Chen L, Miao YJ, Deng PY, Yuan CH, Ma C, Chen X, Zang ML, Wang Q, Li KX, Chang JL, Wang YS, Yang GX, He GY (2014a) Enrichment of provitamin A content in wheat (*Triticum aestivum* L.) by introduction of the bacterial carotenoid biosynthetic genes *CrtB* and *CrtI*. *J Exp Bot* 65:2545–2556
23. Wang K, Liu HY, Du LP, Ye XG (2017) 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
24. Wang XM, Ren X, Yin GX, Wang K, Li JR, Du LP, Xu HJ, Ye XG (2014b) Effects of environmental temperature on the regeneration frequency of the immature embryos of wheat (*Triticum aestivum* L.). *J Integr Agric* 13:722–732

25. Wang YP, Zhen SM, Luo NN, Han CX, Lu XB, Li XH, Xia XC, He ZH, Yan YM (2016) Low molecular weight glutenin subunit gene *Glu-B3h* confers superior dough strength and breadmaking quality in wheat (*Triticum aestivum* L.). *Sci Rep* 6:27182
26. Wu JS, Lu XB, Yu ZT, Han CX, Li XH, Prodanovic S, Yan YM (2017) Effects of *Glu-1* and *Glu-3* allelic variation on wheat glutenic macropolymer content as revealed by size-exclusion high performance liquid chromatography (SE-HPLC). *Genetika* 49: 677–691
27. Xiao MG, Song FJ, Jiao JF, Wang XM, Xu HX, Li HJ (2013) Identification of the gene *Pm47* on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi. *Theor Appl Genet* 126:1397–1403
28. Ye X, Xu HJ, Du LP, He GY, Wang K, Lin ZS (2014) Establishment and application of large-scale transformation systems in wheat. *Scientia Agricultura Sinica* 47:4155–4171 (in Chinese with English abstract)
29. Ye XG, Xu HJ, Xu QF, Du LP, Li ZW (1997) Genetic analysis and combining ability evaluation of the anther culture response in common wheat. *Scientia Agricultura Sinica* 30:49–54 (in Chinese with English abstract)
30. Ye XG, Xu HJ, Zhao LL, Du LP (1998) Studies on improving wheat cultivars by tissue culture. *Acta Agron Sinica* 24:310–314 (in Chinese with English abstract)
31. Yin GX, Wang YL, She MY, Du LP, Xu HJ, Ma JX, Ye XG (2011) Establishment of a highly efficient regeneration system for the mature embryo culture of wheat. *Agricultural Sci China* 10:9–17
32. Yuan J, Guo X, Hu J, Lv ZL, Han FP (2014) Characterization of two *CENH3* genes and their roles in wheat evolution. *New Phytol* 206:839–851
33. Zhang L, Rybczynski JJ, Langenberg WG, Mitra A, French R (2000) An efficient wheat transformation procedure: transformed calli with long-term morphogenic potential for plant regeneration. *Plant Cell Report* 19:241–250
34. Zhang W, Yin MQ, Zhao P, Wang K, Du LP, Ye XG (2018) Regeneration capacity evaluation of some largely popularized wheat varieties in China. *Acta Agron Sinica* 44:208–217 (in Chinese with English abstract)
35. Zhang YL, Wand MJ, Zhang Y, Chu CP, Lin ZS, Xu QF, Ye XG, Chen X, Zhang XS (2012) Development and application of functional markers specific to powdery mildew resistance on chromosome arm 6VS from different origins of *Haynaldia villosa*. *Acta Agron Sinica* 38:1827–1832 (in Chinese with English abstract)
36. Zhao LS, Liu LX, Wang J, Guo HJ, Gu JY, Zhao SR, Li JH, Xie YD (2015) Development of a new wheat germplasm with high anther culture ability by using a combination of gamma-ray irradiation and anther culture. *J Sci Food Agric* 95:120–125
37. Zheng YY, Huang DH, Li JL, Zhang HF, Bao YG, Ni F, Wu JJ (2020) Analysis of the stripe rust resistance in a wheat line CB037 with high regeneration and transformation efficiency. *Acta Agron Sinica* 46:1743–1749 (in Chinese with English abstract)
38. Zhou H, Arrowsmith JW, Fromm ME, Hironaka CM, Taylor ML, Rodriguez D, Pajean ME, Brown SM, Santino CG, Fry JE (1995) Glyphosate-tolerant CP4 and GOX genes as a selectable marker in wheat transformation. *Plant Cell Rep* 15:159–163

Figures

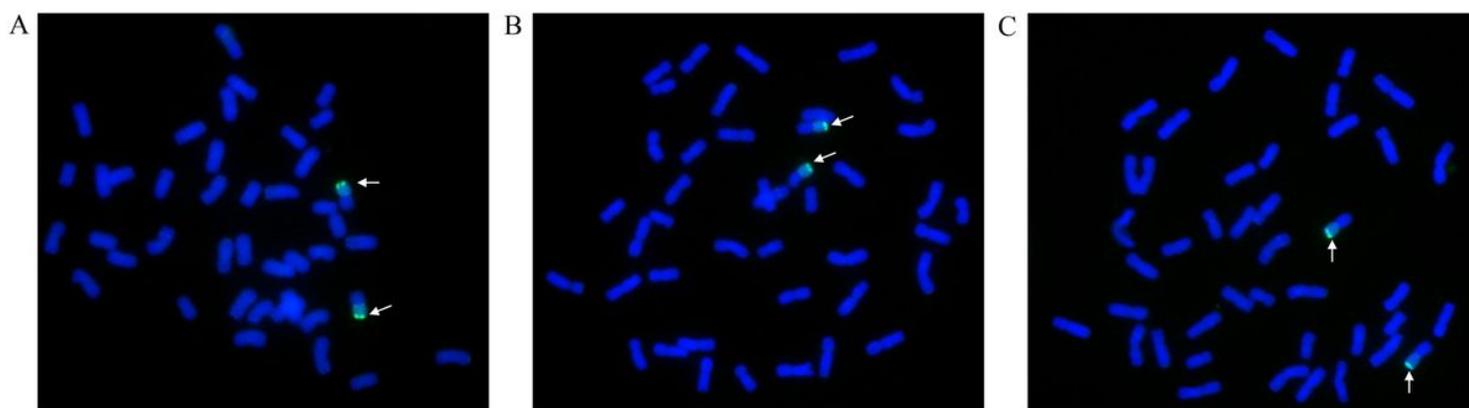


Figure 1

GISH identification of the three near isogenic lines of CB037. A: CB037A; B: CB037B; C: CB037C. The white arrows showed the translocated chromosomes 6DL·6V#2S.

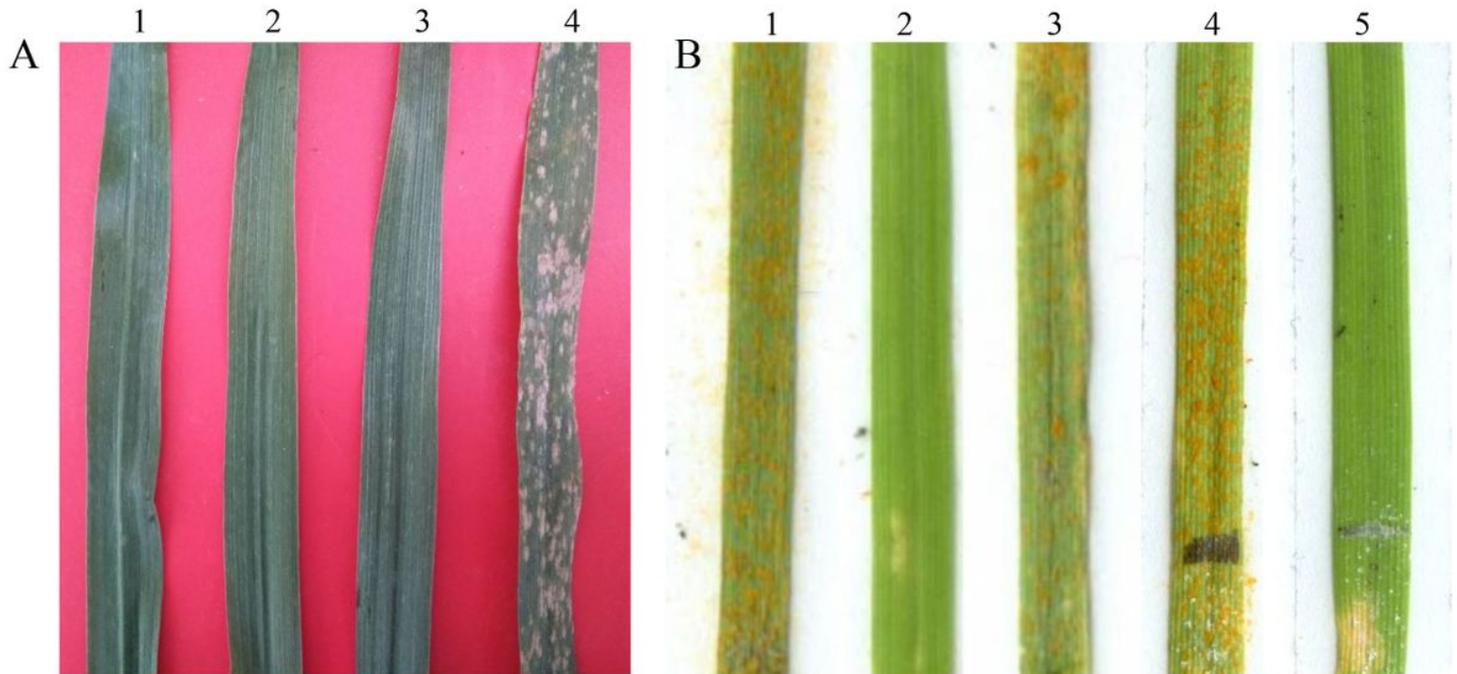


Figure 2

Powdery mildew and stripe rust resistance identification of the three near isogenic lines of CB037. A: the powdery mildew resistance identification, A(1): CB037A, A(2): CB037B, A(3): CB037C, A(4): Fielder; B: the stripe rust resistance identification, B(1): CB037A, B(2): CB037B, B(3): CB037C, B(4): CB037-PstS, B(5): CB037-PstR.

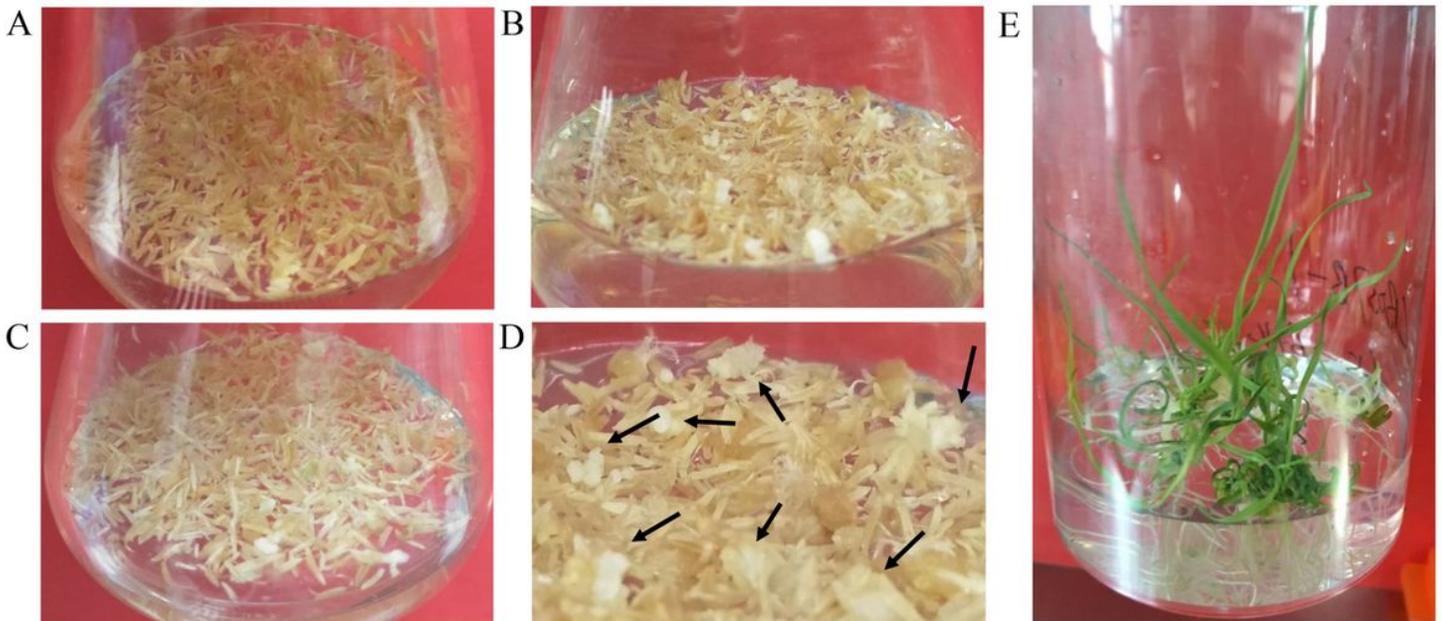


Figure 3

Regeneration ability comparison of the three near isogenic lines of CB037 in anther culture. A: the embryogenic callus induction from CB037A; B and D: the embryogenic callus induction from CB037B, in which the black arrows showed the embryogenic callus; C: the embryogenic callus induction from CB037C; E: the shoot elongation and root formation from CB037B.

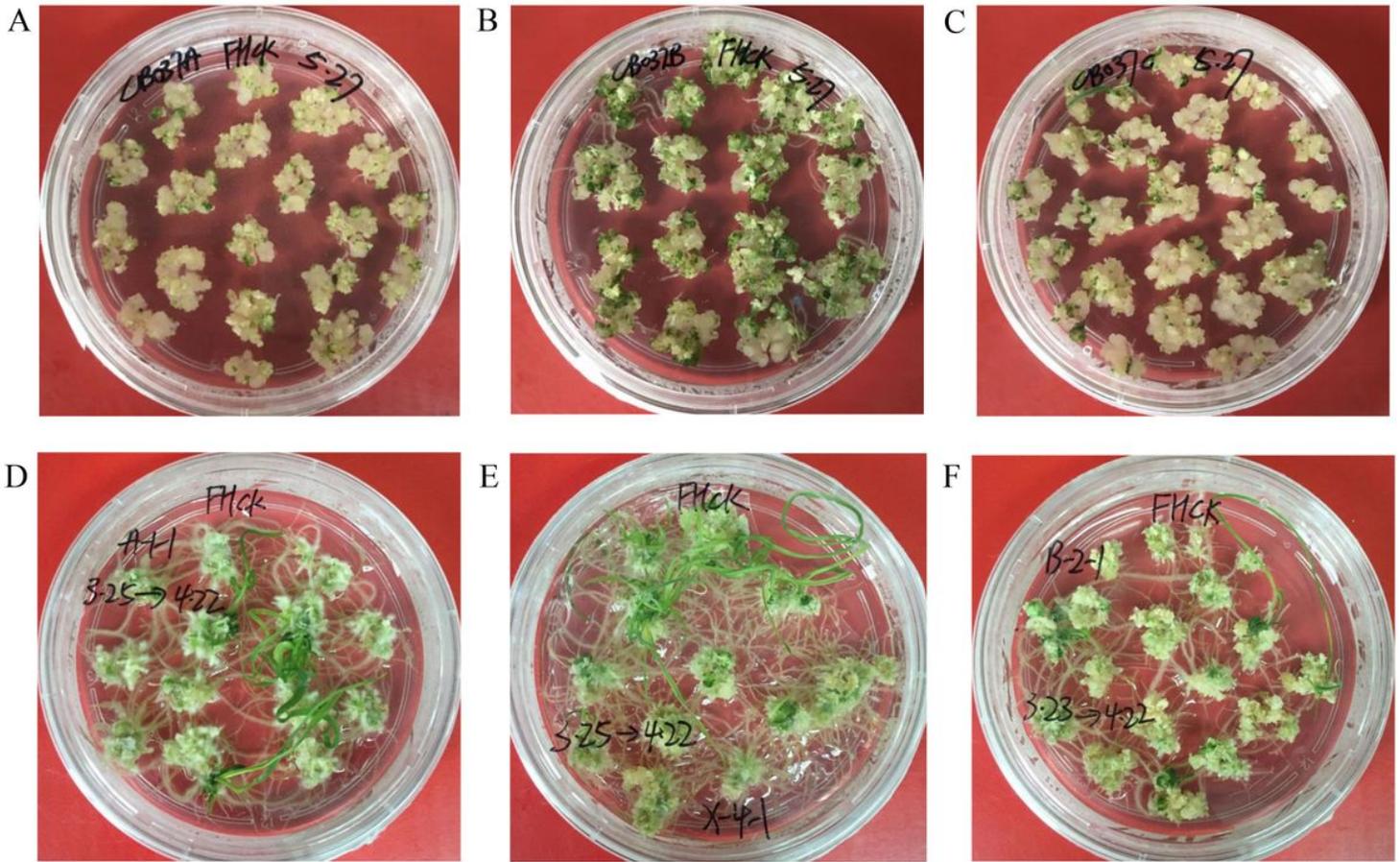


Figure 4

Regeneration ability comparison of the three near isogenic lines of CB037 using immature and mature embryos. A: callus differentiation of CB037A from immature embryos; B: callus differentiation of CB037B from immature embryos; C: callus differentiation of CB037C from immature embryos; D: callus differentiation of CB037A from mature embryos; E: callus differentiation of CB037B from mature embryos; F: callus differentiation of CB037C from mature embryos.



Figure 5

Transformation ability comparison of the three near isogenic lines of CB037 via *Agrobacterium* infection using immature and mature embryos. A: callus differentiation from the transformed immature embryos of CB037A on selection medium; B: callus differentiation from the transformed immature embryos of CB037B on selection medium; C: callus differentiation from the transformed immature embryos of CB037C on selection medium; D: callus differentiation from the transformed mature embryo calli of CB037A on selection medium; E: callus differentiation from the transformed mature embryo calli of CB037B on selection medium; F: callus differentiation from the transformed mature embryo calli of CB037C on selection medium.

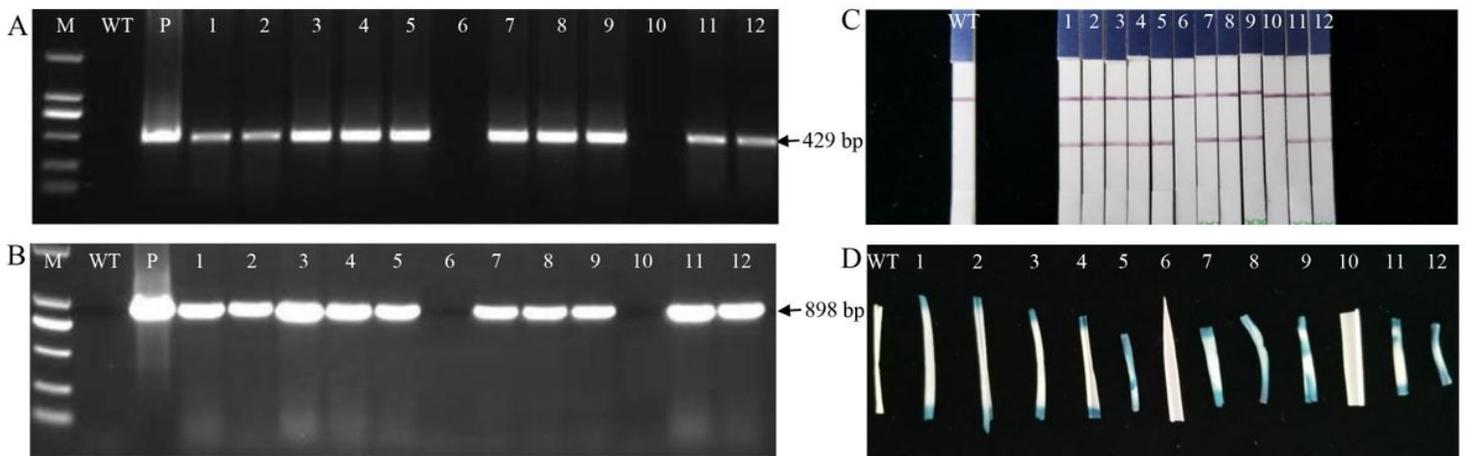


Figure 6

Detection of transgenic wheat plants by PCR for *bar* and *GUS* genes, QuickStix strips for Bar protein, and histochemical staining for β -glucuronidase (GUS) activity. A: detection of the *bar* gene by PCR, 1-12: transgenic plants, P: expression vector pWMB110-*GUS*, WT: wild type CB037; B: detection of the *GUS* gene by PCR, 1-12: transgenic plants, P: expression vector pWMB110-*GUS*, WT: wild type CB037; C: detection of Bar protein by QuickStix strips, 1-12: transgenic plants, WT: wild type CB037; D: detection of the GUS protein by histochemical staining, 1-12: transgenic plants, WT: wild type CB037.

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