Construction of genetic transformation system of Lycium barbarum callus

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Abstract

In order to achieve efficient genetic transformation of *Lycium barbarum* L.and accelerate the process of genetic improvement of *L. barbarum*. The stem segments of *Lycium barbarum* were used as materials, and the Agrobacterium-mediated method was used to determine the LB concentration, infection time and co-culture time under the optimal transformation conditions by three-factor and three-level orthogonal test. The genetic transformation system of callus was established, and the effect of *LbMYB18* gene overexpression on the physiological metabolism of callus was explored by using this system. (1) The optimal transformation conditions were LB concentration of 2%, infection time of 20 min, and co-culture time of 3 d. The callus formation rate was 96.67%, and the GFP fluorescence positive rate was 53.33%. (2) *LbMYB18* gene was successfully integrated and stably expressed, and the expression level was about 5.4 times that of the control group. (3) Physiological analysis showed that the activities of SOD, POD, CAT and the content of MDA in the overexpression lines were significantly higher than those in the control group. The contents of chlorophyll a, chlorophyll b and carotenoids increased significantly. The genetic transformation system of *Lycium barbarum* was established. Overexpression of *LbMYB18* gene can significantly improve the antioxidant capacity of plants and promote the accumulation of photosynthetic pigments. The results provide a technical basis for gene function research and genetic improvement of *Lycium barbarum*.

Introduction

Lycium barbarum L.is an important plant of *Lycium* in Solanaceae. Its fruit is rich in a variety of bioactive components, such as *Lycium barbarum* polysaccharides, carotenoids, flavonoids, etc. It has significant medicinal and health care value and is widely used in medicine, health care and food industries [Nie M Y et al.2025; Yu Y et al.2021; Yu J et al.2023;]. However, with the continuous growth of market demand and the challenges brought by environmental changes, the limitations of traditional Chinese wolfberry in yield, quality and stress resistance have gradually become prominent. Therefore, it has become an urgent need for the sustainable development of wolfberry industry to use modern biotechnology, especially genetic transformation and gene editing technology (Jing F et al.2023), to carry out directional genetic improvement of wolfberry and cultivate new varieties with high yield, high quality and stress resistance.

As a kind of undifferentiated cell mass formed by dedifferentiation in plant in vitro culture, callus has the characteristics of active cell division, relatively uniform genetic background and easy acceptance of exogenous gene integration. It is an important carrier for plant genetic transformation and regeneration system establishment (Ning jing et al. 2026). At present, the callus-mediated genetic transformation system has been successfully established in a variety of plants, such as *Hosta plantaginea*[Zhou H et al.2026], Liriodendron hybrid[Meiping L et al. 2022], indica black rice[Yadav B et al.2026]and so on. In addition, in the evaluation of genetic transformation system, in addition to the transformation efficiency and regeneration ability, the researchers also evaluated the physiological status of transgenic callus by measuring antioxidant enzyme activity and photosynthetic pigment content. Zhang Zhongxing et al [2021]transformed *MhMYB114-Like* into ' Wanglin ' apple (*Malus domestica ' Orin '*) callus. By measuring

the related physiological indexes, the resistance analysis showed that overexpression of *MhMYB114-Like* could significantly improve the resistance of transgenic callus to iron deficiency stress. Yang et al（2026） overexpressed *VvDREB4* gene in grape callus and found that it could significantly alleviate the inhibition of Na_2CO_3 stress on callus growth, enhance the activity of antioxidant enzymes, reduce the accumulation of malondialdehyde, and enhance the tolerance of cells to saline-alkali stress. The above studies have shown that the establishment of genetic transformation technology system based on callus combined with physiological and biochemical index analysis is an effective way to realize plant gene function research and directional genetic improvement.

In *Lycium* plants, researchers have carried out preliminary explorations on callus induction （Karakas P F et al.2020）, proliferation (Xian-Jun Z et al.2017), differentiation (Liang Y et al.2025) and somatic embryogenesis (Kumar S V et al.2022), mainly focusing on basic biological characteristics, regeneration ability and application potential in genetic transformation. Fang Qingchun (2025) found that the breeding efficiency and callus regeneration ability of *Lycium ruthenicum* Murr. could be effectively improved by accurately controlling the disinfection and culture conditions. Zhang Jiaqi et al. (2021) studied the effects of exogenous polyamines on the induction of regenerated organs and vitrification of *Lycium ruthenicum* callus with different properties. Yang Shuai (2025) studied the effects of different treatment media on callus induction and adventitious bud differentiation, and screened out the optimal medium for callus induction and differentiation of *Lycium barbarum* leaves. These studies provide a scientific basis for understanding the growth and development mechanism of *Lycium barbarum*, improving reproductive efficiency and genetic improvement. However, in Ningxia wolfberry, the establishment of callus genetic transformation system and the systematic study of physiological characteristics of transgenic callus are still limited.

In this study, the callus of *Lycium barbarum* was used as the experimental material to construct a stable and efficient genetic transformation system. The establishment of this system can provide core technical support for directional genetic improvement of *Lycium barbarum* in Ningxia and significantly shorten the breeding cycle. At the same time, using callus tissue as the research vector can lay an important foundation for the analysis of functional genes and the molecular mechanism of stress response in *Lycium barbarum*.

1 Materials and Methods

1.1 Experimental materials

Ningxia wolfberry stem segment. The *Lycium barbarum* materials used in the experiment were collected from the experimental farm of Ningxia University. They were located in Xihe Village, Wanghong Town, Yongning County, Yinchuan City, Ningxia Hui Autonomous Region (106° 13' 31"E, 38° 13' 11" N). All of them were healthy and disease-free branches. After picking and taking back to the laboratory for disinfection treatment, they were planted in MS medium (adding 6-BA 0.5 mg / L and NAA 0.05 mg / L).

1.2 Main reagents

Acetyl syringone (AS) solution, cephalosporin (Cef) solution, carbenicillin (Carb) solution, kanamycin (kan+) solution, rifampicin (Rif) solution, 10% sucrose solution, 75% alcohol, 38% sodium hypochlorite solution, Nacl, tryptone (TRYPTONE), yeast extract (YEAST EXTRACT), agar powder. The main medium formulations used in this experiment are as follows (Table 1 and Table 2).

Table 1
Hormone formulations

Reagents	Preparation method
6-BA(1g/L)	Weigh 1 g 6-BA powder, dissolve with a little NaOH, dilute to 1000 mL with distilled water, aliquot and store at 4 ℃.
NAA(1g/L)	Weigh 1 g NAA powder, dissolve with a little 95% ethanol, dilute to 1000 mL with distilled water, aliquot and store at 4 ℃.
2,4-D(1g/L)	Weigh 1 g 2,4-D powder, dissolve with a little 95% ethanol, dilute to 1000 mL with distilled water, aliquot and store at 4 ℃.
AS(100 mg/mL)	Dissolve the powder in 50 mL DMSO in a clean bench, filter through 0.45 μm organic filter, aliquot, seal and store at -20 ℃.
Rif(50 mg/mL)	Dissolve the powder in 20 mL DMSO in a clean bench, filter through 0.45 μm organic filter, aliquot, seal and store at -20 ℃.
Kan(50 mg/mL)	Dissolve the powder in 2 L sterile water in a clean bench, filter through 0.22 μm organic filter, aliquot, seal and store at -20 ℃.
Cef(250 mg/mL)	Dissolve the powder in 20 mL sterile water in a clean bench, filter through 0.22 μm organic filter, aliquot, seal and store at -20 ℃.
Carb(250 mg/mL)	Dissolve the powder in 20 mL sterile water in a clean bench, filter through 0.22 μm organic filter, aliquot, seal and store at -20 ℃

Table 2
Medium Formulations

List of medium names	Medium composition
LB solid medium	1 g NaCl + 1 g TRYPTONE + 0.5 g YEAST EXTRACT + 1 g Agar powder + 100 mL Distilled water + 100 μL Kan + 100 μL Rif
LB liquid medium	1 g NaCl + 1 g TRYPTONE + 0.5 g YEAST EXTRACT + 100 mL Distilled water + 100 μL Kan + 100 μL Rif
Induction medium	41.74 g MS + 6-BA(0.5 mg/L, 500 μL) + 2,4-D□0.5 mg/L, 750 μL□
Suspension medium	200 mL 10% Sucrose + 400 μL AS
Co-cultivation medium	200 mL 10% Sucrose + 400 μL AS
Selection medium	Sucrose(25 g/L) + MS□4.43 g/L□ + Agar□7 g/L□ + 6-BA□0.5 mg/L, 500 μL□ + NAA□0.05 mg/L, 50 μL□ + Cef□300 mg/L, 1000 μL□ + Carb□300 mg/L, 1000 μL□

1.3 Experimental method

1.3.1 Explant disinfection

The stems of fresh *Lycium barbarum* seedlings growing for 1–2 months were taken, the surface dust was washed, and the water was washed in a wide-mouth bottle with gauze for 30 min. In the ultra-clean bench, the stem segments of *Lycium barbarum* seedlings were soaked in 75% ethanol for 30 s, washed with sterile water twice, shaken for 30 s each time, and then soaked in 28% 84 solution for 20 min. During the period of continuous shaking, the stem segments of *Lycium barbarum* seedlings were fully sterilized, washed with sterile water five times, and shaken for 30 s each time.

1.3.2 Preparation of Agrobacterium infection solution

On the ultra-clean bench, a small amount of Agrobacterium solution carrying the target gene (*LbMYB18* cloned by our group previously) was dipped with a gun tip, and the line was drawn on the LB solid medium supplemented with 100 μL Kan + and 100 μL Rif, and then placed in a 28°C incubator for 2days. After the monoclonal colonies appeared, the monoclonal colonies were picked and inoculated in 1 mL LB liquid medium containing 100 μL Kan and 100 μL Rif, and cultured in a shaker at 28°C and 180r·min^{-1} until the bacterial solution was turbid. The turbid bacterial liquid was added to 50 mL LB liquid medium containing 100 μL Kan and 100 μL Rif, and the shaking culture was carried out at 28°C and 180r·min^{-1} for overnight. After centrifugation, the cells were collected and resuspended with a resuspension (200 mL 10% sucrose + 400 μL AS) to the desired concentration.

1.3.3 Orthogonal experimental design of genetic transformation of *Lycium barbarum* stem segments

The cultured stem segments of *Lycium barbarum* were treated with wounds, and infected with the bacterial solution containing the target gene. The infection time was set to 10 min, 20 min and 30 min. At the end of infection, the stem segments were co-cultured in the co-culture medium for 2 d, 3 d and 4 d, respectively, and transferred to the screening medium for dark culture for 4 w.

Three treatment levels (Table 3) were set up, and a total of 9 treatments (Table 4) were used for orthogonal test. Each treatment was repeated three times to screen out the best treatment combination.

Table 3
L_9 (3^3) orthogonal test factor table

Level	A-LB Concentration(%)	B- Infection time(min)	C- Co-cultivation time(d)
1	1.0	10	2
2	1.5	20	3
3	2.0	30	4

Table 4
Treatment combinations of orthogonal experiment

Test No.	A	B	C	Specific protocol
T1	1	1	1	A1 B1 C1
T2	2	2	1	A2 B2 C1
T3	3	3	1	A3 B3 C1
T4	3	2	2	A3 B2 C2
T5	2	1	2	A2 B1 C2
T6	1	3	2	A1 B3 C2
T7	1	2	3	A1 B2 C3
T8	2	3	3	A2 B3 C3
T9	3	1	3	A3 B1 C3

1.3.4 Genetic transformation of *Lycium barbarum* stem segments in Ningxia

The stem segments of L.*barbarum* after disinfection were selected as genetic transformation materials. According to the orthogonal experimental design (Table 4), the stem segments of L.*barbarum* were added to the re-suspended bacterial solution on the ultra-clean bench, and the shaking infection was performed at different times (10, 20 and 30 min). The stem segments of *Lycium barbarum* were taken out, and the bacterial liquid was fully sucked on the filter paper, and inoculated into the induction medium

without antibiotics (pad with filter paper, heavy suspension wetting) for co-culture. After sealing, it was cultured in the tissue culture room at 28°C for 2–4 days. The stem segments after co-culture were degermed first, and then transferred to delayed screening medium after water absorption. Ten stem segments were inoculated in each dish and cultured in dark at 24–26°C for 4 w. The growth of callus and adventitious buds was observed regularly and the data were recorded.

(1) 200 mL 10% sucrose + 400 μL Cef + 400 μL Carb, shaken for 2 min, repeated 5 times;

(2) 200 mL sterile water + 400 μL Cef + 400 μL Carb, washed 3 times

1.3.5 GFP fluorescence detection

A portable dual-band excitation light source (LUYOR-3415, excitation wavelength 470 nm) was used for non-destructive in vivo fluorescence detection of *Lycium barbarum* stem segments transformed by Agrobacterium tumefaciens in petri dishes. Under blue light excitation (~ 470 nm), green fluorescence at 510–530 nm could be detected in the stem incision and callus of transgenic plants due to the expression of GFP reporter gene driven by 35S promoter. The unconverted control material has no visible green fluorescence signal in this band.

1.3.6 RNA extraction and real-time fluorescence quantitative PCR detection of each treatment callus

About 100 mg of fresh or -80°C frozen plant tissues were ground into powder by liquid nitrogen, quickly transferred to a centrifuge tube containing 1 mL of lysis buffer, vortexed and mixed well and allowed to stand at room temperature for 5 min. 0.2 mL chloroform was added and shaken violently for 15 s. After standing for 3–5 min, it was centrifuged at 4°C and 12 000 rpm for 10 min. The upper colorless aqueous phase was mixed with 200 μL anhydrous ethanol and transferred into a RNase-free adsorption column pretreated with 500 μL washing solution. After standing at room temperature for 2 min, it was centrifuged at 4°C and 12 000 rpm for 2 min to discard the waste liquid. A total of 600 μL of ethanol-containing rinse solution was added and centrifuged for 2 min to discard the waste solution. After the cover of volatile ethanol was opened, 50–100 μL of RNase-free ddH_2O was added dropwise to the center of the adsorption column membrane. The solution was allowed to stand at room temperature for 5 min and centrifuged at 4°C and 12 000 rpm for 2 min. The eluent was collected as total RNA.

The extracted total RNA was used as a template, and reverse transcription was performed using a specific reverse transcription system at 42°C for 2 min, 37°C for 15 min, and 85°C followed by 16°C infinite circulation. After the cDNA was obtained, 180 μL sterile water was added to make a working solution. Specific primers were designed based on the conserved sequence of the *LbMYB18* gene of *Lycium barbarum* (see Table 5 for sequence details). The ACT gene of *Lycium barbarum* was used as an internal reference, and the qPCR was performed using the SYBR Green fluorescent dye method. The reaction system consisted of 10 μL of TB, 0.8 μL of primer F and R, 1.6 μL of DNA and 6.8 μL of sterile water. The amplification was carried out at 95°C for 30 s, 95°C for 5 s, 60°C for 30 s (40 cycles of the

latter two steps) and 10°C for 30 min. Each reaction was repeated by three techniques. The specificity of the product was verified by melting curve analysis after amplification.

Table 5
Primer sequences

Gene	Primer sequences(5’-3’)
LbMYB18-2300-F	AGAACACGGGGGACGAGCTCATGGGAAGAGCTCCATGTTGTGATA
LbMYB18-2300-R	ACCATGGTGTCGACTCTAGAATAGTACATCATGAATTTTTCTTCTGTCTTGAT

1.3.7 PCR amplification and agarose gel electrophoresis detection

Genomic DNA was extracted using the Soleibao Plant Genomic DNA Extraction Kit. ≤ 100 mg fresh tissue was grinded into powder in liquid nitrogen. After the liquid nitrogen was volatilized, it was transferred into a centrifuge tube containing 400 μL PA, 20 μL RNase A (10 mg mL Δ1) and 5 μL β-mercaptoethanol, mixed upside down, and allowed to stand at room temperature for 10 min. 140 μL PB was added, mixed, centrifuged at 12000 rpm for 10 min, and the supernatant was taken. Add equal volume of PC and equal volume of absolute ethanol, after mixing, all of them were transferred to the adsorption column, centrifuged at 12000 rpm for 5 min, and the breakthrough solution was discarded. Centrifuging with 600 μL ethanol-containing rinse solution for 1 min, discarding the waste liquid, repeating once; the column was centrifuged at 12 000 rpm for 2 min. The lid was opened at room temperature or 50°C for several minutes, and 50–200 μL of 65°C preheated eluent was added. The cells were allowed to stand at room temperature for 5 min and centrifuged at 12000 rpm for 2 min to collect DNA.

When the genomic DNA extracted by the above method was used as a template for PCR detection, the untransformed plant samples were set as negative controls, and the overexpression vector containing the target gene was used as a positive control. The PCR reaction is carried out according to the process (see above) and the number of cycles is increased to 40.

Component	Volume
Test sample	l
Forward primer	0.5 μL
Reverse primer	0.5 μL
2×Easy Taq Mix	5 μL
ddH_2O	polishing10 μL

1.3.8 Determination of callus phenotype

The size of callus and the length of differentiated buds were measured by vernier caliper, and the number of callus and the number of adventitious buds were counted. The formation rate of callus and adventitious buds and the conversion rate of callus were calculated. The calculation formula is as follows :

Callus formation rate (%) = (number of stem segments formed by callus / total number of stem segments in each treatment) × 100% (1)

Adventitious bud formation rate (%) = (number of adventitious bud formation stem segments / total number of stem segments of each treatment) × 100% (2)

Callus transformation rate (%) = (green fluorescent stem segments / total number of stem segments treated) × 100% (3)

1.3.9 Detection of antioxidant enzyme activity

Malondialdehyde (MDA) content, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activity were measured according to the instructions of Beijing Solarbio Technology Co., Ltd.malondialdehyde content detection kit (BC0020-5 / 4), superoxide dismutase activity detection kit (BC0170-5 / 2), peroxidase activity detection kit (BC0090-5 / 4), catalase activity detection kit (BC4785-10 / 4).

1. 3.10 Determination of chlorophyll and carotenoids

Chlorophyll content was determined by direct extraction method (Mozammel H et al.2025). After the samples were cut into pieces and fully mixed, 0.2 g of the sample was weighed and placed in a centrifuge tube, and a mixture of 45 mL of 1 : 1 pure acetone and pure ethanol was added. The mixture was sealed and extracted overnight in the dark, and intermittently shaken 3–4 times. After the leaf tissue was completely decolored and whitened, the absorbance of the supernatant was measured by spectrophotometer at 646 nm, 663 nm and 470 nm, respectively. The mixed extract treated under the same conditions was used as the blank control. The following formula is calculated.

Chlorophyll a (Chl a) = (12.2663 A663–2.8646 A646) V / 100 W (1)

Chlorophyll b (Chl b) = (20.1646 A646–5.0663 A663) V / 100 W (2)

Carotenoid (Car) = (4.470 A470-0.01 Chl a-0.45 Chl b) V / 100 W (3)

Chl = Chl a + Chl b (4)

In the formula, A is the absorbance value, V is the total volume of the extract (mL), and W is the fresh weight of the leaves (g).

1.4 Data analysis

Microsoft Excel was used for data collation; SPSS 27.0 and Origin 2022 were used to analyze the range analysis and multiple comparison of callus and adventitious bud formation of *Lycium barbarum* stem segments under different treatments.

2 Results and analysis

2.1 Establishment of Agrobacterium-mediated genetic transformation system of *Lycium barbarum* stem segments

2.1.1 Growth of callus and adventitious buds

The growth process of *Lycium barbarum* stem segments was observed every week. After 4 weeks of culture, the growth of *Lycium barbarum* stem segments in each treatment was as shown in Fig. 1. Under different treatments, the stem segments of *Lycium barbarum* grew callus and adventitious buds. In T4, the callus formation of stem segments of *Lycium barbarum* was the most and larger, and the number of differentiated adventitious buds was the most.

2.1.2 Statistics on callus formation and adventitious bud growth of *Lycium barbarum* stem segments under different treatments

After 4 weeks of culture, the test results were statistically analyzed, and the statistical results are shown in Table 6. The callus formation rate of T4 was the highest, which was 96.67%, and the adventitious bud induction rate was the highest, which was 93.33%, which was higher than that of CK. The average size of callus was up to (CK) 10.56 mm, followed by T4 (9.71 mm), and the smallest (T7) 4.41 mm, with a difference of 6.15 mm.

Table 6
Statistical analysis on callus formation and adventitious bud growth of wolfberry stem segments with different treatments

Treatment	Callus formation rate(%)	Callus number(No.)	Average callus size(mm)	Adventitious bud induction rate(%)	Adventitious bud number(No.)	Average adventitious bud length(mm)
T1	46.67	14	7.85	30.00 ± 2.31h	9	5.45
T2	90.00	27	8.41	63.33 ± 2.32cd	19	6.31
T3	86.67	26	7.73	86.67 ± 0.58b7b	26	5.48
T4	96.67	29	9.71	93.33 ± 1.15a	28	10.2
T5	93.33	28	7.81	50.00 ± 0.57e	15	5.53
T6	40.00	12	4.97	36.67 ± 1.73g	11	4.82
T7	53.33	16	4.41	30.00 ± 0.16h	9	4.95
T8	36.67	11	6.16	60.00 ± 2.31d	18	5.75
T9	73.33	22	7.20	66.67 ± 0.58c	20	9.15
CK	90.00	27	10.56	43.33 ± 1.15f	13	5.54

Note: T1–T9 are the same as Table 4. Different lowercase letters within the same column indicate significant difference at $P < 0.05$.

2.1.3 Range analysis of different treatments on callus and adventitious bud formation of *Lycium barbarum* stem segments

(1) Range analysis of callus formation rate of *Lycium barbarum* stem segments after 4 weeks of culture.

The results showed that (Table 7), the order of influence of each factor on callus formation rate was A > B > C, indicating that different LB concentrations had the most significant effect on callus formation rate, followed by infection time, and co-culture time had the least effect. The optimal level combination was A3B2C2 (T4), and the highest callus formation rate was 96.67%.

Table 7
Range analysis for callus formation rate of *Lycium barbarum* stem segments under different treatments

item	A	B	C
k1	0.47	0.71	0.74
k2	0.73	0.80	0.77
k3	0.86	0.54	0.54
R	0.39	0.26	0.22
Note: k1-k3 represent the average induction rate of corresponding factors at level 1 to 3; R is the range, which reflects the influence degree of each factor on the induction rate.			

(2) Range analysis of adventitious bud formation rate of *Lycium barbarum* stem segments after 4 weeks of culture.

It can be seen from Table 8 that the order of the effects of the three factors on the formation rate of adventitious buds was A > B > C, indicating that different LB concentrations had the most significant effect on the formation rate of adventitious buds, followed by the infection time, and the co-culture time had the least effect. The optimal level combination was A3B2C2 (T4), and the highest adventitious bud formation rate was 93.33%.

Table 8
Range analysis for adventitious bud formation rate of *Lycium barbarum* stem segments

item	A	B	C
k1	0.32	0.49	0.64
k2	0.58	0.76	0.73
k3	1.00	0.66	0.52
R	0.68	0.27	0.21
Note: k1-k3 represent the average induction rate of corresponding factors at level 1 to 3; R is the range, which reflects the influence degree of each factor on the induction rate.			

(3) Range analysis of the average length of adventitious buds after 4 weeks of stem segment culture of *Lycium barbarum* L.in each treatment.

According to Table 9, the influence of three factors on the average size of adventitious buds was ranked as A > B > C, indicating that different LB concentrations had the most significant effect on callus transformation rate, followed by infection time, and co-culture time had the least effect. The optimal level combination was A3B2C2 (T4). The maximum length of adventitious buds was 10.20 cm, the minimum was 4.82 cm, and the difference was 5.38 cm.

Table 9
Range analysis for average adventitious bud length of *Lycium barbarum* stem segments

item	A	B	C
k1	5.07	6.71	5.75
k2	5.86	7.15	6.85
k3	8.28	5.35	6.62
R	3.20	1.80	1.10

Note: k1-k3 represent the average induction rate of corresponding factors at level 1 to 3; R is the range, which reflects the influence degree of each factor on the induction rate.

2.2 Identification of overexpression callus

2.2.1 GFP fluorescence detection

Through GFP fluorescence observation, the transgenic callus can stimulate obvious green fluorescence, while the control group has no obvious fluorescence phenomenon (Fig. 2. The statistical results showed that there was no green fluorescence in the blank control; the fluorescence conversion rate of T4 (LB 2%, infection 20min, co-culture 3d) was the highest, reaching 53.33% ± 0.88%. T1 (LB 1%, infection 10min, co-culture 2d) was the lowest, only 13.33% ± 0.33%; the remaining treatments were between 20.00% and 43.33% (Table 10). The results of variance analysis of callus transformation rate under different treatments (see Table 10) showed that there was a significant difference in the transformation rate between different treatments ($P < 0.05$), and the difference between T4 treatment and other treatments was the most significant.

Table 10
GFP transformation rate statistics of different treatments

Treatment	Stem segment number(No.)	Callus transformation rate(%)
T1	30	13.33% ± 0.33 b
T2	30	33.33% ± 1.86 ab
T3	30	43.33% ± 1.76 ab
T4	30	53.33% ± 0.88 a
T5	30	33.33% ± 1.67 ab
T6	30	30.00% ± 0.58 ab
T7	30	20.00% ± 0.58 ab
T8	30	30.00% ± 0.58 ab
T9	30	23.33% ± 0.88 ab

Note: T1–T9 are the same as Table 4. Different lowercase letters within the same column indicate significant difference at $P < 0.05$.

2.2.2 Range analysis of callus conversion rate of *Lycium barbarum* stem segments after 4 weeks of culture

It can be seen from Table 11 that the order of the influence of the three factors on the average size of callus was A > C > B, indicating that different LB concentrations had the greatest influence on the callus conversion rate, followed by the co-culture time, and the infection time had the least influence. The optimal level combination was A3B2C2 (T4), and the highest callus transformation rate was 53.33%.

Table 11
Range analysis of the effects of different treatments on callus transformation rate of *Lycium barbarum* stem segments

item	A	B	C
k1	21.11%	23.33%	30.00%
k2	32.22%	35.55%	38.89%
k3	40.00%	34.44%	24.44%
R	18.89%	12.22%	14.44%

Note: k1-k3 represent the average induction rate of corresponding factors at level 1 to 3; R is the range, which reflects the influence degree of each factor on the induction rate.

2.2.3 qRT-PCR validation and agarose gel electrophoresis detection

In order to further verify the expression of exogenous genes in callus, the expression level of *LbMYB18* gene was detected by real-time fluorescence quantitative PCR. As shown in Fig. 3, the expression of *LbMYB18* gene in the overexpression treatment group was significantly higher than that in the control group ($P < 0.01$), and the expression of T4 was the highest, which was about 5.4 times that of the control group. This indicates that the exogenous gene has been successfully integrated into the genome of *Lycium barbarum* and achieved stable expression. As shown in Fig. 4, the transgenic callus was amplified by PCR using *LbMYB18* specific primers. The positive control showed a single bright band at 948 bp, and the negative control had no band, indicating that the primer specificity was good.

M: DL2000 DNA Marker; CK: Blank control; T1-T9: *LbMYB18*-transformed callus

2.3 Antioxidant enzyme activity analysis of control and overexpression callus

In order to explore the effect of *LbMYB18* gene on the antioxidant capacity of callus, the SOD, POD, CAT activity and MDA content of control callus and overexpressed callus were determined by kit. As shown in Fig. 5, the MDA content and the activities of SOD, POD and CAT in the overexpression treatment group were significantly higher than those in the control group ($P < 0.05$).

a:MDA content; b:POD activity; c:CAT activity; d: SOD activity

2.4 Comparison of chlorophyll and carotenoid contents between control and overexpression calluses

In order to explore the effect of *LbMYB18* gene overexpression on the synthesis and metabolism of photosynthetic pigments in *Lycium barbarum* callus, the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a + Chl b) and carotenoid (Car) were determined by spectrophotometry. The results are shown in Fig. 6.Overexpression of *LbMYB18* significantly increased the photosynthetic pigment level of callus, and the contents of chlorophyll and carotenoid in the overexpression treatment group were significantly higher than those in the control group ($P < 0.05$).

a:Chl a; b: Chl b;c: Total chlorophyll; d: Carotenoids

3 Discussions

3.1 Effects of different treatments on genetic transformation efficiency of *Lycium barbarum* callus

Genetic transformation technology is one of the important research fields of modern plant molecular biology, which is widely used in gene function research and genetic improvement of various plants(Singh K A et al.2025). In recent years, with the rapid development of gene editing technology, genetic transformation technology has been widely used in plants, which not only provides a powerful tool for the study of plant gene function, but also provides a new way for the cultivation of excellent varieties(Wang

Zhijie et al.2025). Through genetic transformation technology, researchers have successfully introduced excellent genes such as insect resistance (Hou Yet al.2025;Corrigendum.2012;), disease resistance(Wang F et al.2026;Cui H et al.2021;), and stress tolerance (Chen L et al.2026;Qiangbo L et al.2023;) into a variety of crops, significantly improving crop yield and quality. In addition, genetic transformation technology has also been used to study the growth and development mechanism of plants, which provides important support for basic research of plant biology (Hu Lu et al.2025).

In this experiment, the effects of three key factors, LB concentration, infection time and co-culture time, on the genetic transformation efficiency of *Lycium barbarum* stem segments were systematically explored through orthogonal experimental design. The results showed that T4 (LB 2%, infection 20 min, co-culture 3d) reached the optimal level in callus formation rate, adventitious bud differentiation rate and GFP positive rate, indicating that this combination of conditions was conducive to the efficient transfer and integration of Agrobacterium T-DNA. Range analysis showed that LB concentration was the primary factor affecting callus formation rate and adventitious bud differentiation rate, which was consistent with the results of Fu Z (2025) and Li Jingjing (2025) in chrysanthemum. The possible mechanism is that the low concentration of LB leads to insufficient amount of Agrobacterium on the incision of explants, resulting in poor transformation effect (Nafisa Muzepal et al.2025). The infection time of 20 min is the best. If the time is too short, it is not conducive to the adhesion of Agrobacterium and T-DNA transfer. If it is too long, it may lead to excessive growth of Agrobacterium and damage plant cells (Liu Leyue et al.2025). The co-culture time of 3 d was better than that of 2 d and 4 d. The insufficient co-culture time may not be enough to complete T-DNA integration, while excessive co-culture time increases the risk of Agrobacterium overgrowth (Sato Lynn et al.2025). The efficient genetic transformation system established in this study provides an important technical platform for gene function research and molecular breeding of *Lycium barbarum*.

3.2 Effects of *LbMYB18* gene on physiological metabolism of callus

Through GFP fluorescence observation and real-time fluorescence quantitative PCR verification, it was confirmed that *LbMYB18* gene had been successfully integrated into the genome of *Lycium barbarum* callus and achieved stable expression. The gene expression level of T4 was 5.4 times that of the control group, and the transformation efficiency was 53.33%, which was significantly higher than that of other treatment groups.

The MYB transcription factor family plays an important role in the regulation of plant secondary metabolism. Different from the reported *AtMYB12* regulating flavonoid synthesis (Guan Fanyuan et al.2024), *VuMYB1* and *VuMYB4* co-regulating anthocyanin formation (Chen J et al.2025), *LbMYB18* affects both antioxidant defense and photosynthetic pigment metabolism in *L.barbarum*, indicating that it has a broader regulatory function. This feature is consistent with the functional diversity of the MYB gene family, and also provides a new reference for the analysis of the unique carotenoid synthesis regulation mechanism of *Lycium barbaru*m. In addition, the dual regulation of *LbMYB18* on antioxidant capacity and photosynthetic pigment accumulation in callus suggested that *LbMYB18* may play an important role in

response to environmental stress, delaying senescence and improving fruit quality, which is worthy of further study at the whole plant level.

The genetic transformation system has a significant effect on the physiology and biochemistry of the plant (Hoang-Chinh N et al.2018). Index analysis showed that overexpression of *LbMYB18* caused synergistic changes in the activation of antioxidant enzyme system and the accumulation of membrane lipid peroxidation products. The activities of SOD, POD and CAT in the overexpression lines were significantly higher than those in the control group, indicating that the gene could activate the enzymatic antioxidant defense system of the cells. In addition, MDA content was also significantly increased, suggesting that *LbMYB18*, as a MYB transcription factor, not only promoted the rapid proliferation and vigorous metabolism of callus, but also led to the production rate of reactive oxygen species (ROS) exceeding the scavenging capacity of antioxidant enzymes, forming a physiological state of high metabolism and high oxidative stress (Wu Rui et al.2022). It also regulates the expression of genes related to ROS production and clearance, and participates in specific oxidative stress signal regulation pathways. Moderate oxidative stress may be beneficial to cell differentiation and regeneration (Zhang Houchen et al.2025). In summary, *LbMYB18* may respond to oxidative stress caused by rapid cell division by directly regulating the expression of antioxidant enzyme-encoding genes. The accumulation of MDA may reflect the normal metabolic renewal of cell membrane during rapid proliferation, rather than severe oxidative damage.

In terms of photosynthetic pigment metabolism, *LbMYB18* overexpression significantly promoted the synthesis and accumulation of chlorophyll a, chlorophyll b and carotenoids. This result indicates that the transcription factor may positively regulate the expression of genes related to photosynthetic pigment biosynthesis, or participate in the regulation of cell differentiation and plastid development (Wang Meimei 2020). The increase of photosynthetic pigment content in callus reflects the enhancement of cell metabolic activity or the physiological transformation to photoautotrophic direction, which is of positive significance for subsequent callus differentiation and plant regeneration. These results indicate that *LbMYB18* has complex multiple functions in the formation of quality traits and the regulation of cell physiological state in *Lycium barbarum*.

4 Conclusion

In this study, the stem segments of *L.barbarum* were used as materials, and the genetic transformation system was established by Agrobacterium-mediated method. The effects of *LbMYB18* gene on antioxidant enzyme activity and photosynthetic pigment indexes of *L.barbarum* callus were investigated by this system. The main results are as follows:

(1) Through orthogonal test, the optimal combination of transformation conditions was determined as LB concentration of 2%, infection time of 20 min, and co-culture time of 3 d. The callus formation rate was 96.67%, and the GFP fluorescence positive rate was 53.33%.

(2) The antioxidant enzyme activity of *LbMYB18* overexpression lines was significantly higher than that of the control, and the MDA content also increased, indicating that the gene can enhance the antioxidant

capacity of callus, but the active cell metabolism may also bring some oxidative damage.

(3) Compared with the control, the overexpression lines showed obvious advantages in the accumulation of photosynthetic pigments, and the contents of chlorophyll a, chlorophyll b and carotenoids were significantly increased. This result indicates that *LbMYB18* has a certain promoting effect on the synthesis of photosynthetic pigments in *Lycium barbarum*.

The genetic transformation system established in this study can be used for subsequent gene function research of *Lycium barbarum*, and the regulatory function of *LbMYB18* gene also provides a reference for the improvement of *Lycium barbarum* quality.

Declarations

CRediT authorship contribution statement

Wenjun Yang: Conceptualization, Methodology, Visualization, Writing. Xiuyun Lei: Softwar and Methodology. Yingjie Liu: Investigation and Visualization. Gaier Yang: Data curation. Formal analysis, Supervision. Linyuan Duan: Resources. Zhijun Song and Xiang Li: Funding acquisition, Project administration, Conceptualization. All authors read and approved the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest in relation to this work.

Research involving Human Participants and/or Animals

This study does not involve human participants, animal subjects, or any data that could identify individuals. Therefore, no ethical approval for human or animal research was required.

Informed consent

Not applicable, as the research did not involve human participants.

Additional statement

All plant materials used in this study were obtained from legal sources. The experiments were conducted in compliance with local biosafety and plant protection regulations. No protected or endangered species were involved.

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Figures

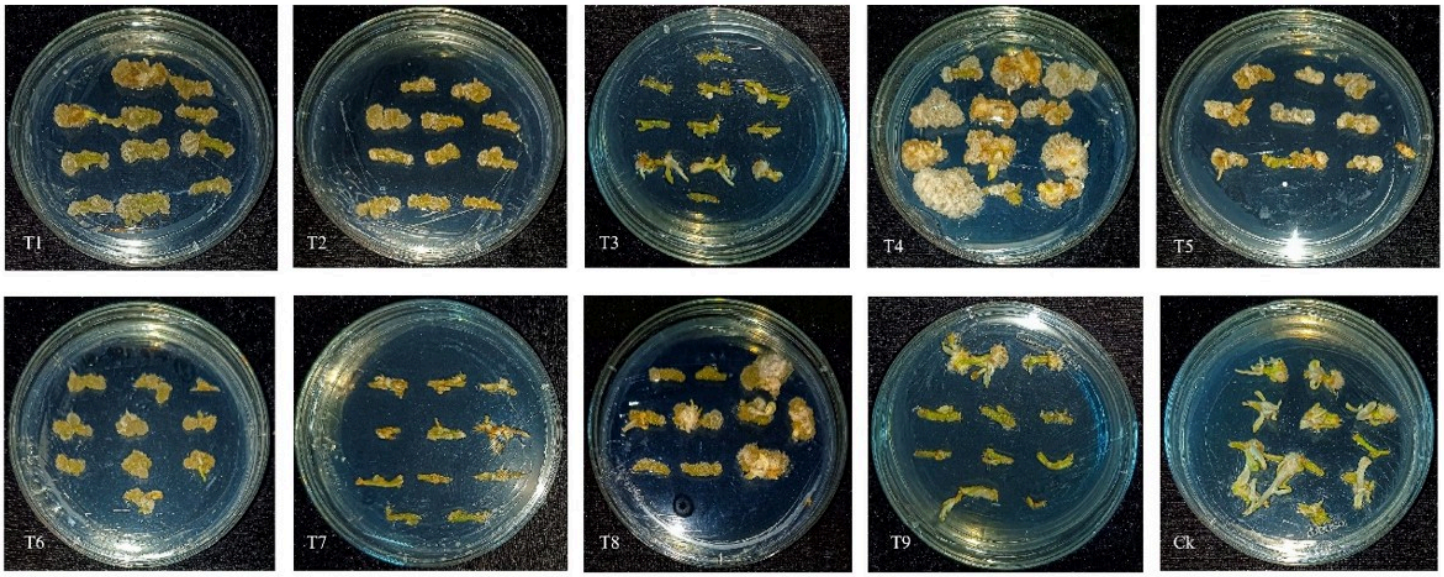

Figure 1

Growth of *Lycium barbarum* stem segments under different treatments after 4-week culture

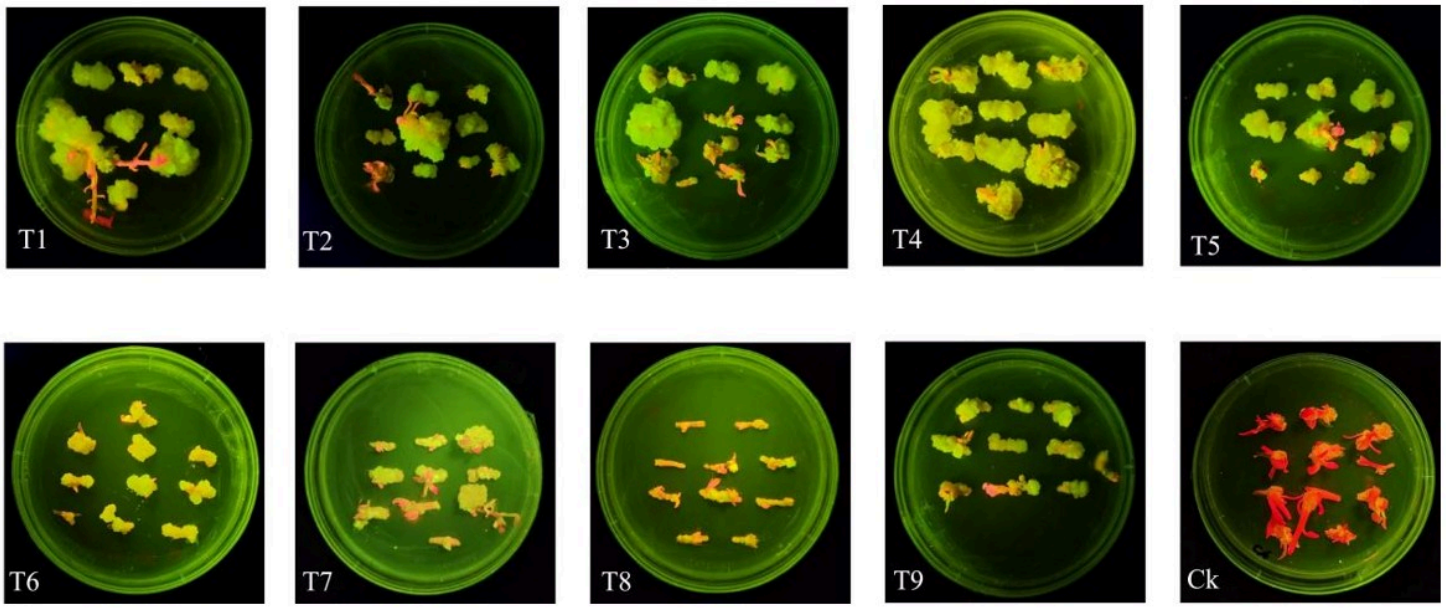

Figure 2

Observation of GFP fluorescence in *Lycium barbarum* callus under different treatments

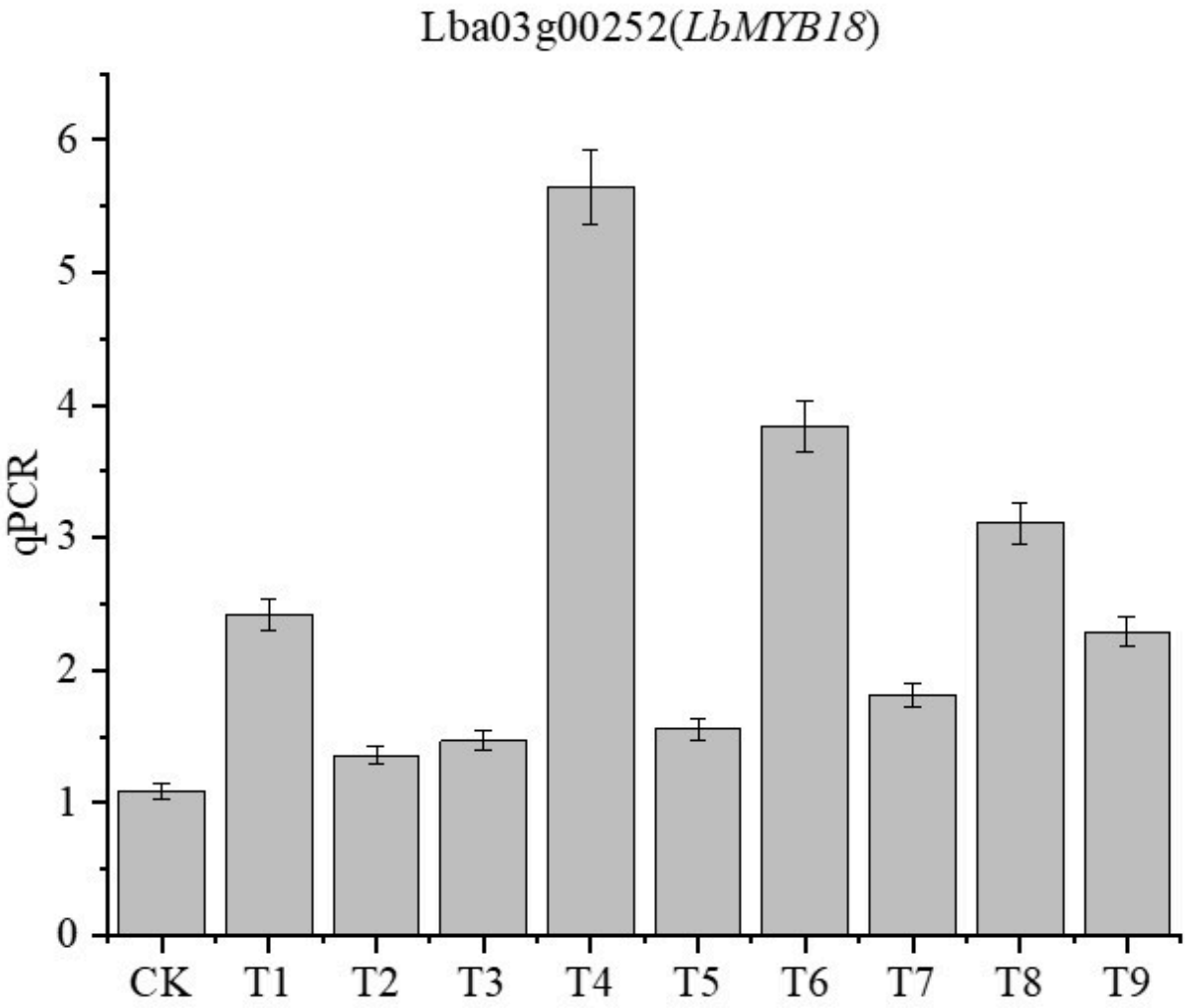

Figure 3

Relative expression of*LbMYB18* gene

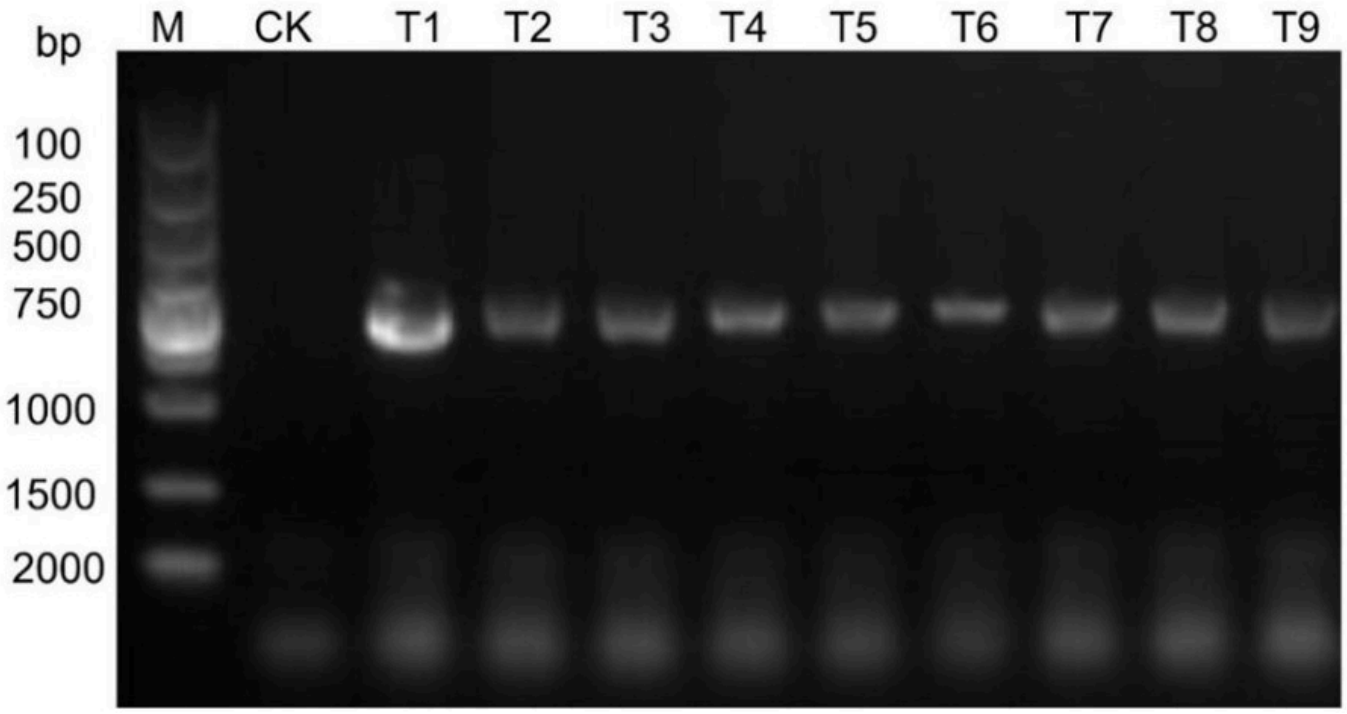

Figure 4

Electrophoresis profile of PCR verification for transgenic *Lycium barbarum* callus

M: DL2000 DNA Marker; CK: Blank control; T1-T9: *LbMYB18*-transformed callus

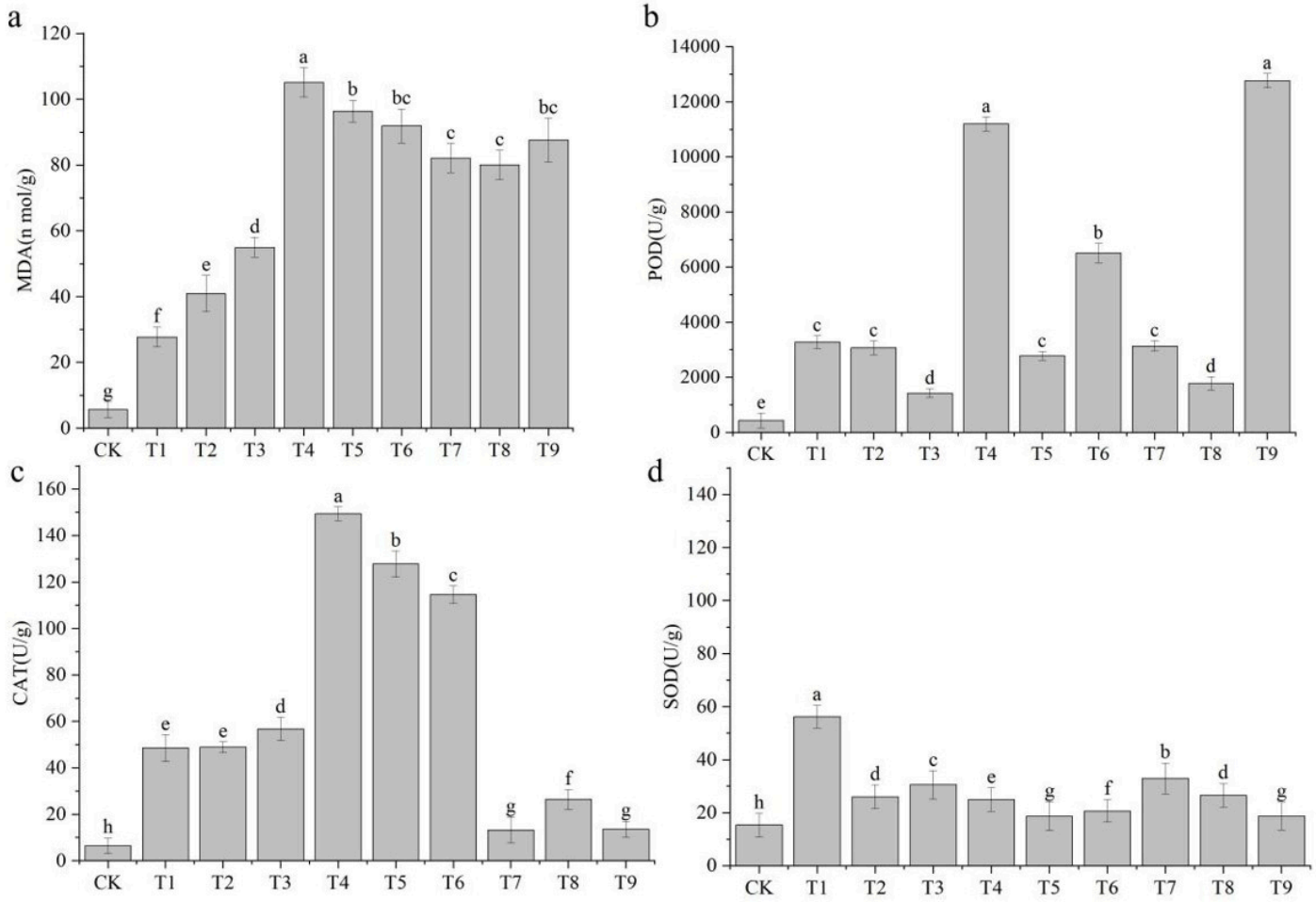

Figure 5

Comparison of antioxidant enzyme activities in control and overexpressed callus

a:MDA content; b:POD activity; c:CAT activity; d: SOD activity

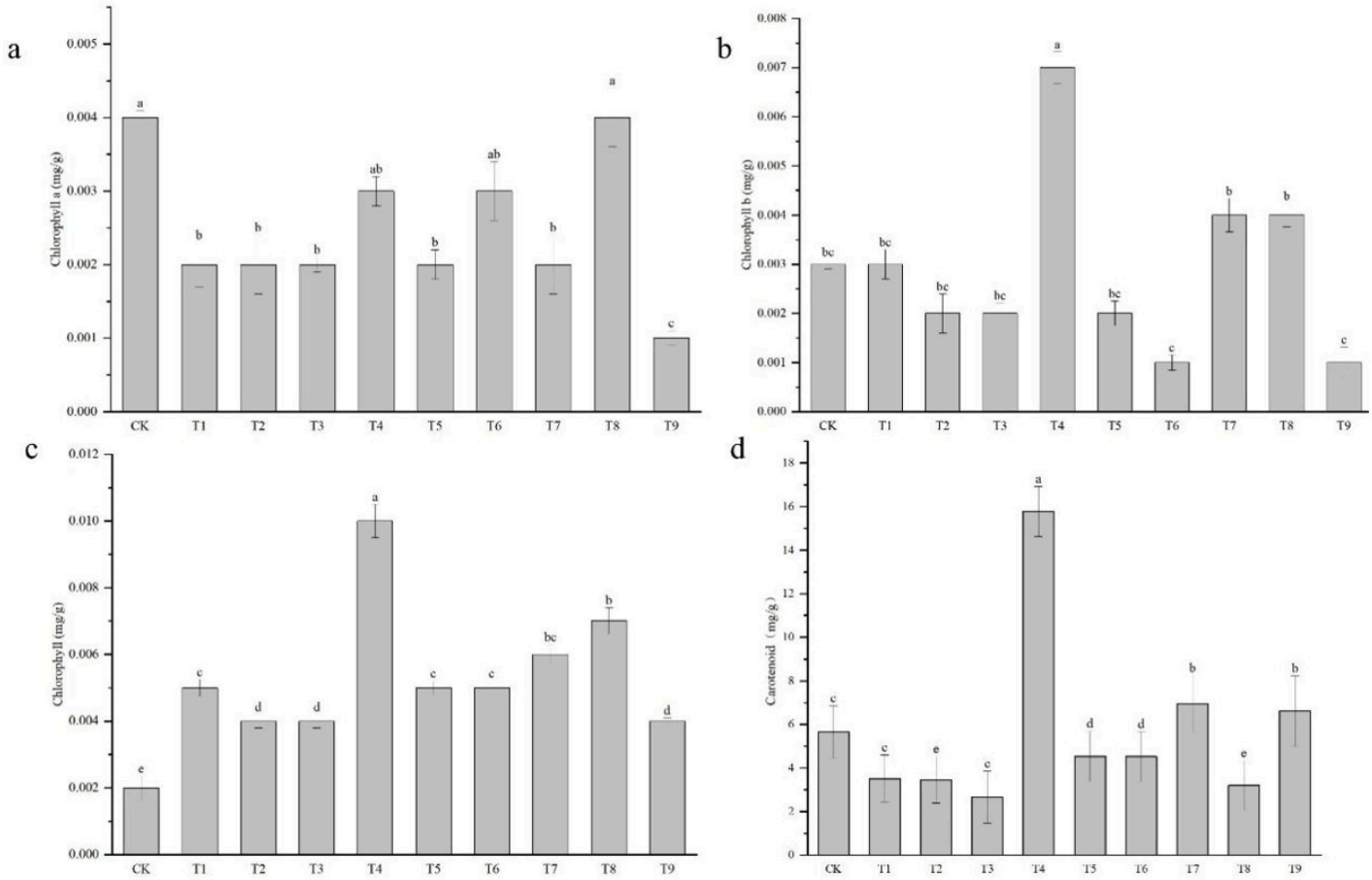

Figure 6

Comparison of chlorophyll and carotenoid contents in control and overexpressed callus

a:Chl a; b: Chl b;c: Total chlorophyll; d: Carotenoids