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CRISPR/Cas9-mediated editing of carotenoid biosynthesis genes alters carotenoid concentrations in kiwifruit

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Abstract

Background CRISPR/Cas9 technology has garnered increasing attention for its simplicity and precision in genome editing, making it an indispensable tool for gene function research and crop genetic improvement. However, the inefficiency and time-consuming nature of genetic transformation continue to pose substantial challenges to its widespread application in woody plants.

Results In this study, we developed a rapid and efficient *Agrobacterium*-mediated transformation system using petioles as explants for kiwifruit. Positive resistant seedlings were obtained within three months by inoculating on MS medium supplemented with 2.0 mg·L^{-1} 6-benzylaminopurine (6-BA), 0.2 mg·L^{-1} naphthaleneacetic acid (NAA), and 10 mg·L^{-1} hygromycin, which was faster than using leaves as explants. Using this system, CRISPR/Cas9-mediated editing of phytoene desaturase (*AcPDS*) and ζ-carotene desaturase (*AcZDS*) achieved an editing efficiency of 20%. Transgenic kiwifruit lines with edited *AcZDS* exhibited a significant reduction in carotenoid content.

Conclusions Overall, we established an efficient *Agrobacterium*-mediated transformation system using petioles as explants, which is applicable for CRISPR/Cas9-mediated gene editing in kiwifruit, thereby facilitating functional gene studies and genetic improvement.

Keywords CRISPR/Cas9, Genetic transformation, Kiwifruit, Phytoene desaturase (PDS), ζ-carotene desaturase (ZDS)

Introduction

Genome editing technology serves as a robust tool for investigating gene function and modifying crops [1]. Among the three genome editing technologies—zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9), the latter has gained more widely adoption due to its superior convenience and efficiency [2, 3]. By incorporating 20 nucleotides complementary to target sequences adjacent to a protospacer-adjacent motif (PAM) into the sgRNA, target sites can be precisely designed. Guided by the sgRNA, the Cas9 nuclease

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cleaves the target site into double-strand breaks (DSBs). These DSBs are typically repaired via the error-prone non-homologous end joining (NHEJ) pathway, leading to insertions, deletions, or substitutions of nucleotides [4]. Alternatively, if homologous donor templates are present during DSB formation, the homology-directed repair (HDR) pathway can also facilitate DSB repair [5]. In recent years, CRISPR/Cas9 technology has been successfully applied to genome editing in various plants, including Arabidopsis [6], tobacco [7], rice [8], soybean [9], maize [10], tomato [11], orange [12], watermelon [13], and grape [14, 15].

Carotenoids are tetraterpene pigments that exhibit yellow, orange, red, and purple colors and are widely distributed in plants [16]. These compounds are essential for photosynthetic organisms alongside chlorophylls and play crucial roles in photosynthesis and photoprotection. In non-photosynthetic organs, carotenoids function as photoprotectors, antioxidants, color attractants, and precursors of plant hormones [17]. Carotenoids are synthesized in plastids of all photosynthetic organisms as well as in fruits, flowers, seeds and reserve roots [18]. The biosynthetic pathway of carotenoids has been extensively characterized in plants [19]. Briefly, the initial stage of biosynthesis involves the condensation of two geranylgeranyl diphosphate molecules by phytoene synthase (PSY) to produce phytoene. Subsequently, phytoene undergoes a series of four desaturation reactions mediated by phytoene desaturase (PDS), zeta-carotene isomerase (Z-ISO), zeta-carotene desaturase (ZDS), and carotenoid isomerase (CRTISO), leading to the formation of lycopene [20]. Double cyclization of lycopene can produce α-carotene (called α-branch) or β-carotene (named β-branch). Further modifications transform α-carotene into zeaxanthin and lutein, and β-carotene into β-cryptoxanthin, zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin. Mutations in the PDS gene can result in albino or dwarf phenotypes in plants, making it a valuable target for validating CRISPR/Cas9 gene-editing technology [21]. Previous studies have utilized CRISPR/Cas9 for functional analysis of the PDS gene in kiwifruit, reporting an editing efficiency of less than 10% [22]. Despite these efforts, there remains a critical need to enhance the editing efficiency of the CRISPR/Cas9 system.

Kiwifruit (*Actinidia chinensis*), a rapidly emerging crop in the 21 st century, has garnered significant attention in recent years due to its high nutritional value [23, 24]. The rapid advancement of bioinformatics technology has led to the release of multiple genomic datasets for kiwifruit, facilitating a deeper exploration of gene functions [25–28]. CRISPR/Cas9 has been utilized to explore gene function by creating gene-edited materials, typically through *Agrobacterium*-mediated transformation. Although this technique has been successfully applied to kiwifruit [29], improving transformation efficiency remains a critical step. In this study, we established an efficient genetic transformation system for kiwifruit using petioles as explants, thereby bypassing the lengthy callus induction and resistant bud induction processes, which significantly reduced the time required. Building on this foundation, we employed CRISPR/Cas9-mediated genome editing to target and modify the carotenoid synthesis genes PDS and ZDS, achieving both high transformation and editing efficiencies. Our findings provide valuable technical insights for leveraging gene editing technology to enhance the quality of kiwifruit.

Materials and methods

Plant materials

The sterile cultured kiwifruit seedlings (*Actinidia chinensis*, cv. 'Hongyang', with a genome size of approximately 610 Mb) established in our laboratory were used as the material for genetic transformation. These seedlings were maintained in our laboratory and subcultured on solid MS (Murashige and Skoog) medium supplemented with 1 $mg{\cdot}L^{-1}$ 6-benzylaminopurine (6-BA) and 0.1 $mg{\cdot}L^{-1}$ naphthalene-acetic acid (NAA), at a temperature of 25 ± 2 ℃ with a 16/8 h light/dark photoperiod [31].

Screening of induction explants, culture medium and hygromycin concentration

Petioles or leaves from 'Hongyang' sterile culture seedlings were used as explants for callus or bud induction on MS medium supplemented with different concentrations of 6-BA (3, 1 $mg{\cdot}L^{-1}$) and NAA (2, 0.2 $mg{\cdot}L^{-1}$), and cultured in the dark at 25 ℃. To optimize the transgenic system, the effect of five concentration levels of hygromycin (Hyg) (0, 5, 10, 15, and 20 $mg{\cdot}L^{-1}$) was investigated. After 30 days of culture, induced callus or bud formation was observed, and the browning rate and germination rate were recorded. The browning rate was calculated as (number of browning explants/total number of explants) × 100%, and the budding rate was calculated as (number of buds/total number of explants) × 100%.

Genetic transformation mediated by *Agrobacterium tumefaciens*

After activation, *A. tumefaciens* EHA105 harboring the recombinant plasmid was inoculated into YEP medium supplemented with 50 $mg{\cdot}L^{-1}$ kanamycin (Kan) and 50 $mg{\cdot}L^{-1}$ rifampicin (Rif). The culture was grown until the OD_{600} reached 0.6, after which it was adjusted to approximately 0.5 using an infection buffer containing 100 $\mu mol{\cdot}L^{-1}$ acetosyringone (AS). The pre-cultured petiole segments were then immersed in the A. tumefaciens suspension for 10 min and co-cultured on induction MS medium containing 100 $\mu mol{\cdot}L^{-1}$ AS for 3 days in the

dark. Subsequently, the petioles were transferred to differentiation medium containing 10 mg·L^{-1} hygromycin (Hyg) and 200 mg·L^{-1} Timentin (Tim) and cultured under shade for 14 d to induce callus formation or bud development. Then, the cultures were incubated at 25 °C with a 16/8 h light/dark, and the medium was changed every 20–30 d.

Gene cloning, target sites selection

To accurately identify suitable target sites for gene editing, we cloned *AcPDS1*, *AcPDS2*, *AcZDS1* and *AcZDS2* from kiwifruit. Total RNA was extracted from the leaves of 'Hongyang' using an RNA Extraction Kit (Biomed, Beijing, China), and cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). Primers were designed using the software Primer 5.0 (Table S1). The PCR amplification was performed with Phusion DNA polymerase (Thermo Scientific, Wuhan, China).

After sequence comparison, the target sites were selected from the conserved regions of *AcPDS1* and *AcPDS2* using the online tools CRISPRdirect (http://crispr.dbcls.jp/) and targetDesign (http://skl.scau.edu.cn/targetdesign/). Primers were then designed according to the method described by Xing et al. [43]. To minimize potential off-target effects, the target site with the highest score was chosen based on several criteria, including an intra-exon GC content ranging between 45% and 70%, mismatch tolerance, PAM compatibility, and variations in promoter preference for sgRNA start base selection.

Construction for CRISPR/Cas9

The binary vectors pHSE401 and pCBC-DT1T2, which were constructed by Chen's Lab [43], were used for vector construction. First, the expression cassette containing two target sequences and sgRNA was amplified with two pairs of primers (DT1-AcPDS-F0/DT2-AcPDS-R0 and DT1-AcPDS-BsF/DT2-AcPDS-BsR) with pCBC-DT1T2 as the template. The amplification was performed using Phusion High-Fidelity DNA Polymerase (NEB) according to the manufacturer's instructions. The PCR conditions were 94 °C for 2 min, followed by 30 cycles of 94 °C for 15 s, 60 °C for 30 s, and 68 °C for 1 min, with a final extension at 68 °C for 5 min. Second, the purified DT1T2-PCR product was inserted into the pHSE401 vector to construct a series of binary vectors pHSE-PDS using the Goldengate ligation kit (NEB, USA). Positive clones were confirmed by Sanger sequencing and subsequently transformed into *A. tumefaciens* EHA105 for further transformation into kiwifruit.

The PTG/Cas9 vectors were kindly provided by Dr. Xie and were used for ZDS editing, following the method described by Xie [30]. Briefly, the target site primers were designed for target genes *AcPDS* and *AcZDS*. PCR amplification was performed using the pGTR vector as the template to obtain the long fragments of *AcPDS*-PTG and *AcZDS*-PTG, which contain tRNA and gRNA scaffolds. Subsequently, these two long fragments were individually inserted into the *BsaI*-digested pRGEB32 vector using T4 DNA ligase (Takara, Japan).

Detection of Transgenic plants and mutations

Genomic DNA was extracted from the leaves of each transgenic line using a micro DNA Extraction Kit (Aidlab, Beijing, China), followed by PCR amplification using the U6-26p-F and U6-29p-R primers (Table S2). A recombinant plasmid served as the positive control, while the wild type was used as the negative control.

Primers targeting regions approximately 200 bp upstream and downstream of the target site were designed for amplifying the target sequences. PCR was performed using polymerase amplification and cloned into PCR-Blunt II-TOPO vector (Life Technologies). Twelve monoclonal colonies were selected from each positive transgenic line for sequencing. The sequences were analyzed and compared to detect specific additions, deletions and substitutions. The mutation rate was calculated as the ratio of mutated clonal colonies versus total sequenced colonies.

Carotenoid extraction and measurement

The extraction and determination of carotenoids were performed following the method outlined by Xia et al. [31]. In brief, 1.0 g of the powdered sample was mixed with 5 mL of an acetone solution containing 0.1% butylated hydroxytoluene and sonicated for 60 min. After centrifugation, the supernatant was filtered through a 0.22 μm filter for subsequent analysis. Total carotenoid content was measured using a UV-1200 spectrophotometer (Mapada, Shanghai, China) at a wavelength of 450 nm. Individual carotenoid components were identified and quantified using an Agilent 1260 HPLC system (Palo Alto, CA, USA) equipped with a variable wavelength detector (VWD) and a YMC C30 column (250 mm × 4.6 mm, 5 μm). The mobile phase was composed of methyl tert-Butyl Ether (30:70, v/v) at a flow rate of 0.5 mL/min and a column temperature of 25 °C. The injection volume was 20 μL, and the detection wavelength was set to 450 nm. Qualitative and quantitative determinations of the carotenoid components were based on the retention time and peak area of the standards.

Data analysis

Data were expressed as mean ± SD with three replicates. Statistical analysis was performed using IBM SPSS Statistics software (SPSS Inc., Chicago, USA). Significant differences among groups were determined by one-way ANOVA followed by Student's *t*-test (**$P < 0.01$).

Table 1 Effects of different explants and induction medium on the bud regeneration

Name of Medium	6-BA (mg·L^{-1})	NAA (mg·L^{-1})	Explant	Browning rate (%)	Budding rate	Bud No.	Duration (week)
M1	2	0.2	leaf	1.5	84%	4–6	10–12
			petiole	1.3	85%	4–6	1–2
M2	3	1.0	leaf	1.8	88%	7–8	10–12
			petiole	1.1	87%	4–6	4–5

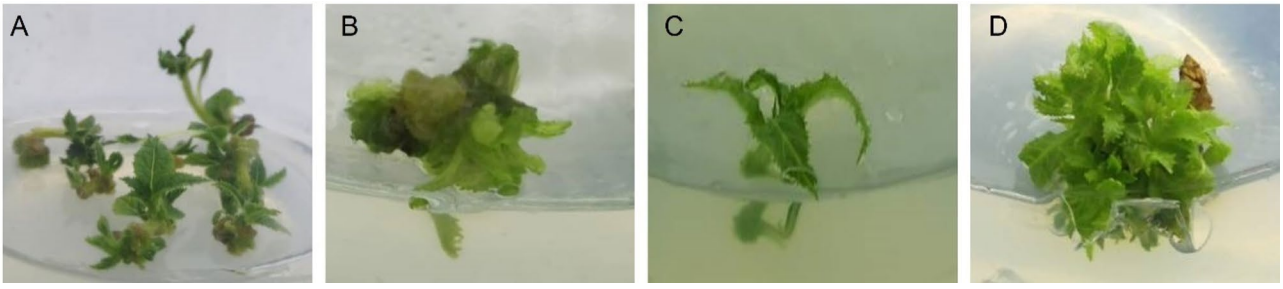

Fig. 1 The phenotype of regenerating shoots induced on different medium. **A**, **B** The petiole induced on M1 and M2 medium; **C**, **D** The leaf induced in M1 and M2 medium

Table 2 Effects of hygromycin concentration on shoot regeneration

Hyg concentration (mg·L^{-1})	Inoculated number	Browning rate (%)	budding rate (%)
0	55	4	88.5
5	55	58	53
10	55	76	24
15	55	86.5	9.5
20	55	100	0

Results

Explant and medium selection for Kiwifruit genetic transformation

To optimize the kiwifruit bud regeneration system, two types of explants, leaf and petiole, were utilized to induce calli or buds on two modified MS medium containing different hormone concentration combinations as shown in Table 1, named M1 and M2, respectively (Table 1). The browning rate of both explants inoculated on the respective media was found to be less than 1.8%, and the budding rate exceeded 84%, indicating compliance with regeneration requirements. However, there was a disparity in duration between the two methods. When utilizing leaves as explants, a substantial amount of callus formation occurred easily, followed by a prolonged induction period of 10–12 weeks for bud regeneration. Each leaf disc yielded 4–6 regenerated buds. Differently, when employing petiole as explant, only minimal callus appeared at both ends of the petiole and bud differentiation took place within 1–2 weeks after inoculation. Each petiole could produce 4–6 regenerated buds (Fig. 1). Generally, leaves can induce more regenerating shoots but require a longer period; whereas petioles produce indefinite regenerating shoots in a shorter duration on M1 medium, thereby significantly shortening the cycle length. Therefore, the petioles were selected as explants for genetic transformation using M1 medium.

Screening antibiotic concentration for genetic transformation

Hygromycin concentration was further optimized for genetic transformation. Regeneration buds exhibited high sensitivity to hygromycin, with growth inhibition and browning occurring when the concentration exceeded 10 mg·L^{-1}, resulting in a browning rate exceeding 75% (Table 2, Supplemental Figure S1). Therefore, a concentration of 10 mg·L^{-1} hygromycin was selected for screening kiwifruit petioles.

After optimization, we successfully established an efficient genetic transformation system for kiwifruit petiole. The procedure was as follow: after 3 days of pre-culture, petioles was immersed in *A. tumefaciens* solution containing recombinant plasmid at OD_{600} = 0.6 for 10 min, followed by inoculation in MS medium supplemented with 2.0 mg·L^{-1} 6-BA, 0.2 mg·L^{-1} NAA and 100 μ mol·L^{-1} AS for co-culture in darkness for 3 d (Fig. 2A, B). Subsequently, the infected petioles were transferred to the same medium containing 10 mg·L^{-1} hygromycin and cultured in the dark for two weeks to induce bud formation (Fig. 2C, D). Regenerated buds were observed after continuous culture for 20 d (Fig. 2E, F).

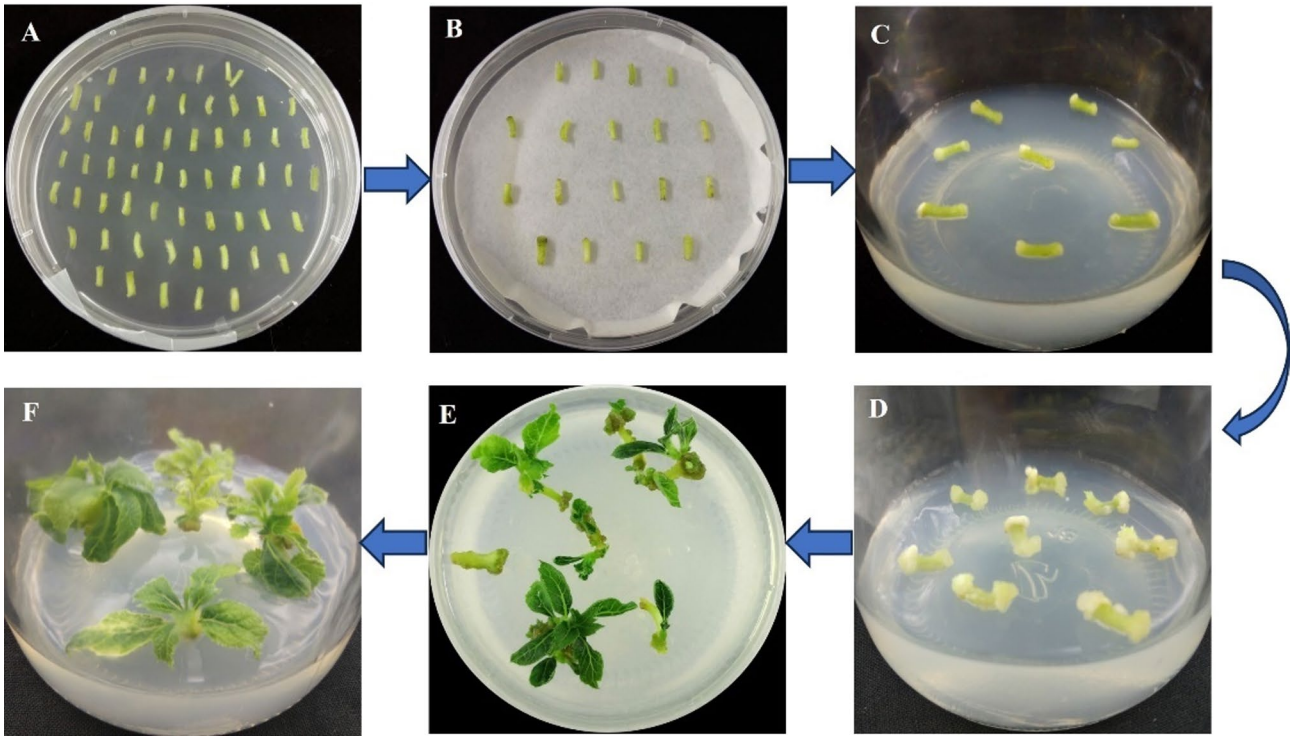

Fig. 2 Procedures for genetic transformation of kiwifruit petiole. **A** Petiole preculture; **B** Coculture with *Agrobacterium*; **C** Antibiotic screening culture; **D** Cultured in the dark for 14 d; **E** Bud differentiation induced in the light; **F** Subculture of resistant buds

Selection of SgRNA targets and construction of CRISPR/Cas9 Recombinant plasmids

To precisely design target sites for constructing CRISPR/Cas9-mediated gene editing vector, we cloned two PDS genes, *AcPDS1* and *AcPDS2*, and sequenced their cDNA and DNA. Sequences analysis revealed that *AcPDS1* and *AcPDS2* have a high degree of similarity with 93.34% identity in nucleotide sequences. Both genes possess 14 exons and 13 introns (Figure S2). Subsequently, six specific sgRNA target sites were selected within the conserved regions of both *AcPDS*s adjacent to PAM with GC content more than 40% using CRISPRdirect (http://crispr.dbcls.jp/) and targetDesign (http://skl.scau.edu.cn/targetdesign/). The chosen target sites are located on the first, fourth, fifth, sixth, seventh, and twelfth exons, respectively (Fig. 3A). Subsequently, three binary vectors, named pHSE-PDS-1, pHSE-PDS-2, and pHSE-PDS-3 containing two sgRNA expression cassettes were constructed using the Goldengate ligation Kit (Fig. 3B).

Phenotypes of CRISPR/Cas9-*AcPDS* Transgenic kiwifruit

The pHSE401-*AcPDS* recombinant plasmid, containing two sgRNA cassettes, Cas9, and the hygromycin marker, was introduced into explants through *Agrobacterium*-mediated transformation. Following a 2–3 week culture period, regenerated buds emerged with smaller leaves exhibiting an albino or partially albino phenotype. Notably, the growth of the albino plants exhibited significantly slower shoot regeneration compared to the wild type and was accompanied by dwarfing (Fig. 4A), likely attributed to delayed chlorophyll synthesis resulting in developmental delays. A total of 16 resistant buds were obtained from 150 transformed petioles, among which 13 were confirmed positive transgenic buds using PCR analysis (Fig. 4B), achieving a transgenic efficiency of 81.25%.

CRISPR/Cas9-mediated *AcPDS* editing

To assess editing efficiency, positive transgenic lines were analyzed for mutations by sequencing the *AcPDS*. A total of four editing lines (L5, L6, L10, and L13) (Fig. 5A) were identified with either substitution or deletion mutations at target sites, resulting in a mutation rate of 23.07%. L10 exhibited heterozygous editing with a base change from A to G, leading to an amino acid alteration from threonine to alanine and displaying a mosaic albino phenotype. Line L13 showed biallelic editing with a C-to-T base substitution and an A deletion causing frameshifting and the formation of an early stop codon, resulting in a complete albino phenotype. Single-base substitutions occurred in lines L5 and L6, resulting in pale green phenotypes (Fig. 5B).

CRISPR/Cas9-mediated *AcZDS* editing

To further validate the applicability of the system, we constructed an sgRNA/Cas9-based editing construct for the carotene dehydrogenase gene (*AcZDS*) (Supplemental

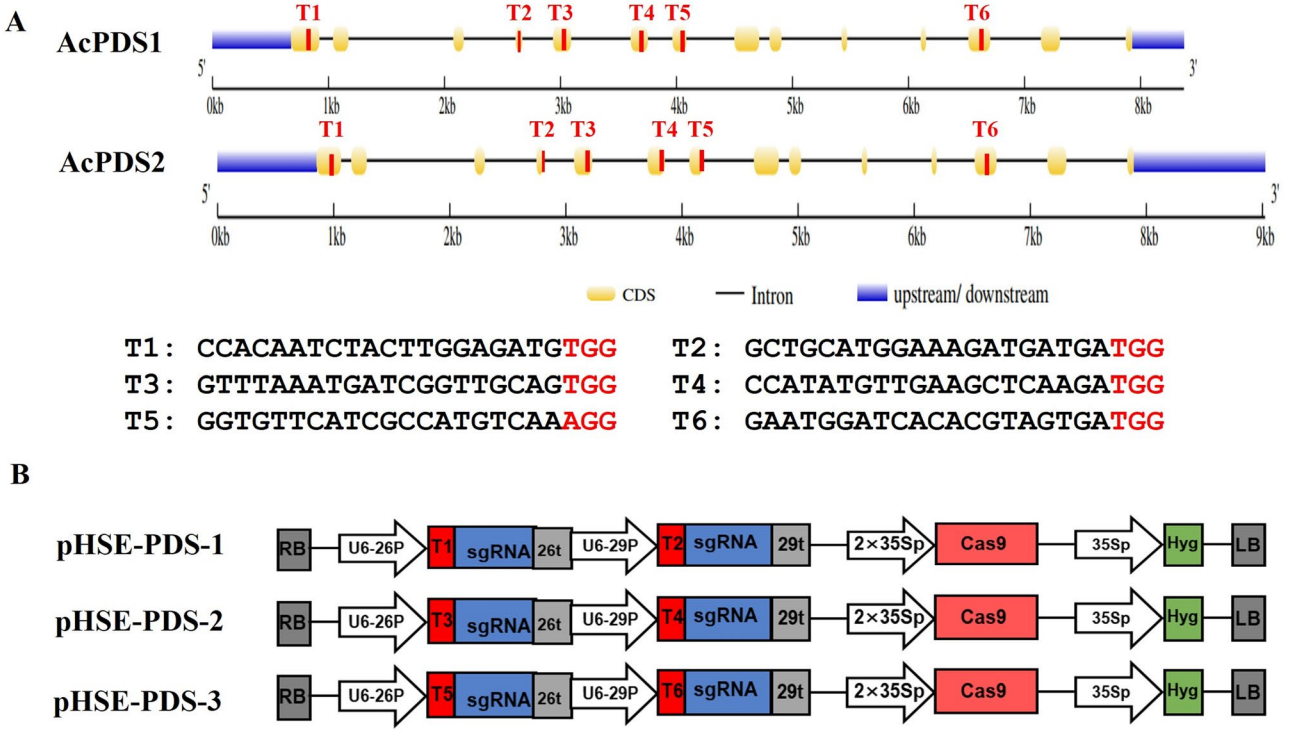

Fig. 3 Construction of editing vectors. **A** Gene structures of *AcPDS1* and *AcPDS2*. T1-T6 were six selected target sites. **B** CRISPR/Cas9 binary vector structures. Construct of editing vectors was confirmed by PCR production of sgRNA expression cassette

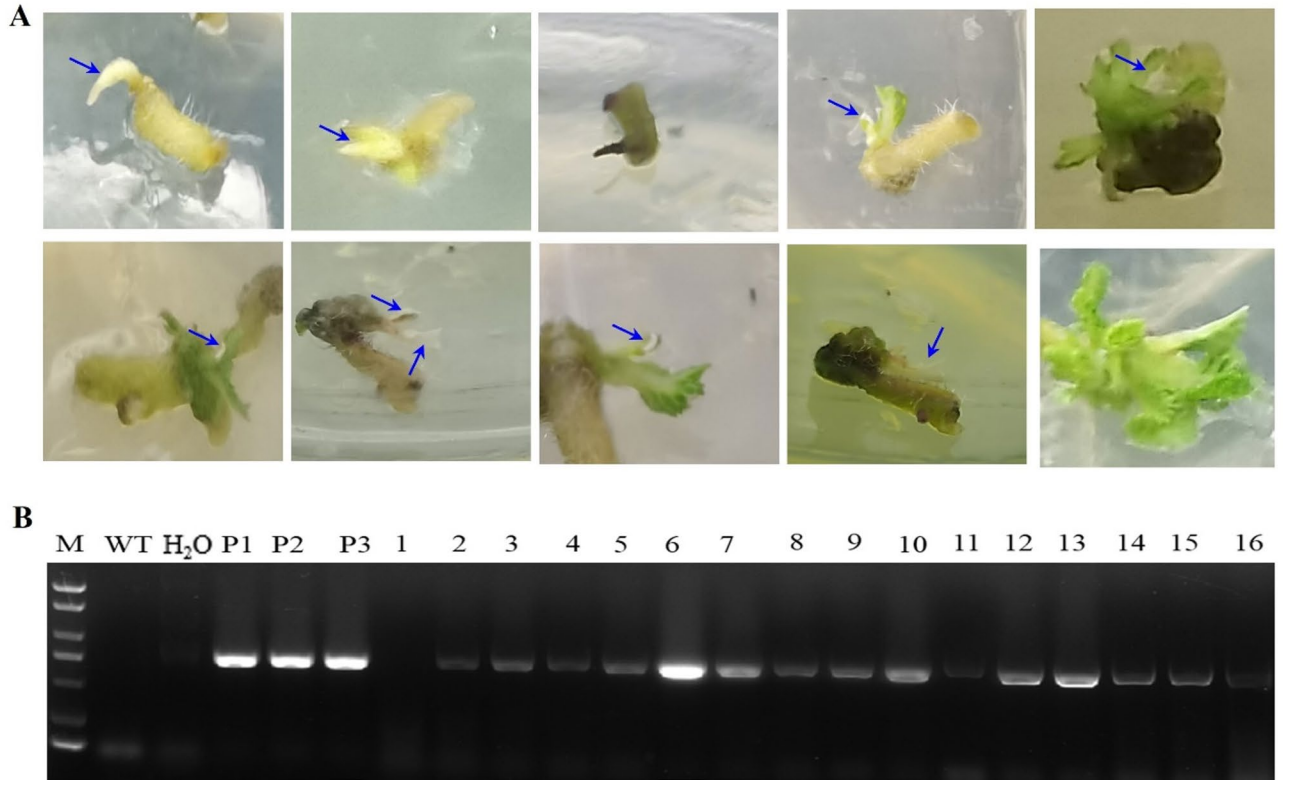

Fig. 4 Phenotype of the obtained regenerated kiwifruit buds transformed with pHSE401-*AcPDS*. **A** Growth status of regenerated buds; **B** Amplification of the pHSE401 vector fragment to confirm positive transgenic kiwifruit lines. M: DL2000 marker; WT: wild type; P1: pHSE-PDS1; P2: pHSE-PDS2; P3: pHSE-PDS3; 1–16: independent transgenic kiwifruit lines

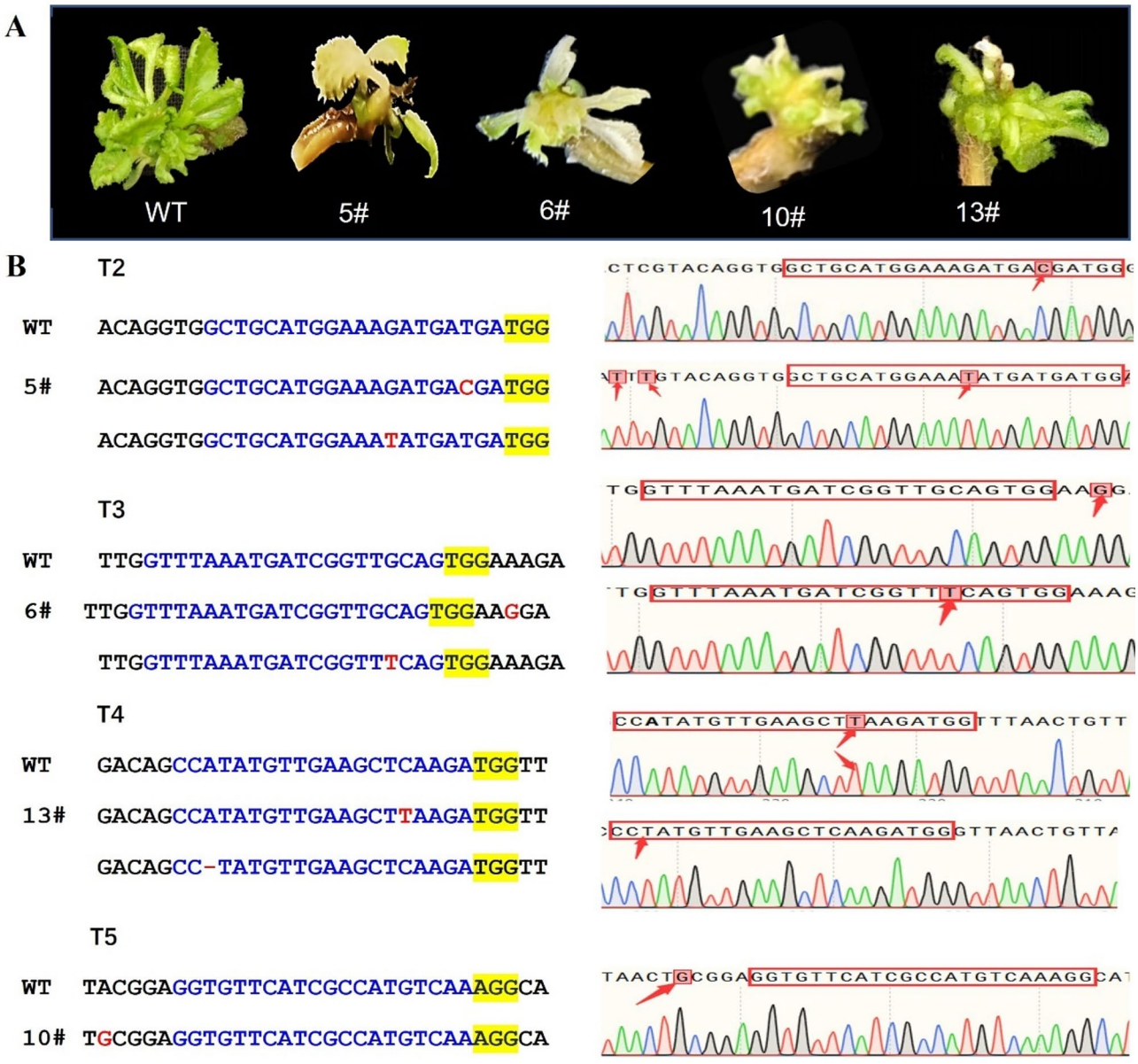

Fig. 5 Sequence analysis of *AcPDS* editing lines. **A** The phenotype of editing lines. **B** Sequencing chromatograms of the mutated target sites. Dashes indicate deletion, target sequences are shown in light blue and the PAM sequence (NGG) is highlighted in yellow

Figure S3), designated as pHSE401-*AcZDS*, and generated transformed kiwifruit lines. From 250 infected petioles, we obtained eight resistant buds, and four of these were confirmed as positive lines by PCR, achieving a transformation efficiency of 50%. DNA was extracted from the positive plants for sequencing of the *AcZDS* gene, and sequence comparison revealed that one positive line had been successfully edited. Specifically, the base A at the T2 site was converted to base G, leading to a threonine-to-alanine substitution, which resulted in a mosaic albinism phenotype (Fig. 6).

AcZDS editing using PTG/Cas9 system

In addition, we constructed a PTG/Cas9-based *AcZDS* editing vector, pRGEB32-*AcZDS*. Through genetic transformation, we obtained 19 pRGEB32-*AcZDS*-resistant kiwifruit buds from 660 infected explants, resulting in a transgenic efficiency of 2.88%. Among these, 5 positive lines were identified, with a transgene efficiency of 26.31%. Sequencing analysis revealed three mutant lines (10#, 13#, and 15#), achieving a mutation efficiency of 60%. Line 10 exhibited homozygous editing with a single base deletion (T deletion), causing a frameshift that leads to abnormal gene function, potentially explaining the albinism observed in this line. Line 13 underwent

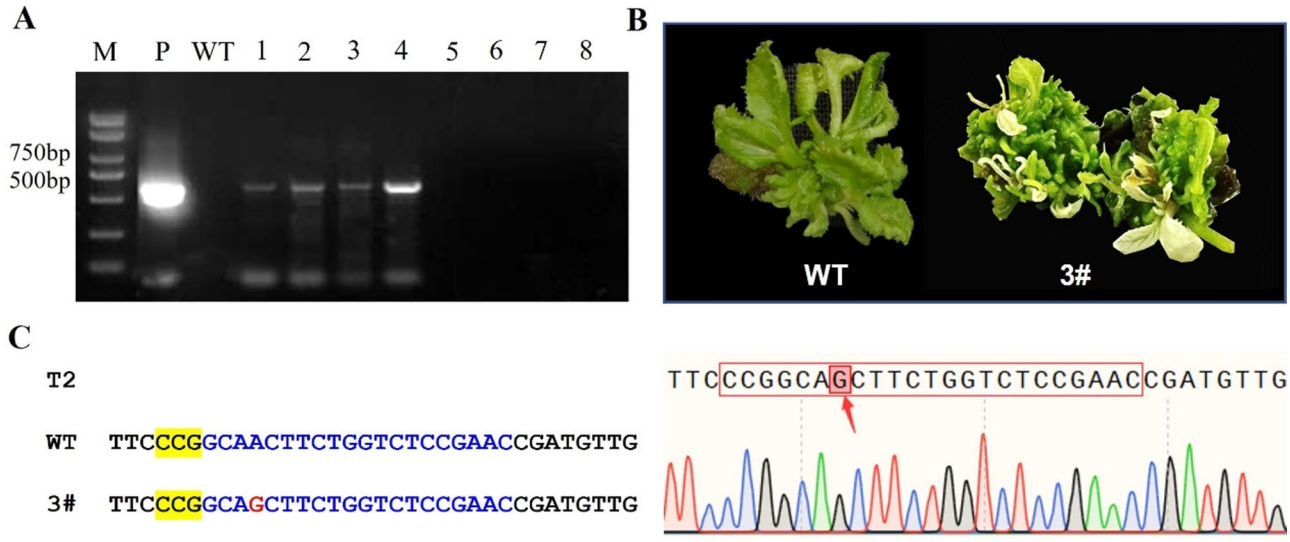

Fig. 6 Positive detection and phenotype of edited kiwifruit lines transformed with pHSE401-*AcZDS*. **A** PCR detection of positive transgenic kiwifruit lines. **B** The phenotype of the editing lines. **C** Sequencing chromatograms showing mutations at the target site regions

biallelic editing, with C mutated to G and G mutated to C, resulting in arginine being replaced by glycine and proline, respectively. Line 15 displayed heterozygous editing, where a single base substitution did not alter the amino acid sequence, leading to a chimeric mutation phenotype (Fig. 7). These results indicate that the petiole genetic transformation system we established is suitable for application in various gene editing systems.

Carotenoid content in leaves of *AcZDS*-edited Transgenic plants

To investigate the influences of *AcZDS* editing on carotenoid accumulation, we quantified the content of total carotenoids, zeaxanthin, lutein, and β-carotene using an HPLC system. Our results showed a significant reduction in the content of total carotenoids (38%), zeaxanthin (56%), lutein (63%), and β-carotene (57%) compared to WT plants (Fig. 8), suggesting that *AcZDS* editing has impaired its functional role in carotenoid biosynthesis. However, due to the high lethality rate caused by severe albino phenotypes in *AcPDS* editing lines, carotenoid content could not be determined.

Discussion

CRISPR/Cas9 technology is increasingly used in crop bio-breeding; however, its application in kiwifruit has been constrained due to the low transformation efficiency. In this study, we developed a rapid and efficient *Agrobacterium*-mediated transformation system for kiwifruit using petioles as explants. Using this system, we successfully achieved CRISPR/Cas9-mediated editing of the *AcPDS* and *AcZDS* in kiwifruit, with an editing efficiency reaching 20%. Transgenic kiwifruit lines carrying CRISPR/Cas9-*AcZDS* exhibited a significant reduction in carotenoid content. This work provides a critical foundation for the application of genome editing technology in kiwifruit and contributes to advancing genetic improvement of kiwifruit.

An efficient petiole-based *Agrobacterium*-mediated transformation system in kiwifruit

Agrobacterium-mediated genetic transformation remains the most widely used method for plant genetic modification, despite the lack of effective transformation systems for many woody plants. Transformation efficiency is influenced by several factors, including explant type, regeneration conditions, and transformation conditions. In kiwifruit transformation, the leaf disc method has been predominantly employed [32], yielding numerous transgenic materials, such as overexpression lines of *AcBCH*, *AcMADS32* [33]. However, low transformation frequency and lengthy processes remain critical challenges that need urgent solutions. Recently, callus-based transformation methods have been developed for gene function analysis such as with *AcGST* [34], while petiole-based transformation systems are still underexplored. Petioles, as an alternative source of micropropagation explants, expose more vascular tissues at their cut ends, potentially enhancing *Agrobacterium* infection. Previous studies have demonstrated successful callus induction from petioles of the Hayward variety, but failed to achieve transgenic plants [35]. In this study, we utilized petioles from 'Hongyang' seedlings as explants and established an efficient petiole-based genetic transformation system by optimizing the induction medium and antibiotic concentration. This system enables direct bud regeneration after a brief period of minimal callus induction, significantly shortening the transformation period.

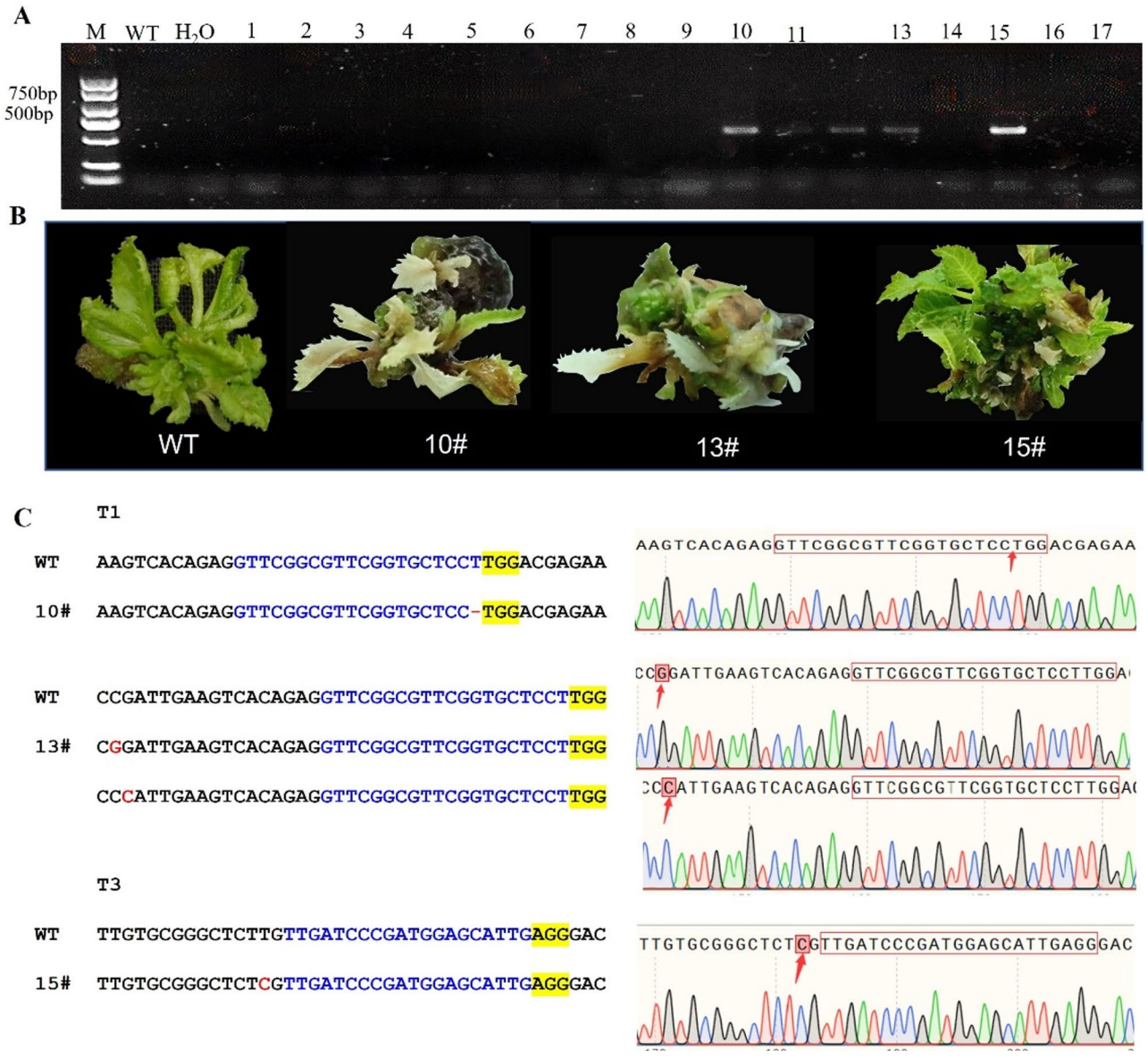

Fig. 7 Sequence analysis of mutants transformed with PTG-*AcZDS* vector in 'Hongyang' kiwifruit. **A** PCR detection of positive transgenic kiwifruit lines. **B** The phenotype of editing lines. **C** Sequencing chromatograms showing mutations at target site regions. Dashes indicate for deletion sites, target sequences are displayed in light blue and the PAM sequence (NGG) is highlighted in yellow

CRISPR/Cas9 system mediated by binary vector and PTG vector

CRISPR/Cas9 is a robust tool for validating gene function through the knockout or knockdown of gene expression. This technology has been successfully applied to modify various horticultural crops, including tomato [36], strawberry [37], citrus [38, 39], banana [40] and apple [41, 42]. Initially, vectors were designed to target single sites. To improve success rates, binary vectors or multiple editing vectors were subsequently constructed [43]. Moreover, sgRNA expression cassettes and Cas9 were placed in separate vectors to enhance transformation efficiency [44]. Recently, vectors incorporating repeated tRNA sequences have been introduced, significantly reducing off-target effects, thereby enhancing specificity [24]. However, the unique characteristics of different plant species necessitate experimental validation of vectors applicability. In this study, two CRISPR/Cas9 vectors were used to transform kiwifruit to investigate the applicability of different vector designs to kiwifruit. Among them, the dual-targeting vector pHSE401 used AtU6 to drive sgRNA and CaMV 35 S to drive Cas9, while the PTG multi-targeting vector pRGEB32 used OsU3 to drive sgRNA and Ubi to drive Cas9.

The phytoene desaturase (PDS), a key enzyme in the carotenoid biosynthetic pathway, is commonly used as a

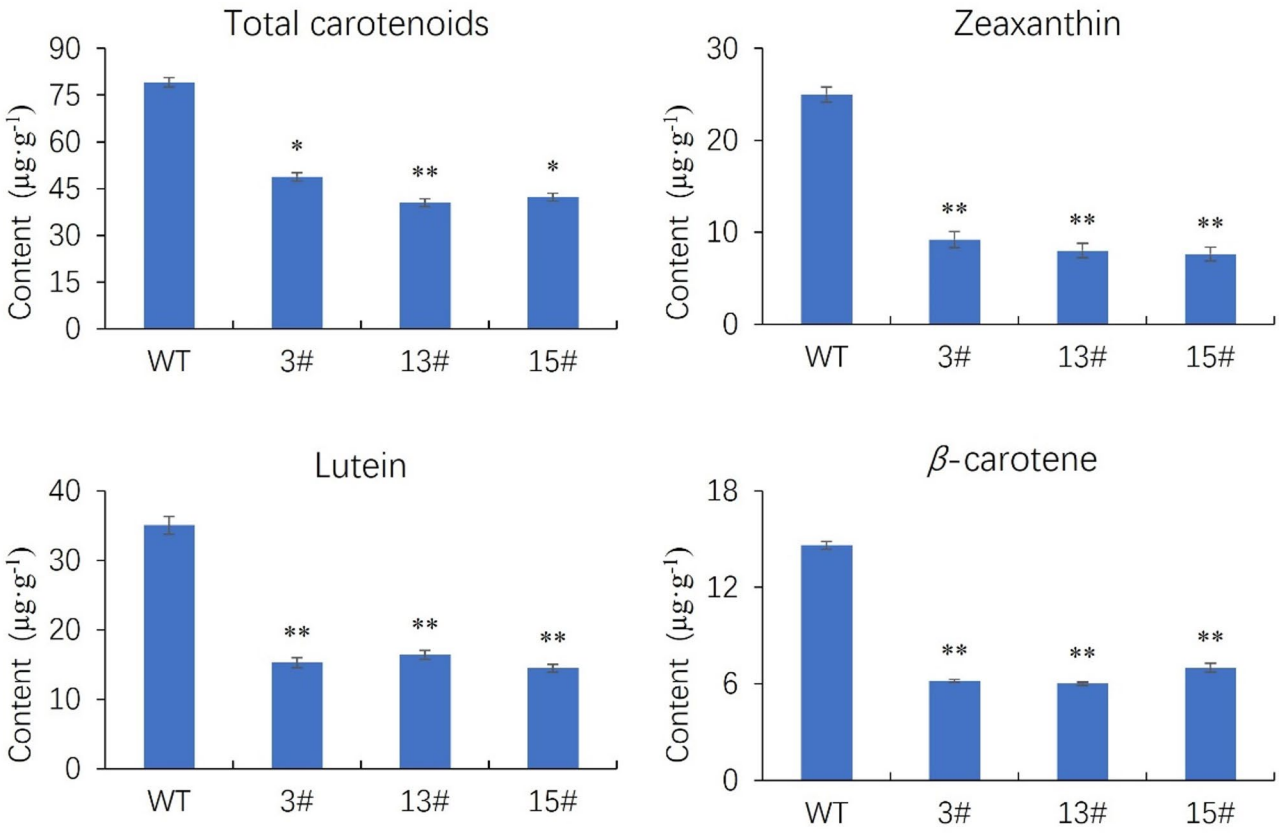

Fig. 8 Carotenoid content in leaves of *AcZDS*-edited transgenic plants. Data are expressed as mean ± SD with three biological replicates. Significant differences were determined by Student's *t*-test (**$P < 0.01$, $n = 3$)

target gene for establishing gene editing systems due to its easily identifiable albino phenotype [45]. Given the highly heterozygous genetic background of kiwifruit, we isolated two PDS genes (*AcPDS1* and *AcPDS2*) from 'Hongyang' kiwifruit and selected conserved regions as target sites to construct a binary Cas9 vector, pHSE401-*AcPDS*. The pHSE401 binary vector contains sgRNA expression guided by the *Arabidopsis* U6 promoter and Cas9 protein driven by the CaMV 35 S promoter [43]. Through stable transformation in kiwifruit, we obtained transgenic kiwifruit plants with an average editing efficiency of 23.07%, as determined by analysis of five target sites within the AcPDS gene. This level of efficiency is comparable to that observed in *Arabidopsis*, rice, and watermelon [13, 43, 46] and is notably higher than previously reported systems in kiwifruit, which ranged around 8% [22, 29]. The enhanced editing efficiency may be attributed to the use of dual sgRNAs, which can improve targeting specificity [47, 48]. Moreover, the percentages of biallelic editing were much lower than those observed in rice [49, 50]. Due to the prolonged juvenile phase of perennial woody plants, which impedes the timely evaluation of target phenotypes and slows down the breeding process, the induction of biallelic and homozygous mutations becomes crucial in plant genetic breeding [39].

Recently, a polycistronic tRNA-gRNA system has been developed for Cas9-based multiplex RNA-guided editing in plants. This system relies on endogenous tRNA processing mechanisms [30], where tRNA precursors can be cleaved at specific sites to remove excess 5′ and 3′ sequences. Multiple gRNAs can be assembled with flanking tRNA sequences into a single polycistronic tRNA-gRNA gene (PTG), requiring only one U3 or U6 promoter, thereby significantly simplifying the construction of RNA-guided Cas9 systems. The effectiveness of PTG constructs in multiplex genome editing has been demonstrated in rice [30] and maize [51], showing that the PTG-based system is more efficient in mutagenesis compared to single sgRNAs.

In this study, we evaluated the effectiveness of two gene editing systems, PTG/Cas9 and CRISPR/Cas9. In terms of transformation efficiency, the PTG/Cas9 system used in this study achieved a transgene efficiency of 26–50%, which was lower than that of the sgRNA/Cas9 system (44–81%). This difference may be attributed to the relatively large size of the PTG multi-targeting vector, which could hinder vector delivery in kiwifruit. Regarding

editing efficiency, the CRISPR/Cas9 system showed an editing efficiency of 23–44%, while the PTG/Cas9 system exhibited a comparable efficiency of 25–60%. Similar results were observed in a study on grape, where the editing efficiency using the PTG/Cas9 system was slightly lower than that obtained with the CRISPR/Cas9 system [15]. This discrepancy may be related to variations in promoter preferences across different plant species [52, 53]. The "OsU3-tRNA-sgRNA/AtUb10-Ros1 system" demonstrated higher efficiency than the AtU6 driven system in editing the genome of tobacco plants [52]. In this study, the sgRNA was driven by the OsU3 promoter in the PTG/Cas9 system, whereas in the CRISPR/Cas9 system, it was driven by the AtU6 promoter. In grape, the U6 promoter has been shown to exhibit stronger activity than the U3 promoter [15]. Cas9 expression in the PTG/Cas9 system was controlled by the OsUbi promoter, while in the CRISPR/Cas9 system, it was driven by the CaMV 35 S promoter. The Ubi promoter is typically expressed at lower levels than the CaMV 35 S promoter in dicotyledonous plants. Therefore, different promoters may result in different editing efficiency.

Editing of *AcPDS*, *AcZDS* affects carotenoid biosynthesis in Transgenic kiwifruits

Through *Agrobacterium*-mediated transformation using petiole explants, we successfully generated transgenic kiwifruit lines harboring mutations in the *AcPDS* gene. These transgenic lines exhibited albino and dwarf phenotypes, consistent with observations in other plants such as *Arabidopsis* [54], poplar [55], apple [41, 42], and banana [56]. Mutations in the PDS gene leading to these phenotypes result in lethality and prevent the mutation from being transmitted to subsequent generations [13]. This lethality is likely due to disruptions in the carotenoid and gibberellic acid biosynthesis pathways, which are critical for plant growth and development [57]. Furthermore, we successfully obtained CRISPR/Cas9-mediated *AcZDS*-edited transgenic kiwifruit lines. These transgenic lines showed a significant reduction in carotenoid content (Fig. 8), indicating impaired carotenoid biosynthesis. Unfortunately, due to the knockout of the ZDS gene, the plants showed an albino phenotype with slow growth and high mortality, often dying before they grew up, thus preventing further experimental studies such as subsequent photosynthesis or fruit characterization.

Conclusions

In this study, we developed an efficient genetic transformation system using the petioles of 'Hong yang' as explants. This system enables the rapid regeneration of shoots after inducing only a small amount of callus within a short period, thereby significantly shortening the transformation cycle. Moreover, *AcPDS* and *AcZDS*-edited transgenic plants were successfully generated using both CRISPR/Cas9 and PTG/Cas9 systems via this transformation approach, achieving an editing efficiency of over 20%. These results indicate the potential applicability of our system in kiwifruit genetic improvement.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-07112-6.

Supplementary Material 1.

Authors' contributions

X.H. and L.D. conceived and designed the experiments; L.X., D.Y., and L.Y. performed the experiments with assistance of Z.H., L.X.; Z.X., and Li Y. analyzed the data and constructed the Figures; L.X. and D.Y., wrote the draft; H.X. and D.L. reviewed and edited the manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Jiao R, Gao C. The CRISPR/Cas9 genome editing revolution. J Genet Genomics. 2016;43(5):227–8. https://doi.org/10.1016/j.jgg.2016.05.004.
2. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339(6121):819–23. https://doi.org/10.1126/science.1231143.
3. Gaj T, Gersbach CA, Barbas CF. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397–405. https://doi.org/10.1016/j.tibtech.2013.04.004.
4. Liu W, Zhu X, Lei M, Xia Q, Botella JR, Zhu JK, Mao Y. A detailed procedure for CRISPR/Cas9 mediated gene editing in *Arabidopsis thaliana*. Sci Bull. 2015;60(15):1332–47. https://doi.org/10.1007/s11434-015-0848-2.
5. Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V. Plant genome editing made easy: targeted mutagenesis in model and crop plants using the crispr/cas system. Plant Methods. 2013;9(1):39. https://doi.org/10.1186/1746-4811-9-39.
6. Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in arabidopsis, tobacco, sorghum and rice. Nucleic Acids Res. 2013;41(20):e188. https://doi.org/10.1093/nar/gkt780.
7. Li JF, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J. Multiplex and homologous recombination-mediated genome editing

in Arabidopsis and *Nicotiana benthamiana* using guide RNA and Cas9. Nat Biotechnol. 2013;31(8):688–91. https://doi.org/10.1038/nbt.2654.
8. Zhang H, Zhang J, Wei P, Zhang B, Gou F, Feng Z, Mao Y, Yang L, Zhang H, Xu N. The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. Plant Biotechnol J. 2014;12(6):797–807. https://doi.org/10.1111/pbi.12200.
9. Jacobs TB, LaFayette PR, Schmitz RJ, Parrott WA. Targeted genome modifications in soybean with CRISPR/Cas9. BMC Biotechnol. 2015;15:16. https://doi.org/10.1186/s12896-015-0131-2.
10. Liang Z, Zhang K, Chen K, Gao C. Targeted mutagenesis in *Zea mays* using TALENs and the crispr/cas system. J Genet Genomics. 2014;41(2):63–8. https://doi.org/10.1016/j.jgg.2013.12.001.
11. Brooks C, Nekrasov V, Lippman ZB, Van Eck J. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol. 2014;166(3):1292–7. https://doi.org/10.1104/pp.114.247577.
12. Peng A, Chen S, Lei T, Xu L, He Y, Wu L, Yao L, Zou X. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene *CsLOB1* promoter in citrus. Plant Biotechnol J. 2017;15(12):1509–19. https://doi.org/10.1111/pbi.12733.
13. Tian S, Jiang L, Gao Q, Zhang J, Zong M, Zhang H, Ren Y, Guo S, Gong G, Liu F, Xu Y. Efficient CRISPR/Cas9-based gene knockout in watermelon. Plant Cell Rep. 2017;36(3):399–406. https://doi.org/10.1007/s00299-016-2089-5.
14. Wang X, Tu M, Wang D, Liu J, Li Y, Li Z, Wang Y, Wang X. CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. Plant Biotechnol J. 2018;16(4):844–55. https://doi.org/10.1111/pbi.12832.
15. Ren C, Liu Y, Guo Y, Duan W, Fan P, Li S, Liang Z. Optimizing the CRISPR/Cas9 system for genome editing in grape by using grape promoters. Hortic Res. 2021;8(1):52. https://doi.org/10.1038/s41438-021-00489-z.
16. Maoka T. Carotenoids as natural functional pigments. J Nat Med. 2020;74(1):1–16. https://doi.org/10.1007/s11418-019-01364-x.
17. Giuliano G. Plant carotenoids: genomics meets multi-gene engineering. Curr Opin Plant Biol. 2014;19:111–7. https://doi.org/10.1016/j.pbi.2014.05.006.
18. Park H, Kreunen SS, Cuttriss AJ, DellaPenna D, Pogson BJ. Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation, and photomorphogenesis. Plant Cell. 2002;14(2):321–32. https://doi.org/10.1105/tpc.010302.
19. Rosas-Saavedra C, Stange C. Biosynthesis of carotenoids in plants: enzymes and color. Subcell Biochem. 2016;79:35–69. https://doi.org/10.1007/978-3-319-39126-7_2.
20. Bai C, Capell T, Berman J, Medina V, Sandmann G, Christou P, Zhu C. Bottlenecks in carotenoid biosynthesis and accumulation in rice endosperm are influenced by the precursor-product balance. Plant Biotechnol J. 2016;14(1):195–205. https://doi.org/10.1111/pbi.12373.
21. Zhou J, Li D, Wang G, Wang F, Kunjal M, Joldersma D, Liu Z. Application and future perspective of CRISPR/Cas9 genome editing in fruit crops. J Integr Plant Biol. 2020;62(3):269–86. https://doi.org/10.1111/jipb.12793.
22. Wang Z, Wang S, Li D, Zhang Q, Li L, Zhong C, Liu Y, Huang H. Optimized paired-sgRNA/Cas9 cloning and expression cassette triggers high-efficiency multiplex genome editing in kiwifruit. Plant Biotechnol J. 2018;16(8):1424–33. https://doi.org/10.1111/pbi.12884.
23. Park YS, Im MH, Ham KS, Kang SG, Park YK, Namiesnik J, Leontowicz H, Leontowicz M, Katrich E, Gorinstein S. Nutritional and pharmaceutical properties of bioactive compounds in organic and conventional growing kiwifruit. Plant Foods Hum Nutr. 2013;68(1):57–64. https://doi.org/10.1007/s11130-013-0339-z.
24. Wang S, Qiu Y, Zhu F. Kiwifruit (*Actinidia* spp.): a review of chemical diversity and biological activities. Food Chem. 2021;350:128469. https://doi.org/10.1016/j.foodchem.2020.128469.
25. Han X, Zhang Y, Zhang Q, Ma N, Liu X, Tao W, Lou Z, Zhong C, Deng XW. Two haplotype-resolved, gap-free genome assemblies for *Actinidia latifolia* and *Actinidia chinensis* shed light on the regulatory mechanisms of vitamin C and sucrose metabolism in kiwifruit. Mol Plant. 2023;16(2):452–70. https://doi.org/10.1016/j.molp.2022.12.022.
26. Tang W, Sun X, Yue J, Tang X, Jiao C, Yang Y, Niu X, Miao M, Zhang D, Huang S, et al. Chromosome-scale genome assembly of Kiwifruit *Actinidia Eriantha* with single-molecule sequencing and chromatin interaction mapping. Giga Sci. 2019;8(4). https://doi.org/10.1093/gigascience/giz027.
27. Xia H, Deng H, Li M, Xie Y, Lin L, Zhang H, Luo X, Lv X, Wang J, Liang D. Chromosome-scale genome assembly of a natural diploid kiwifruit (*Actinidia chinensis* var. *deliciosa*). Sci Data. 2023;10(1):92. https://doi.org/10.1038/s41597-023-02006-4.
28. Wang Y, Dong M, Wu Y, Zhang F, Ren W, Lin Y, Chen Q, Zhang S, Yue J, Liu Y. Telomere-to-telomere and haplotype-resolved genome of the Kiwifruit *Actinidia Eriantha*. Mol Hortic. 2023;3(1):4. https://doi.org/10.1186/s43897-023-00052-5.
29. Herath D, Voogd C, Mayo-Smith M, Yang B, Allan AC, Putterill J, Varkonyi-Gasic E. CRISPR-Cas9-mediated mutagenesis of kiwifruit BFT genes results in an evergrowing but not early flowering phenotype. Plant Biotechnol J. 2022;20(11):2064–76. https://doi.org/10.1111/pbi.13888.
30. Xie K, Minkenberg B, Yang Y. Boosting CRISPR. /Cas9 multiplex editing capability with the endogenous tRNA-processing system. Proc Natl Acad Sci U S A. 2015;112(11):3570–5. https://doi.org/10.1073/pnas.1420294112.
31. Xia H, Zhou Y, Lin Z, Guo Y, Liu X, Wang T, Wang J, Deng H, Lin L, Deng Q, et al. Characterization and functional validation of β-carotene hydroxylase AcBCH genes in *Actinidia chinensis*. Hortic Res. 2022;9:uhac063. https://doi.org/10.1093/hr/uhac063.
32. Wang T, Karunairetnam S, Wu R, Wang Y, Gleave AP. High efficiency transformation platforms for Kiwifruit (*Actinidia* spp.) functional genomics. Acta Hort. 2012;929:143–8. https://doi.org/10.17660/ActaHortic.2012.929.20.
33. Xia H, Lin Z, He Z, Guo Y, Liu X, Deng H, Li M, Xie Y, Zhang M, Wang J, et al. AcMADS32 positively regulates carotenoid biosynthesis in Kiwifruit by activating AcBCH1/2 expression. Int J Biol Macro Mol. 2023;242(Pt 3):124928. https://doi.org/10.1016/j.ijbiomac.2023.124928.
34. Zhang X, Zhang K, Guo Y, Lv X, Wu M, Deng H, Xie Y, Li M, Wang J, Lin L, et al. Methylation of AcGST1 is associated with anthocyanin accumulation in the kiwifruit outer pericarp. J Agric Food Chem. 2023;71(48):18865–76. https://doi.org/10.1021/acs.jafc.3c03029.
35. Oliveira MM, Pais MS. Plant regeneration from protoplasts of long-term callus cultures of *Actinidia Deliciosa* var. Deliciosa cv. Hayword (kiwifruit). Plant Cell Rep. 1991;9(11):643–6. https://doi.org/10.1007/bf00231807.
36. Pan C, Ye L, Qin L, Liu X, He Y, Wang J, Chen L, Lu G, Corrigendum. CRISPR/Cas9-mediated efficient and heritable targeted mutagenesis in tomato plants in the first and later generations. Sci Rep. 2017;7:46916. https://doi.org/10.1038/srep46916.
37. Gou Y, Li Y, Bi P, Wang D, Ma Y, Hu Y, Zhou H, Wen Y, Feng J. Optimization of the protoplast transient expression system for gene functional studies in strawberry (*Fragaria vesca*). Plant Cell Tissue Organ Cult. 2020;141(1):41–53. https://doi.org/10.1007/s11240-020-01765-x.
38. Jia H, Wang N. Targeted genome editing of sweet orange using Cas9/sgRNA. PLoS One. 2014;9(4):e93806. https://doi.org/10.1371/journal.pone.0093806.
39. Xu Y, Zhang L, Lu L, Liu J, Yi H, Wu J. An efficient CRISPR/Cas9 system for simultaneous editing two target sites in *Fortunella hindsii*. Hortic Res. 2022;9:uhac064. https://doi.org/10.1093/hr/uhac064.
40. Kaur N, Alok A, Shivani, Kumar P, Kaur N, Awasthi P, Chaturvedi S, Pandey P, Pandey A, Pandey AK. CRISPR/Cas9 directed editing of lycopene epsilon-cyclase modulates metabolic flux for β-carotene biosynthesis in banana fruit. Metab Eng. 2020;59:76–86. https://doi.org/10.1016/j.ymben.2020.01.008.
41. Charrier A, Vergne E, Dousset N, Richer A, Petiteau A, Chevreau E. Efficient targeted mutagenesis in Apple and first time edition of Pear using the CRISPR-Cas9 system. Front Plant Sci. 2019;10:40. https://doi.org/10.3389/fpls.2019.00040.
42. Osakabe Y, Liang Z, Ren C, Nishitani C, Osakabe K, Wada M, Komori S, Malnoy M, Velasco R, Poli M, et al. CRISPR-Cas9-mediated genome editing in Apple and grapevine. Nat Protoc. 2018;13(12):2844–63. https://doi.org/10.1038/s41596-018-0067-9.
43. Xing HL, Dong L, Wang ZP, Zhang HY, Han CY, Liu B, Wang XC, Chen QJ. A CRISPR/Cas9 toolkit for multiplex genome editing in plants. BMC Plant Biol. 2014;14:327. https://doi.org/10.1186/s12870-014-0327-y.
44. Zhang A, Liu Y, Wang F, Li T, Chen Z, Kong D, Bi J, Zhang F, Luo X, Wang J, et al. Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the *OsRR22* gene. Mol Breed. 2019. https://doi.org/10.1007/s11032-019-0954-y.
45. Cazzonelli CI, Pogson BJ. Source to sink: regulation of carotenoid biosynthesis in plants. Trends Plant Sci. 2010;15(5):266–74. https://doi.org/10.1016/j.tplants.2010.02.003.
46. Zhang B, Yang X, Yang C, Li M, Guo Y. Exploiting the CRISPR/Cas9 system for targeted genome mutagenesis in *Petunia*. Sci Rep. 2016;6:20315. https://doi.org/10.1038/srep20315.
47. Chen C, Fenk LA, de Bono M. Efficient genome editing in *caenorhabditis elegans* by CRISPR-targeted homologous recombination. Nucleic Acids Res. 2013;41(20):e193. https://doi.org/10.1093/nar/gkt805.

48. Liu X, Yang J, Song Y, Zhang X, Wang X, Wang Z. Effects of SgRNA length and number on gene editing efficiency and predicted mutations generated in rice. Crop J. 2022;10(2):577–81. https://doi.org/10.1016/j.cj.2021.05.015.
49. Biswas S, Tian J, Li R, Chen X, Luo Z, Chen M, Zhao X, Zhang D, Persson S, Yuan Z. Investigation of CRISPR/Cas9-induced SD1 rice mutants highlights the importance of molecular characterization in plant molecular breeding. J Genet Genomics. 2020;47(5):273–80. https://doi.org/10.1016/j.jgg.2020.04.004.
50. Ishizaki T. CRISPR/Cas9 in rice can induce new mutations in later generations, leading to chimerism and unpredicted segregation of the targeted mutation. Mol Breed. 2016;36(12):165. https://doi.org/10.1007/s11032-016-0591-7.
51. Qi W, Zhu T, Tian Z, Li C, Zhang W, Song R. High-efficiency CRISPR/Cas9 multiplex gene editing using the Glycine tRNA-processing system-based strategy in maize. BMC Biotechnol. 2016;16(1):58. https://doi.org/10.1186/s12896-016-0289-2.
52. Zhang JD, Xing JX, Mi QL, Yang WW, Xiang HY, Xu L, Zeng WL, Wang J, Deng LL, Jiang JR, et al. Highly efficient transgene-free genome editing in tobacco using an optimized CRISPR/Cas9 system, pOREU3TR. Plant Sci. 2023;326:11523. https://doi.org/10.1016/j.plantsci.2022.111523.
53. Kor SD, Chowdhury N, Keot AK, Yogendra K, Chikkaputtaiah C, Sudhakar Reddy P. RNA pol III promoters-key players in precisely targeted plant genome editing. Front Genet. 2023;13:989199. https://doi.org/10.3389/fgene.2022.989199.
54. Wolabu TW, Park JJ, Chen M, Cong L, Ge Y, Jiang Q, Debnath S, Li G, Wen J, Wang Z. Improving the genome editing efficiency of CRISPR/Cas9 in Arabidopsis and medicago truncatula. Planta. 2020;252(2):15. https://doi.org/10.1007/s00425-020-03415-0.
55. Bae EK, Choi H, Choi JW, Lee H, Kim SG, Ko JH, Choi YI. Efficient knockout of the phytoene desaturase gene in a hybrid Poplar (*Populus alba* × *Populus glandulosa*) using the CRISPR/Cas9 system with a single gRNA. Transgenic Res. 2021;30(6):837–49. https://doi.org/10.1007/s11248-021-00272-9.
56. Kaur N, Alok A, Shivani, Kaur N, Pandey P, Awasthi P, Tiwari S. CRISPR/Cas9-mediated efficient editing in phytoene desaturase (PDS) demonstrates precise manipulation in banana cv. Rasthali genome. Funct Integr Genomics. 2018;18(1):89–99. https://doi.org/10.1007/s10142-017-0577-5.
57. Qin G, Gu H, Ma L, Peng Y, Deng XW, Chen Z, Qu LJ. Disruption of phytoene desaturase gene results in albino and dwarf phenotypes in Arabidopsis by impairing chlorophyll, carotenoid, and gibberellin biosynthesis. Cell Res. 2007;17(5):471–82. https://doi.org/10.1038/cr.2007.40.

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