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Development and Utilization of the *DsRed2* Visual Reporter Gene System Driven by Soybean *GmUbi* Promoter

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Abstract

Screenable reporter genes are essential tools for plant genetic engineering and transgenic breeding. Red fluorescent protein represented by *DsRed2* has distinct advantages including no requirement for exogenous substrates, non-destructive detection and low plant autofluorescence interference, collectively conferring strong potential for transgenic screening applications. The *ubiquitin* promoter is well established as a broadly active constitutive promoter; however, the capacity of the soybean-endogenous *GmUbi* promoter to drive *DsRed2* expression as an effective visual reporter system has not been systematically characterized. In this study, we constructed two expression vectors carrying *DsRed2* driven by the CaMV 35S promoter and *GmUbi* promoter respectively. Soybean hairy root transformation was first performed to evaluate promoter activity via fluorescence quantitative analysis. The results showed that the fluorescence intensity of hairy roots mediated by *GmUbi* promoter was significantly higher than that of the 35S promoter, and the red fluorescence gradually enhanced over time due to continuous accumulation of DsRed2 protein. Subsequently, the GmUbi-DsRed2 expression cassette was transformed into *Arabidopsis thaliana* and soybean. Obvious red phenotypes were visually observed in siliques, seeds and floral organs of transgenic *Arabidopsis*, as well as in flowers, leaves, pods and seed coats of transgenic soybean. The color difference caused by DsRed2 accumulation allowed direct discrimination between transgenic and non-transgenic materials by naked eyes throughout plant growth and seed germination stages. Taken together, these results demonstrate that the soybean *GmUbi* promoter drives robust transcriptional activity in both *Arabidopsis* and soybean. The GmUbi-DsRed2 reporter system provides a simple, rapid, and non-destructive strategy for the visual screening of transgenic events, offering broad utility in plant genetic transformation and related biotechnological research.

Keywords: *Arabidopsis thaliana*; soybean; *GmUbi* promoter; *DsRed2*; reporter gene

Introduction

Screenable marker genes and reporter genes are a class of functional genes that can be stably expressed in recipient cells and produce intuitively detectable signals. They have become indispensable basic tools in the fields of plant genetic engineering, molecular biology research, and transgenic crop breeding. In the practice of crop genetic improvement, the application value of screenable marker genes and reporter genes is reflected in the following aspects: screening and identification of transgenic cells or plants, detection of promoter activity, and spatiotemporal dynamic visual tracking of target genes

At present, the most widely used screenable marker genes or reporter genes in plant genetic engineering are mainly divided into four categories: enzymatic reporter genes, antibiotic resistance genes, pigment-related genes (such as anthocyanin and betalain), and fluorescent protein reporter genes. Enzymatic reporter genes are represented by the β-glucuronidase gene (*GUS*) and the luciferase gene (*LUC*). As a classic reporter gene, the *GUS* gene encodes β-glucuronidase, which can catalyze substrates such as X-Gluc to produce blue precipitates (Jefferson 1989). It possesses the advantages of simple detection methods, low cost, and stable signals. However, the detection process of the GUS is destructive; it cannot realize continuous observation and dynamic tracking of living plants, and the reaction relies on exogenous substrates. The luciferase can catalyze the oxidation of luciferin to generate bioluminescence. It features high sensitivity and is suitable for quantitative analysis of gene expression and in vivo imaging. Nevertheless, the LUC detection requires exogenous substrates, and fluorescence detection instruments, resulting in high operational costs (Smirnova and Ugarova 2017). Antibiotic resistance reporter genes identify transgenic plants by screening the resistance of transformed cells to specific antibiotics. They are widely applied in plant genetic transformation due to their high screening efficiency and simple operation. However, the residual resistance genes may pose potential risks to the ecological environment (Mmbando 2023). In addition, during antibiotic screening, the expression of resistance genes may exert certain toxicity to plant cells and affect the normal growth and development of plants. Moreover, the screening results need to be verified by subsequent molecular detection, making intuitive identification of transgenic plants unachievable. With the unique advantages of visualization and non-invasive detection, pigment-related genes have developed rapidly in plant genetic engineering, molecular breeding, and gene function verification in recent years. By encoding proteins involved in

pigment synthesis, these genes endow recipient cells or tissues with specific colors, enabling rapid screening and tracking without expensive instruments or chemical reagents. This significantly improves research efficiency and reduces costs. Among them, anthocyanin synthesis regulatory genes (such as *AtMYB75*) and the RUBY reporter system of the betalain synthesis pathway are the two most intensively studied and widely applied types (Fan et al. 2020a; He et al. 2020; Grützner et al. 2021; Wang et al. 2023; Chen et al. 2024a; Tang et al. 2025; Tan et al. 2025). Their research advances have promoted the innovation of plant biotechnology.

The discovery and application of fluorescent protein reporter genes have brought revolutionary breakthroughs to plant genetic engineering. Fluorescent protein genes possess unique advantages such as no requirement for exogenous substrates, *in vivo* detectability, non-destructiveness to cell structures, and the capability of real-time tracking of gene expression dynamics, making them one of the most widely used types of reporter genes at present. Since the green fluorescent protein (GFP) gene was successfully cloned and applied to heterologous expression in 1992, a series of fluorescent proteins with distinct spectral characteristics have been successively developed via genetic engineering modification, providing abundant tools for multicolor fluorescence imaging and co-expression analysis of multiple genes (Seo et al. 2024). The application performance of fluorescent proteins is closely associated with their core properties, including spectral characteristics, photostability, cytotoxicity, tissue penetrability, and background interference (Lippincott-Schwartz and Patterson 2003). Among the fluorescent proteins commonly used in plant genetic engineering, red fluorescent protein (RFP) has an emission wavelength ranging from 580 to 630 nm. As a long-wavelength fluorescence, it causes minimal photodamage to plant cells and does not significantly affect plant growth and development even under long-term excitation. The autofluorescence of plant cells is mainly concentrated in the blue and green light bands, while the autofluorescence signal in the red light band is extremely weak and almost negligible (Cranfill et al. 2016; Wannier et al. 2018). This enables the fluorescence signal of RFP to penetrate plant tissues such as leaves and stems, achieving efficient detection and non-destructive identification in intact whole plants. Among various red fluorescent protein (RFP) variants, DsRed (Discosoma sp. red fluorescent protein) ranks as one of the earliest cloned and practically applied RFPs. It has an excitation wavelength of 558 nm and an emission wavelength of 583 nm, producing intense red fluorescence and possessing distinct advantages for experimental use (Bevis and Glick 2002; Nahalkova and Fatehi 2003; Shaner et al. 2004). As an improved variant of DsRed1, DsRed2 features accelerated protein maturation. When expressed under the control of a strong promoter, DsRed2

can generate visible red fluorescence in specific plant tissues without the aid of optical detection instruments (Nishizawa et al. 2006; Huai et al. 2023).

The expression level of genes is determined by promoters. As the "switch" of gene expression, the transcriptional activity of promoters governs the expression abundance and spatiotemporal specificity of downstream genes. Promoters commonly used in plant genetic engineering are classified into constitutive promoters, tissue-specific promoters and inducible promoters. Constitutive promoters can continuously drive gene expression in all plant tissues and developmental stages, enabling efficient expression of DsRed in various soybean organs including leaves, stems and roots, and facilitating whole-plant fluorescence detection. Thus, they are the preferred promoter type for the expression of transgenic reporter genes.

At present, the most widely applied constitutive promoters in plant transgenesis include the Cauliflower Mosaic Virus 35S (CaMV 35S) promoter, *ubiquitin* (*Ubi*) promoter, and rice *actin 1* (*Actin1*) promoter. As one of the strong promoters applied in plant genetic engineering, the CaMV 35S promoter exhibits broad species adaptability, which can efficiently initiate gene expression in dicotyledons and partial monocotyledons. The maize *ubiquitin* promoter (*ZmUbi*) is also a constitutive promoter. In monocotyledons, the activity of *ZmUbi* is 8–15 times higher than that of the 35S promoter (Christensen et al. 1992). In tomato, the editing efficiency of *Cas9* driven by the *ZmUbi* promoter is threefold that of the 35S promoter (Čermák et al. 2017), indicating that *ZmUbi* promoter possesses favorable expression activity in both monocotyledons and dicotyledons. Additionally, the potato-derived *StUbi* promoter mediates higher constitutive accumulation of β-glucuronidase than the 35S promoter (Miroshnichenko et al. 2020). In this study, the soybean *ubiquitin* promoter was selected to drive the expression of the red fluorescent reporter gene, *DsRed2*, aiming to verify whether the *GmUbi*-derived *DsRed2* expression can reach a level visible to the naked eye in plants. Herein, an efficient method for rapid evaluation of promoter activity was firstly established via *Agrobacterium rhizogenes*-mediated hairy root transformation. Subsequently, the *DsRed2* expression cassette driven by the *GmUbi* promoter was transformed into *Arabidopsis thaliana* and soybean, and transgenic positive events could be directly distinguished by naked eyes without auxiliary optical instruments.

Methodology

Construction of *DsRed2* expression vectors driven by different promoters

The CaMV 35S promoter was used as control to drive the *DsRed2* gene. The *DsRed2* gene was amplified with the primer set Red1/2 (the primer sequence used in this study was listed in the Supplementary Table S1) with KOD One PCR Master Mix -Blue- (TOYOBO biotech, Japan) using the plasmid pRed1305 as template (Fan et al. 2020b). The PCR product was digested with *Nco*I

(NEB Biotech, US), and the binary expression vector pCAMBIA3301 was digested with *Nco*I and *Pml*I. The two DNA fragments were recovered from agarose gel electrophoresis, and subsequently ligated using T4 DNA ligase (NEB Biotech, US). The ligation mixture was transformed with 100 μL chemical competent cells DH5α at 42 ℃ for 45 s. The transformed bacteria solution was incubated at 37 ℃ for 60 min, and then spread on solid LB medium with 50 mg/L kanamycin. Randomly selected transformants were verified by restriction digestion and Sanger sequencing.

Among strong constitutive promoters for mediating transgene expression, the ubiquitin promoter is the most widely adopted. So in this study the *polyubiquitin* (*GmUbi*) promoter of soybean was cloned to drive the *DsRed* gene (Grant et al. 2017). The *GmUbi* promoter was amplified with the primer set GmUbi1/2 using the genomic DNA of soybean variety Williams 82 as template (Chen et al. 2022). The PCR product and the vector pB35Red were digested with *Eco*RI and *Nco*I. The same verification method was adopted for the construction of this vector.

Transformation of soybean hairy roots, evaluation of fluorescent intensity

The seeds of soybean variety Williams 82 were surface-sterilized with 75% ethanol for 1 minute, rinsed three with sterile water, and then sown in vermiculite. When the cotyledons were fully expanded (about after 7 d of cultivation), the seedlings were excised at approximately 0.5 cm below the cotyledon node. The wounds on the hypocotyls were inoculated with *Agrobacterium rhizogenes* strain K599 harboring the expression vector. Subsequently, the treated seedlings were transferred to sterilized moist vermiculite, covered with a highly transparent plastic bag, and incubated in a growth chamber (Fan et al. 2020c). For each expression vector, 20 soybean seedlings were used for hairy root transformation.

Evaluation of fluorescent intensity with hairy roots

After 14 d of hairy root transformation, soybean seedlings were taken out from vermiculite and the vermiculite attached to roots was rinsed off. Photographs were captured under natural light and green excitation light respectively using a Tanon-5200Multi imaging system (Tanon Co., Ltd., China) with excitation wavelength of 540 nm and emission wavelength of 600 nm, and all images were stored in TIFF format. Fluorescence intensity of red fluorescent roots was quantified with ImageJ software (Gunadi et al. 2016; Zhang et al. 2026). Ten random points were selected on fluorescent roots via the Multi-point tool, and another ten points were chosen from the blank surrounding area to determine background fluorescence intensity. The final mean fluorescence intensity (MFI) was calculated as original mean fluorescence intensity of the region of interest minus background mean fluorescence intensity. Two red fluorescent roots were sampled from each independent transformed composite plant. The fluorescence intensity of *DsRed* gene driven by 35S and

GmUbi promoters in hairy roots was determined. A total of 10 composite plants (20 hairy roots) transformed with pB35Red@K599 and 11 composite plants (22 hairy roots) transformed with pBGmURed@K599 were measured.

Transformation of Arabidopsis thaliana with pBGmURed expression vector

Seeds of *A. thaliana* ecotype Col-0 were sown in nutrient soil mixed with vermiculite at a ratio of 1:3. Plants were cultivated under a 16 h light/22 °C and 8 h dark/18 °C photoperiod. Upon flowering, flower buds of fully bloomed Col-0 plants were infected with *Agrobacterium tumefaciens* strain GV3101 harboring plasmid pBGmURed via the floral dip method.(Clough and Bent 1998).

Transformation of Soybean with pBGmURed Expression Vector and Field Cultivation Management of Transgenic Soybean

The vector pBGmURed was electrotransformed into *A. tumefaciens* strain EHA105. Wounded cotyledonary-node explants of soybean cultivar Williams82 were used as materials and infected with EHA105. The soybean genetic transformation procedure was performed according to the method described by Olhoft et al.(Olhoft et al. 2003). Ammonium glufosinate was applied for resistance screening in shoot induction medium (SIM), shoot elongation medium (SEM) and rooting medium (RM) (Hada et al. 2016).

Transgenic T0 soybean lines were planted in the experimental field of Liaocheng University (36°N, 116°E) on June 26, 2025. T1 generation seeds were harvested via self-pollination, and the agronomic traits of transgenic plants were investigated and evaluated

Results

Construction expression vector and acquisition of transgenic lines

Verified by restriction digestion and Sanger sequencing, the CaMV 35S promoter and *GmUbi* promoter were successfully cloned to drive the *DsRed2* gene. The newly constructed vectors were designated as pB35Red and pBGmURed, respectively (Fig. 1).

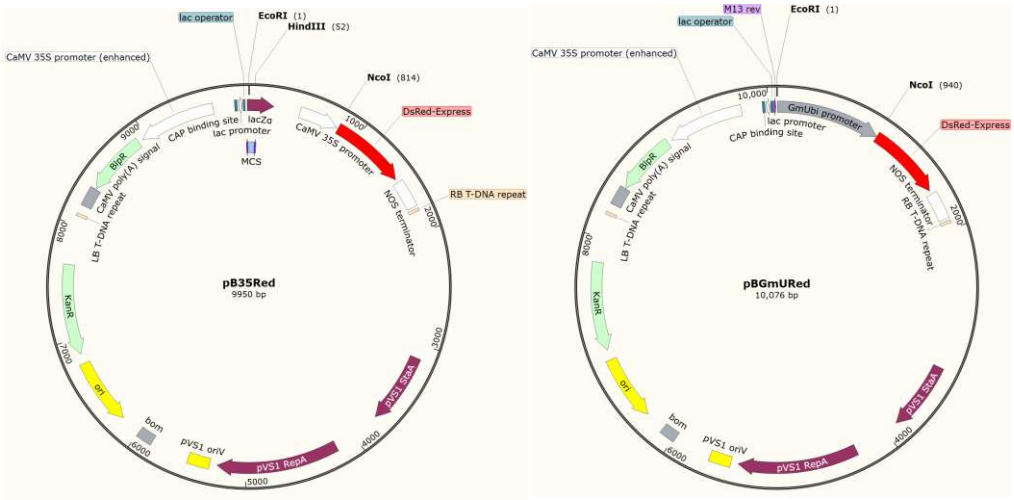


Fig. 1 The binary pB35Red and pBUbiRed vector map.

3.2. Visual comparison by naked Eye and evaluation of fluorescent intensity of soybean hairy roots

At 14 d after soybean hairy root transformation with pBGmURed, red roots were visible under natural light (Fig. 2A), and stronger red fluorescent signals were observed under green excitation light (Fig. 2B). In contrast, transgenic roots could not be distinguished under natural light 21 d after transformation with pB35Red, and all hairy roots appeared white (Fig. 2C). Red fluorescent roots could be identified under green excitation light (Fig. 2D), while their fluorescence intensity was markedly lower than that of pBGmURed transformed hairy roots.

After 21 d (Fig. 3A-B) and 28 d (Fig. 3C-D) of transformation of soybean hairy roots with pBGmURed, the growth status of hairy roots was re-observed. The red fluorescence of hairy roots deepened gradually over time, indicating that continuous accumulation of DsRed protein enhanced the red coloration in root tissues

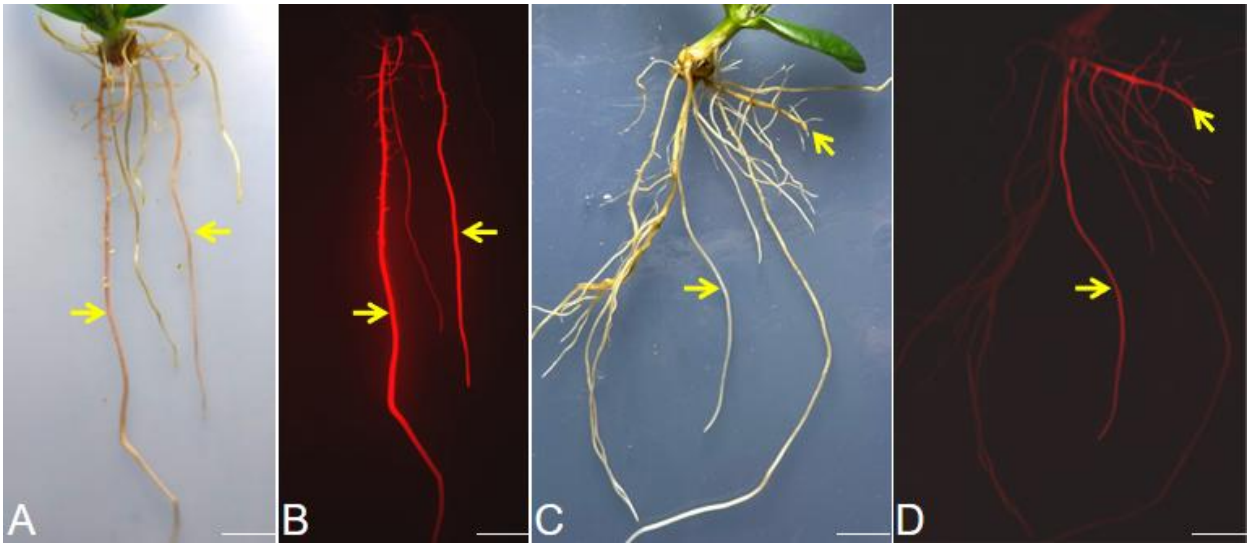


Fig. 2 The hairy roots of *Agrobacterium*-mediated hairy root transformation in soybean after 14 d , wtih the *DsRed* driven by the 35S promoter (A/B) and *GmUbi* promoter (C/D) under natural light and green excitation light with excitation at 540 nm, emission at 600 nm, respectively. Bars, 1cm.

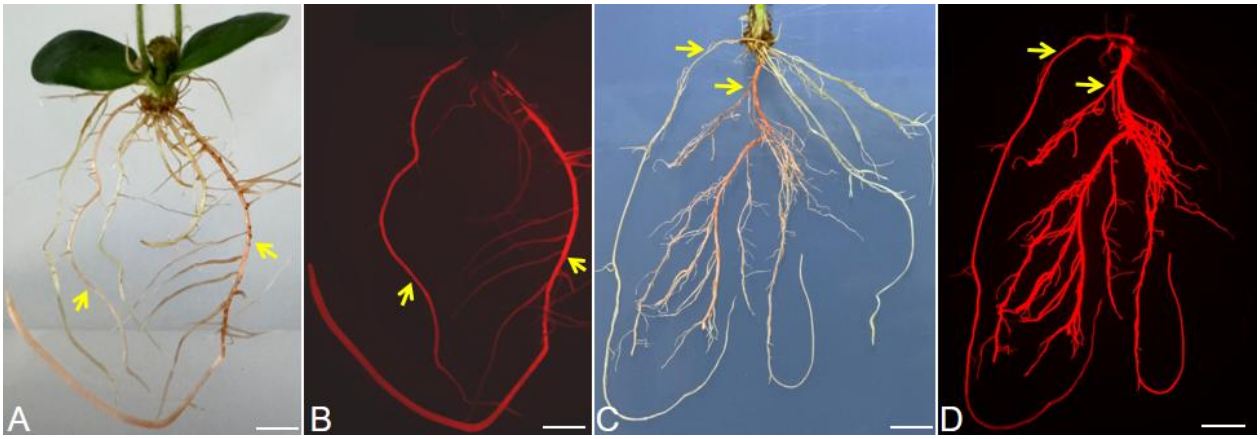


Fig. 3 The soybean hairy roots with pBGmURed after 21 d (A/B) and 28 d (C/D) of transformation. B and D were pictured under green excitation light with excitation at 540 nm, emission at 600 nm, respectively. Bars, 1cm.

After 28 d of soybean hairy root transformation with pB35Red and pBGmURed, the images were captured under green excitation light. The fluorescence intensity of the *DsRed2* gene driven by the 35S and *GmUbi* promoters was quantified respectively. The MFI of *DsRed2* driven by the 35S promoter was 35.545, while that driven by the *GmUbi* promoter reached 79.85 (Fig. 4, Supplementary Fig. 1). The fluorescence intensity was consistent with the visual color difference of hairy roots. Roots transformed with pBGmURed exhibited deeper red color compared with those transformed with pB35Red (Supplementary Fig. 1). These results demonstrated that *GmUbi* promoter could drive robust *DsRed2* expression in hairy roots, producing visible red fluorescence to distinguish transgenic roots from non-transgenic ones.

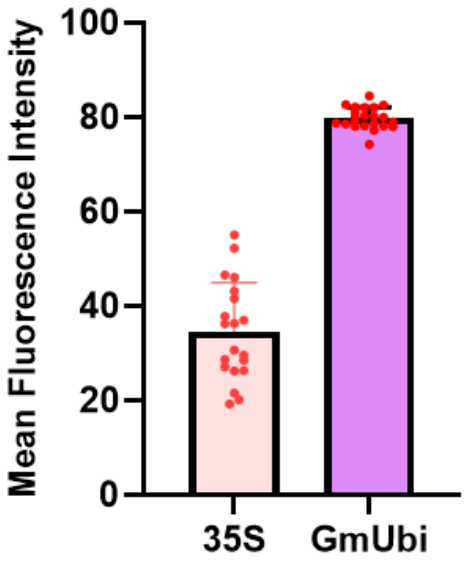


Fig. 4 The MFI of transgenic hairy roots after 28 d of soybean hairy root transformation.35S and GmUbi were showed the MFI of transgenic hairy roots from pB35Red and pBGmURed, respectively.

The phenotypic characteristics of transgenic *A. thaliana*

Fourteen days after *A. thaliana* transformation, strong red fluorescence were detected in partial seeds inside siliques of *A. thaliana* using a handheld fluorescence lamp under green excitation light (Fig. 5A, B), which showed the successful transformation of the *DsRed2* gene and confirmed that the fluorescence of DsRed2 protein could penetrate the cell layers of *Arabidopsis* siliques. After seed maturation, two types of seeds with red and yellow-green color were isolated from siliques. Under green excitation light, red seeds emitted obvious red fluorescence, whereas yellow-green seeds showed no fluorescence signal (Fig. 5C, D). The results revealed that the accumulation of DsRed2 protein led to red seed coat color, proving that *GmUbi* promoter possesses strong transcriptional driving activity.

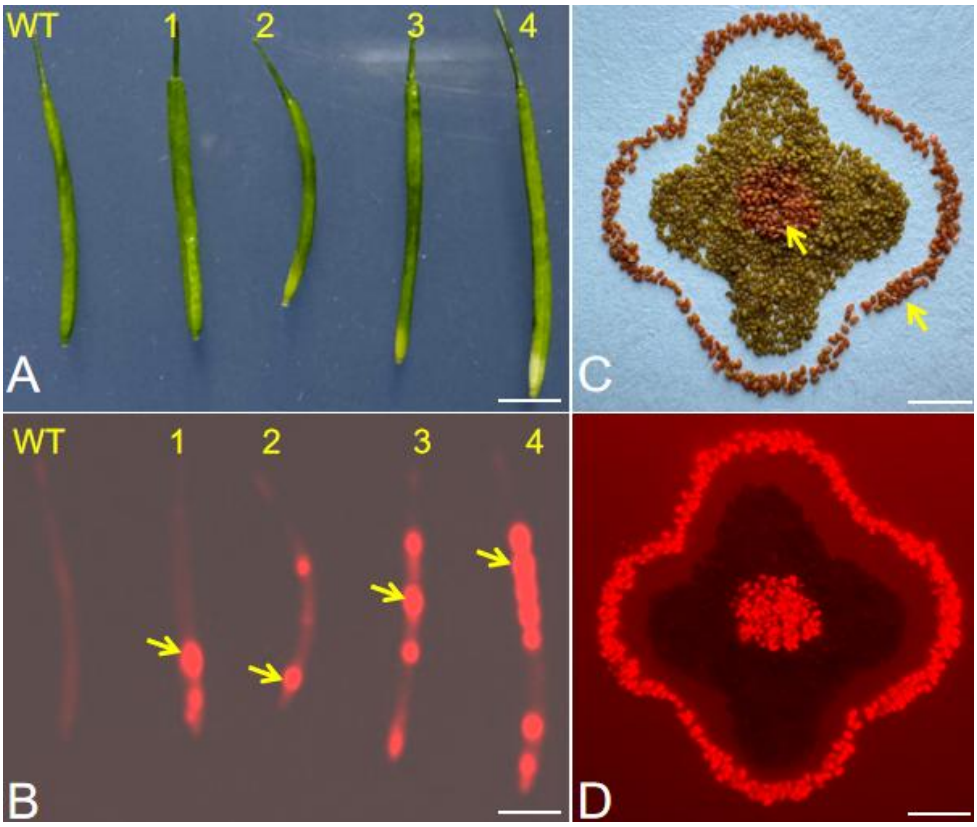


Fig. 5 Strong red fluorescence signals were observed in *A. thaliana* siliques 14 d after transformation (A, B) and mature seeds (C, D). (A, C) were pictured under natural light. (B, D) were pictured under green excitation light with excitation at 540 nm, emission at 600 nm. Yellow arrows, the seeds emitting red fluorescence. Bars, 0.5cm.

Seeds were harvested from *A. thaliana* plants subjected to floral dip transformation and sown on 1/2 MS medium. Seven days after germination, the roots of seedlings derived from red seeds (emitting red fluorescence) exhibited distinct red coloration, while the roots of seedlings grown from yellowish-green seeds remained white. Under green excitation light, strong red fluorescence was clearly detected in seedlings with red roots, whereas no red fluorescence was observed in seedlings with white roots (Fig. 5A, B). These results indicate that the red fluorescence from the roots was entirely derived from the DsRed2 protein, eliminating the interference of autofluorescence. These *Arabidopsis* seedlings were transplanted into nutrient soil for cultivation. At the flowering stage, the plants emitting red fluorescent displayed a red-green phenotype with pale red petals, while non-fluorescent plants showed dark green foliage and white petals (Fig. 5C, D). This finding demonstrates that the accumulation of DsRed2 protein in *Arabidopsis* tissues, especially in petals, produces a visible red hue to the naked eye. It is confirmed that the *DsRed2* reporter gene driven by the soybean *GmUbi* promoter can be visually identified without the aid of optical instruments.

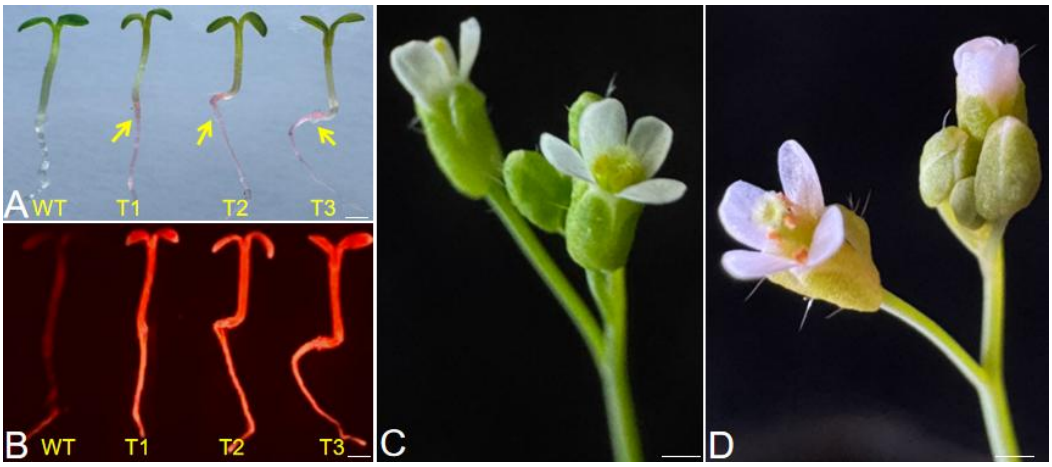


Fig. 5 The seedlings (A, B) and flowers (C, D) of *A. thaliana* transformated with pBGmURed. A and C were pictured under natural light. B and D were pictured under green excitation light with excitation at 540 nm, emission at 600 nm. Bars, 0.5cm.

The phenotype of transgenic soybean transformated with pBGmURed

After transforming soybean cultivar Williams82 with *A. tumefaciens* strain EHA105 harboring plasmid pBGmURed, a total of 26 regenerated soybean lines were obtained on ammonium glufosinate-selective medium. Among them, 18 lines emitted red fluorescence and were identified as positive transgenic soybean plants. At the flowering stage, the flowers of these positive transgenic plants showed distinct red color visible to the naked eye, while the flowers of non-transgenic plants without red fluorescence and wild-type soybean remained white color (Fig. 6). When the *DsRed2* transgenic soybeans developed to the pod-setting stage, young leaves remained green, while leaves in the middle and lower parts of the plants gradually turned brown (Supplimentary Fig. 1). After harvesting, the pods and the seed coats of transgenic lines displayed bright red (Fig. 6).



Fig. 6 The flowers, pods, and seeds of soybean. (A, C) soybean wildtype Williams82. (B, D) transgenic line 4 transformated with pBGmURed. Bars, 1cm.

After harvesting T1 transgenic soybean seeds, obvious differences in seed coat color were observed among different transgenic lines (Fig. 7A, B). The seed coats of multiple lines including Line 4 and Line 10 displayed bright red coloration. Line 7 exhibited red spots on seed coats, while only faint red pigment was detected in Line 6. ross-sectioning revealed that seeds with red seed coats also exhibited visible red or orange coloration in the inner tissues (Fig. 7C, D). When these seeds were sown, the cotyledons and hypocotyls displayed an obvious pale red color visible to the naked eye at the hypocotyl hook stage (Fig. 7E). In contrast, the cotyledons and hypocotyls of wild-type soybeans remained green. This clear color difference enables easy distinction between transgenic and non-transgenic seedlings.

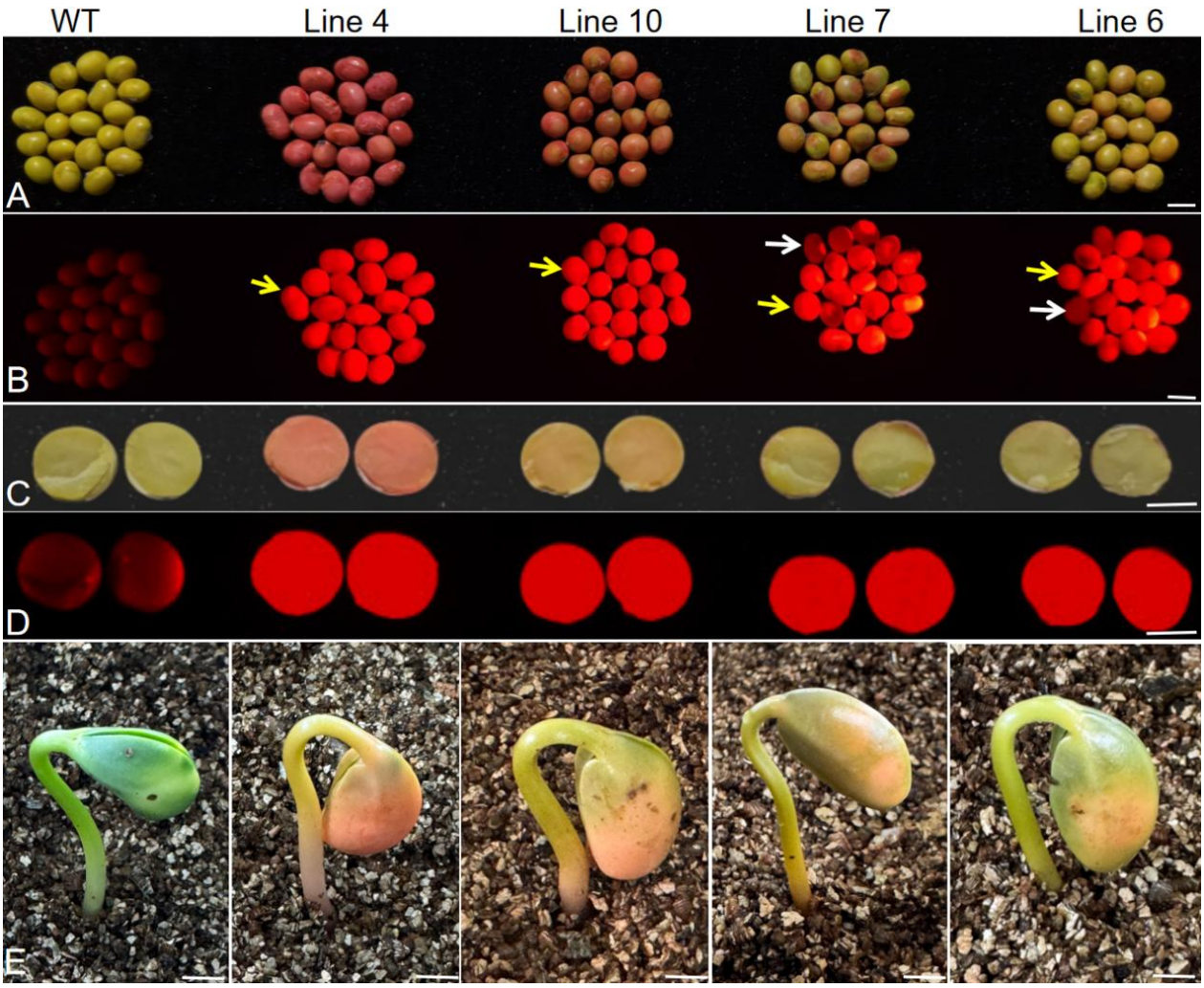


Fig. 7 The seeds (A/B) and seedlings (E) of wildtype (WT) and transgenic soybeans (Line 4, 10, 7, and 6). C and D: the cross-section of the seeds. B and D were pictured under green excitation light with excitation at 540 nm, emission at 600 nm. Bars, 0.5cm.

Discussion

Numerous approaches are available to quantify promoter activity in plants, among which the reporter gene assay is widely adopted. Common reporter genes encompass those used in the Dual-

Luciferase Assay, fluorescent marker genes such as *GFP* and *RFP*, as well as the *GUS* reporter gene. Alternatively, the transcriptional quantification *in vivo* can be applied to characterize the intrinsic transcriptional driving capacity of target promoters inside plant cells. Such as in cotton transformation, the GFP gene is driven by the CaMV 35S promoter. No GFP fluorescence is detectable during the early stages of embryonic development. Detectable GFP signals first emerge in a narrow region at the cotyledonary node of embryos approximately 13 d post-anthesis. As embryogenesis proceeds, the green fluorescent intensity progressively increases and spreads across the entire cotyledons and hypocotyl (Sunilkumar et al. 2002). In *Arabidopsis* transformation, seed-specific napin and cruciferin promoters were employed to drive the expression of fluorescent protein genes. This system enables rapid visual screening of transgenic seeds and facilitates the evaluation of adverse impacts of T-DNA insertion or exogenous transgene integration on seed development and germination (Stuitje et al. 2003). Fluorescent reporter proteins offer significant advantages for promoter activity analysis, real-time in vivo monitoring, non-destructive sampling, and visualization of spatiotemporal expression dynamics. In contrast, this approach effectively overcomes the limitations inherent to conventional GUS histochemical staining, including poor tissue penetration, false-negative results, and irreversible tissue damage associated with destructive sampling (Chiu et al. 1996; Sunilkumar et al. 2002).

Fluorescent protein genes are widely employed as reporter genes in plant genetic transformation, and some studies have documented their application in naked-eye phenotypic screening of primary transformants and their progeny via visible color labeling. For instance, in transgenic soybean plants where the *DsRed2* gene is driven by the 35S promoter, the color of soybean seeds can be readily distinguished by the naked eye, whereas obvious color development is not observed in vegetative and reproductive organs including leaves, flowers and pods (Nishizawa et al. 2006). Transgenic peanuts with the *DsRed2* gene driven by the 35S promoter exhibit visible pale red color in the two seed cotyledons after seed coat removal, which can be clearly distinguished by the naked eye. However, no macroscopically observable red fluorescence was detected in leaves, stems, flowers and other plant organs (Huai et al. 2023). These findings demonstrate that the 35S promoter-driven *DsRed2* reporter confers insufficient visible coloration for phenotypic identification, particularly during the early developmental stages of transgenic plants. Transformation of soybean with *DsRed2* driven by the *S-adenosylmethionine synthetase* (*SAMS*) gene promoter fused with two consecutive viral enhancers (2×Enh) derived from the CaMV 35S promoter generated visually red soybeans observable by the naked eye (Yan et al. 2025).

In the screening of strong endogenous promoters from soybean, the expression levels of promoters of *RPS28*, and *EIF1* showed no significant difference compared with *GmUbi* promoter, an

endogenous promoter widely used in soybean genetic transformation (Chen et al. 2022). In the present study, the *DsRed2* gene was driven by the *GmUbi* promoter for separate genetic transformation of *A. thaliana* and soybean. About 14 d post-transformation in *Arabidopsis*, intense fluorescent signals were detected in siliques using a portable fluorescent lamp. Flowers of T1 generation transgenic *Arabidopsis* exhibited pale red coloration visible to the naked eye, and harvested seeds also bore pale-red seed coats. Transgenic soybeans shared analogous phenotypic characteristics: their flowers turned visibly pale red, while soybean seeds developed an even deeper red pigmentation. After germination of T1 soybean seeds, cotyledons and hypocotyls of resulting seedlings showed pale red pigmentation, enabling straightforward naked-eye discrimination between transgenic and non-transgenic counterparts. Collectively, these results verify that the *GmUbi* promoter possesses stronger transcriptional driving activity than the CaMV 35S promoter in both *Arabidopsis* and soybean.

In this study, the fluorescence intensity of hairy roots 21 d after transformation was higher than that at 12 d. This phenomenon may be attributed to the gradual accumulation of fluorescent protein, which continuously enhances the red fluorescent signal. In *DsRed2* transgenic soybean plants obtained in this experiment, young leaves appeared green, while leaves gradually turned brown with aging. This color change resulted from both chlorophyll degradation and the accumulation of red fluorescence in leaf tissues. The above results are consistent with previous studies. the intensity of fluorescent protein is strictly positively correlated with its accumulation level. As time elapses, fluorescent proteins keep accumulating in plant cells, leading to a progressive increase in fluorescent signals (Mann et al. 2012; Pasin et al. 2014; Cook et al. 2024).

Three genes involved in the betalain biosynthetic pathway were assembled into a single expression cassette, designated RUBY. Expression of this cassette in plants enables the biosynthesis of betalain from tyrosine, resulting in bright red pigmentation in plant tissues. Recently, numerous studies have reported the application of RUBY as a reporter gene (Chen et al. 2024b; Bi et al. 2025; Alok et al. 2026). Nevertheless, the three genes of the betalain biosynthetic pathway possess relatively long sequences and occupy a large proportion of the vector backbone. This issue is particularly prominent in gene-editing vectors, which also carry the lengthy *Cas9* gene. Inserting the RUBY expression cassette into such editing vectors will substantially increase the total vector size, thereby compromising the efficiency of multiple gRNA assembly and plant transformation. In contrast, the *DsRed2* gene features a short sequence with low vector occupancy, which greatly facilitates vector construction.

In conclusion, the *GmUbi* promoter was used to drive the expression of red fluorescent protein in this study. This system enables rapid identification of positive transgenic events during *A. thaliana* transformation. In the progeny with trait segregation, transgenic and non-transgenic plants can be distinguished by visible color changes with the naked eye. For transgenic soybean plants obtained via genetic transformation, obvious color alterations were observed in flowers, leaves and other organs. The harvested T1 seeds can be screened for the presence of transgenic elements by detecting fluorescence signals. Moreover, transgenic events can be readily identified by visible phenotypic changes during the germination of T1 seeds. Collectively, these results demonstrate that the *GmUbi* promoter exhibits strong transcriptional activity in both *A. thaliana* and soybean. Using the *DsRed2* gene driven by this promoter as a reporter gene confers distinct advantages for the screening of transgenic plants.

Conclusions

This study demonstrated that the soybean *GmUbi* promoter is a highly efficient constitutive promoter with strong transcriptional activity in both *A. thaliana* and soybean. Compared with the widely used CaMV 35S promoter, *GmUbi* drove significantly higher expression of the *DsRed2* gene, producing intense and visually recognizable red fluorescence in plant tissues. Fluorescence signals gradually increased over time due to continuous accumulation of red fluorescent protein, which was consistent with previous findings. The *DsRed2* reporter gene dirved by *GmUbi* promoter enabled direct, non-destructive and naked-eye screening of transgenic materials throughout the whole growth period, including transformed hairy roots, plant organs, mature seeds and germinating seedlings. In addition, the *DsRed2* gene has a short coding sequence, which confers obvious advantages over the RUBY reporter system in vector construction, especially for gene editing vectors. In conclusion, the *GmUbi* promoter-driven *DsRed2* reporter system provides a simple, rapid and reliable visual screening tool. It shows great application potential for routine genetic transformation, promoter activity analysis and gene editing research in soybean, *Arabidopsis* and other plant species.

Supplementary Materials: All primers used in this study were listed in Table S1. The pictures of wild-type (WT) and transgenic soybean (Line 4) at the podding stage were showed in Fig. S1.

Author Contributions: QZ: YH, YD, FZ, and YB performed the experiments (plant transformation, planting the transgenic lines, taking the images and data analysis), YF and QZ wrote the original draft. YF and LL provided resources, supervised the work, and read and edited the manuscript. LL and SL conceived the study, designed the study, supervised the work and read and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of Interest: The authors declare no competing interests.

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