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CRISPR/Cas9-mediated knockout of *E4* gene promotes maturation in soybean

Shuiqing Wu [a,b,1], Li Chen [b,1], Mengwei Guo [b,c,1], Yupeng Cai [b], Yang Gao [d], Shan Yuan [b], Shi Sun [b], Yuxian Zhang [a], Wensheng Hou [b,**], Tianfu Han [a,b,*]

[a] College of Agriculture, Heilongjiang Bayi Agricultural University, Daqing, 163316, China
[b] Ministry of Agriculture Key Laboratory of Soybean Biology (Beijing), Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, 100081, China
[c] College of Agriculture, Northeast Agricultural University, Harbin, 150030, China
[d] State Key Laboratory of Crop Germplasm Innovation and Molecular Breeding, Syngenta Biotechnology (China) Co., Ltd, Beijing, 100081, China

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ABSTRACT

Soybean is a broadly popular and extensively cultivated crop, however, many high-yield and high-quality varieties require specific growth conditions, restricting their widespread adoption. The appropriate light conditions and photoperiod must be attained for these varieties to thrive in new environments. In this study, we employed CRISPR/Cas9 to design two sgRNAs aimed at knocking out the maturity-related gene *E4* in a major American soybean variety called "Jack", which belongs to maturity group MGII. *E4* gene is primarily involved in the photoperiodic flowering and maturity in soybean, making it an ideal candidate for genetic manipulation. We successfully obtained 1 homozygous *E4*-SG1 mutant type with 1-bp insertion, and 4 homozygous *E4*-SG2 mutants type with 2-bp deletion, 7-bp deletion, 61-bp deletion, and 1-bp insertion, respectively. The homozygous *e4* mutant plants contained early termination codons devoid of transgenic elements. Additionally, no potential off-target sites of the *E4* gene were detected. A comparative analysis revealed that, unlike the wild-type, the maturity time of homozygous *e4* mutants was early under both short-day and long-day conditions. These mutants offer novel germplasm resources that may be used to modify the photoperiod sensitivity and maturity of soybean, enhancing its adaptability to high-latitude regions.

1. Introduction

Soybean (*Glycine* max (L.) Merr.) is an important crop rich in protein and oil, that is critical for ensuring food safety in China and across the globe. As a short-day crop, soybean growth is severely affected by day length, constraining the suitable planting areas for different varieties. Consequently, many elite soybean varieties with high-yields, good quality, and strong resistance cannot be widely grown. This issue may be addressed by altering a line of interest's appropriate growth period or maturation rate.

Dozen of soybean maturity-related genes have been identified (Bernard, 1971; Buzzell, 1971; Buzzell and Voldeng, 1980; Bonato and Vello, 1999; Cober and Voldeng, 2001; Cober et al., 2010; Ray et al., 1995; Kong et al., 2014; Samanfar et al., 2016), among which *E1* (Xia et al., 2012), *E2* (Watanabe et al., 2011), *E3* (Watanabe et al., 2009), *E4* (Liu et al., 2008), *E6* (Fang et al., 2021), *E9* (*GmFT2a*) (Kong et al., 2010; Sun et al., 2011; Zhao et al., 2016), *E10* (*GmFT4*) (Samanfar et al., 2016), *J* (Lu et al., 2017; Yue et al., 2017) have been localized and cloned. The *E*-series genes exhibit diverse effects on reproduction stage traits, and the interactions between these loci play a crucial role in soybean breeding. Previous research has reported that the *E4* gene encodes *GmPHYA2*, which is a homolog of *Phytochrome A* (*PHYA*) in *Arabidopsis*. Soybean possesses three additional homologous *PHYA* genes, *GmPHYA1*, *GmPHYA3*, and *GmPHYA4*. E3 and E4 regulate E1 by directly binding to and stabilizing the LUX protein, a key component of the evening complex (Lin et al., 2022). Furthermore, these proteins can directly bind to E1 and its homologs to stabilize the E1 protein (Xia et al., 2012; Xu et al., 2015; Lin et al., 2022).

The flowering delay conferred by *E4* can be mitigated by strict control under conditions with a lower redtofar-red quantum ratio (R: FR), similar

* Corresponding author. College of Agriculture, Heilongjiang Bayi Agricultural University, Daqing, 163316, China.
** Corresponding author.
E-mail addresses: houwensheng@caas.cn (W. Hou), hantianfu@caas.cn (T. Han).
[1] These authors contributed equally to this work.

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to the flowering time observed under various natural light-quality long-term conditions (Liu et al., 2008; Watanabe et al., 2009). Varieties with recessive *e4* alleles exhibit high latitude adaptability and are not sensitive to photoperiod, making them prime candidates for breeding efforts. In recent study, sequencing and marker analysis were employed to detect four new and independent dysfunctional genes, *e4-oto*, *e4-tsu*, *e4-kam*, and *e4-kes*, all of which exhibit single base deletions in the first or second exons (Tsubokura et al., 2013). The functional deficiency of the *E4* gene may play an important role in the photoperiod-insensitive evolution of early flowering and photoperiod-insensitive varieties adapted to high-latitude regions (Tsubokura et al., 2013). In addition to their roles in the pre-flowering stage, the *E4* loci may influence the post-flowering stage (Jiang et al., 2014; Xu et al., 2015; Liu et al., 2020). This further supports the hypothesis that *E4* dysfunction may be pivotal for the creation of early maturity and photoperiod-insensitive varieties that are capable of thriving in high-latitude regions.

In recent years, gene editing technologies have been successfully utilized to regulate flowering time and regionally enhance soybean varieties (Cai et al., 2018a, 2018b, 2020a, 2020b; Han et al., 2019; Chen et al., 2020; Wan et al., 2022; Zhao et al., 2022). A previously created *e1* mutant displayed reduced photoperiod sensitivity, altered growth habits, and fewer branches (Han et al., 2019; Wan et al., 2022). Cai et al. (2018a, 2018b, 2020a) analyzed knockout lines and lines containing a single base substitution of *GmFT2a*. Their findings revealed a significant delay in flowering, which was particularly pronounced in the knockout lines compared to those with a single base substitution from C to G. Meanwhile, targeted *GmFT5a* knockout revealed that *GmFT2a* and *GmFT5a* jointly regulate soybean flowering. Under short-day (SD) conditions, the effect of *GmFT2a* was more pronounced than that of *GmFT5a*, while the inverse was observed under long-day (LD) conditions (Cai et al., 2020b). Better understanding of the influences of these genes is the key to future soybean breeding.

In this study, we utilized the CRISPR/Cas9 system in conjunction with *Agrobacterium tumefaciens*-mediated genetic transformation technology to introduce our expression vector into the Jack soybean variety. We aimed to knock out the *E4* gene and analyze the potential impacts on flowering and maturity time. These mutants provide novel materials for selecting early maturing receptors, thus promoting the development of genetic improvement in soybeans and providing a foundation for improving soybean growth trait improvement. Taken together, these results provide guidance for soybean gene function research, molecular breeding, and variety layout.

2. Materials and methods

2.1. Plant materials and growth conditions

The soybean variety Jack served as the control and was used for all genetic transformations during this study. Seeds were harvested from both the control and *E4*-CRISPR/Cas9 T_0 plants, and then sown under natural-day (ND) conditions in Beijing, China. Subsequently, the T_2 homozygous *e4* mutant plants were sown in a greenhouse maintained at 27 °C and 50% relative humidity, under either LD (16 h light/8 h dark) or SD (12 h light/12 h dark).

2.2. SgRNA design and construction of the CRISPR/Cas9 expression vector

Two sgRNAs (20 bp) followed by 5′-NGG (PAM, protospacer adjacent motif) were designed using the web tool CRISPR-P (http://crispr.hzau.edu.cn/CRISPR2/) and termed *E4*-SG1 and *E4*-SG2. The primers for the *E4*-SG1 (5′-GAGATCAAGACGTAGTGCTA-3′) and *E4*-SG2 (5′-TCCAGTTCGGTGCGCATCTC-3′) sgRNAs were synthesized and integrated downstream of the AtU6 promoter in the vector. The CRISPR/Cas9 expression vectors were transformed into *Agrobacterium tumefaciens* strain EHA105 via electroporation and utilized for subsequent soybean transformation. The *bar* gene driven by the CaMV35S promoter served as a screening marker in this experiment. The structure of *E4*-CRISPR/Cas9 expression vector was shown in Fig. 1. The sequence and other information regarding soybean endogenous gene *E4* (*Glyma.20G090000*) sequence were downloaded from the Phytozome database (Phytozome https://phytozome-next.jgi.doe.gov/). Sequences were synthesized by Qingke (Beijing, China).

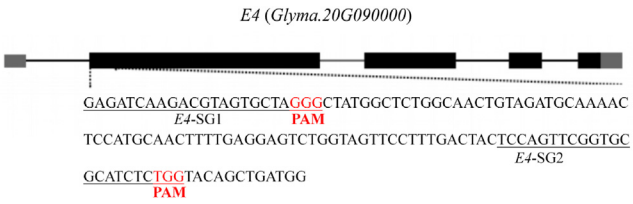

Fig. 1. *E4* gene structured with target sites. Untranslated regions were represented by grey stripes, introns by grey lines, and exons by black stripes. Underlining indicated the target sites and red represents PAM sequences.

2.3. Agrobacterium tumefaciens-mediated transformation

The soybean variety Jack was used for genetic transformation according to the previously established protocols (Chen et al., 2018). The main steps are as follows: Soybean seeds germinate for 16 h, then the cotyledon nodes was scratched, and explants were infected. Then, the explants transferred to co-culture medium. After 5 days, the explants transferred to recovery medium. After 21 days, the explants transferred to screening medium. After 21 days, the clustered buds were transferred to elongation medium. The elongated clustered buds were transplanted to rooting medium, and then the plants were transplanted.

2.4. Screening for homozygous and "transgene-free" mutants

Genomic DNA was extracted from the leaves of each experimental plant using NuClean Plant Genomic DNA Kit. Regions spanning the target sites were amplified through PCR using 2 × Taq Plus MasterMix (CW Biotech) with the *E4* forward (5′-TCTTGAGAAGGTGTTGGA-3′) and reverse (5′-TAAGGACAGGGTTAAGAAGCA-3′) primers, purified, and sequenced. Sequence peaks were used to identify mutation types. The heterozygous mutations displayed overlapping peaks between the target sites and end while the wild-type (WT) and homozygous mutants showed no overlapping peaks at the target sites. Homozygous mutants were then identified by aligning their sequences with the WT.

2.5. Statistical analysis

The flowering time of each soybean plant was recorded as days from emergence to the R1 stage (beginning bloom) and R7 stage (physiological maturity) (Fehr et al., 1971). A minimum of 8 soybean plants per genotype were recorded for quantitative analysis. Statistical analyses were performed using Microsoft Excel. A one-way analysis of variance (ANOVA) with a least significant difference (LSD) test was conducted to compare the significance of differences between controls and treatments at the 0.05 probability level. All histograms were created with GraphPad Prism. Flowering time is shown as mean values ± standard error.

2.6. Real-time qPCR of gene expression

The expression levels of *GmFT2a*, *GmFT5a*, *GmFT4*, and *GmFT1a* were

Table 1
The edited rate of T_0 plants in two target sites.

The Target site	No. of T_0 plants	No. of T_0 edited plants	Edited rate
E4-SG1	13	2	15.39%
E4-SG2	16	9	56.25%

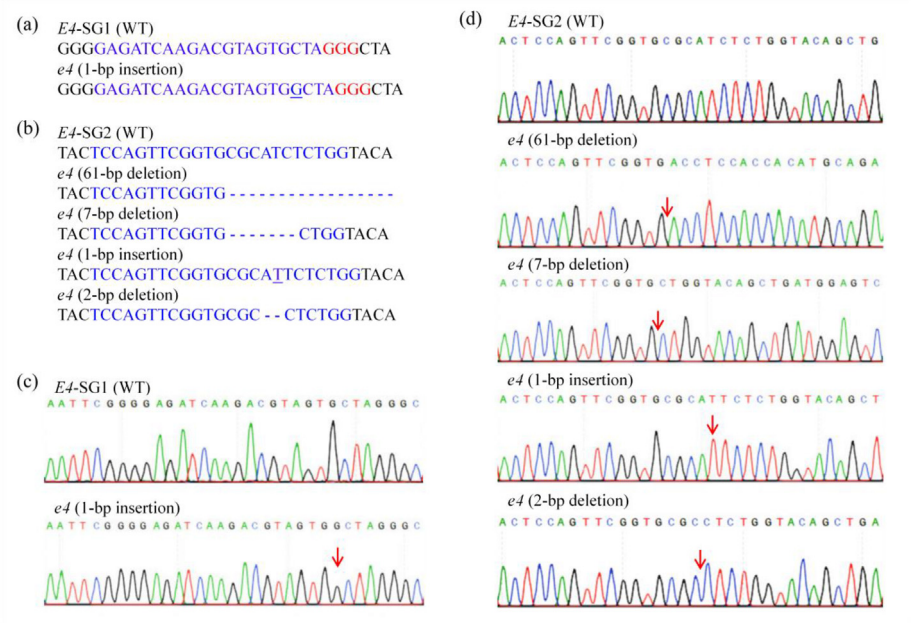

Fig. 2. Homozygous targeted mutagenesis of *E4*. (a, b) Sequences of WT and representative mutation types induced at target sites *E4*-SG1 and *E4*-SG2. The underlines represented base insertion. The short dashes represented base deletion. (c, d) Sequence peaks of WT and representative mutation types at target sites *E4*-SG1 and *E4*-SG2. Red arrowheads indicated mutation locations.

Table 2
The mutagenesis of *E4* in the T_1 generation.

T_1 with *e4* mutations	No. of plants sequenced	No. of homozygous *e4* mutants	No. of heterozygous *e4* mutants	No. of plants with no mutation
E4-SG1-2	17	2	12	3
E4-SG1-5	10	0	6	4
E4-SG2-2	8	0	0	8
E4-SG2-3	10	0	6	4
E4-SG2-4	8	1	4	3
E4-SG2-5	8	0	0	8
E4-SG2-6	15	3	10	2
E4-SG2-9	8	0	8	0
E4-SG2-10	8	2	6	0
E4-SG2-13	9	2	2	5
E4-SG2-16	8	4	3	1

comparatively analyzed in all WT plants and T_2 homozygous *e4* mutants grown under both LD and SD conditions. 18 WT and 36 *e4* mutants were grown and divided into LD and SD treatments, respectively. Every 5 days, beginning at 10 days after emergence (DAE), fully developed trifoliate leaves were sampled 4 h after lights were turned on each morning. Samples were immediately frozen in liquid nitrogen and total RNA was isolated using FastPureR Plant Total RNA Isolation Kit (Vazyme Biotech). Single-stranded cDNA was synthesized through reverse transcription. All qPCR reactions were repeated using 3 biological and three technical replicates. Data were analyzed using the $2^{-\triangle\triangle Ct}$ method and the mRNA level of *GmActin* (*Glyma18g52780*) served as an internal control.

3. Results

3.1. CRISPR/Cas9-mediated E4 mutants

In this study, the CRISPR/Cas9-mediated genome-editing tool was utilized to knock out the endogenous soybean gene *E4*. We selected 2 target sites in the first exon of *E4*, *E4*-SG1, and *E4*-SG2 (Fig. 1), and utilized *Agrobacterium tumefaciens*-mediated transformation to insert the corresponding sgRNA/Cas9 vectors into the soybean variety Jack.

Leaf tissue DNA sequencing of each generated plant enabled us to analyze CRISPR/Cas9-induced mutations at each target site. We identified T_0 transgenic plants harboring the T-DNA of the sgRNA/Cas9 vectors and determined that 15.39% (2 of 13) and 56.25% (9 of 16) had heterozygous-targeted mutations at *E4*-SG1 and *E4*-SG2 sites, respectively (Table 1). Subsequently, all plants of the T_0 generation were allowed to self-pollinate, and seeds were collected and planted as the T_1 generation.

We next used PCR and DNA sequencing to detect *E4*-targeted mutations in the T_1 generation. A total of 27 plants were obtained from two T_0 plants with edits at the *E4*-SG1 site. Of these, we detected 2 homozygous plants with a 1-bp insertion at the *E4*-SG1-2 site (Fig. 2a and c), 12 heterozygous plants mutated at the *E4*-SG1-2 site, and 6 heterozygous plants mutated at the *E4*-SG1-5 site (Table 2). A total of 82 plants with mutations at the *E4*-SG2 target site were obtained from the T_0 generation. Of these, we detected 12 homozygous plants, 1 homozygous plant with 61-bp deletion at the *E4*-SG2-4 site, 3 homozygous plants with 2-bp deletion at the *E4*-SG2-6 site, 2 homozygous plants with 61-bp deletion at the *E4*-SG2-10 site, 2 homozygous plants with 1-bp insertion at the *E4*-SG2-13 sites, and 4 homozygous plants with 7-bp deletion at the *E4*-SG2-

Table 3
Potential off-target analysis at the *E4* target sites in the T_1 generation.

Target	Potential target	Target sequence	No. of mismatch	Position	No. of plants sequenced	No. of plants with off-targets
E4-SG1	*Glyma.20G031100*	GAGACCAACACGAAGTGCTTCGG	4	exon	2	0
	Glyma.02G185100	GTGATCAAGAACTTGTGCTAGAG	4	exon	2	0
E4-SG2	*Glyma.10G141400*	TCCAGTTCGGTGCGTGTCTCTGG	2	exon	12	0
	Glyma.09G203500	TCCACTTCGGTGGGAATCTCAAG	3	exon	12	0
	Glyma.01G019200	TCCACTTCGGTGGGGATCTCAAG	3	exon	12	0

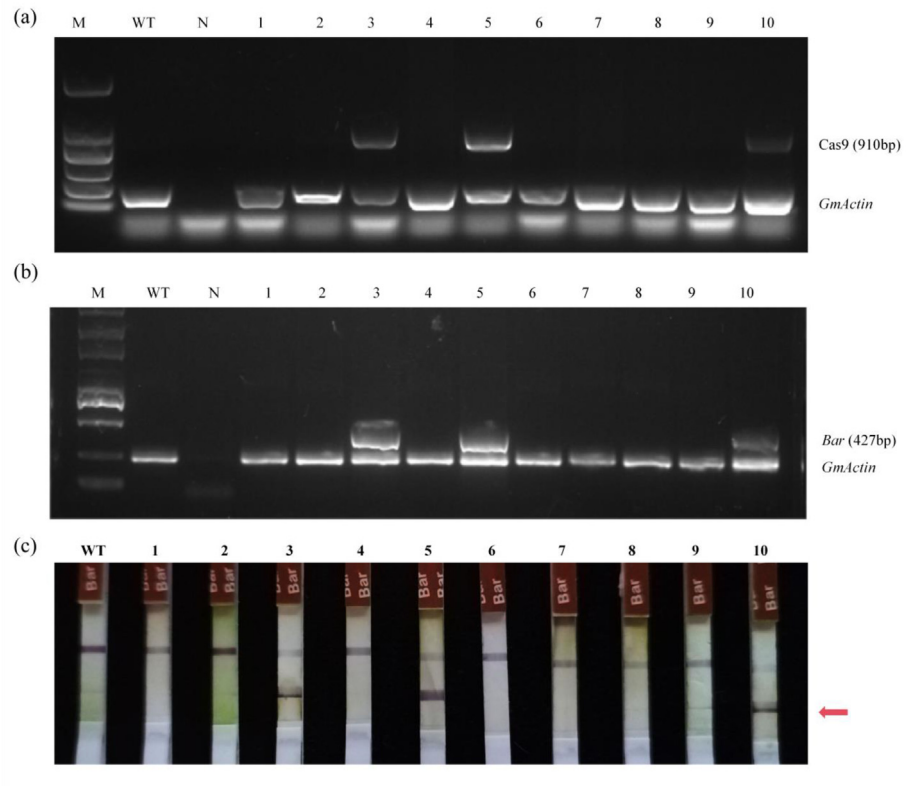

Fig. 3. Trans-free mutant identification. (a) Gel image of PCR products analyzed for T-DNA elements. Cas9 portion of the coding sequence. Bar (427bp), *Bar* gene coding sequence. *GmActin* was used as a control. M indicated Marke. WT, wild-type. N, ddH_2O. (b) Detection of the selectable marker gene bar via Bar test strip. Red arrowhead indicated bar gene positivity.

16 site (Table 2, Fig. 2b–d). These variants have all undergone code shift mutations, resulting in premature translation termination codons.

3.2. Screening of "transgene-free" homozygous e4 mutants

To detect the potential off-target CRISPR/Cas9 variants, we consulted the CRISPR-P website (http://crispr.hzau.edu.cn/CRISPR2/) and selected 5 target sites most likely to deviate. These sites were only 2–4 bp mismatches (Table 3). All homozygous *e4* mutants were detected through a site-specific genomic PCR and sequencing analysis, these homozygous *e4* mutants revealed no potential off-target (Table S1). Comparison of potential off target sites as shown in Fig. S1.

To obtain homozygous *e4* mutant soybean plants devoid of transgenic elements containing sgRNA/Cas9 vectors, we utilized PAT strip test for Bar gene detection and Cas9 specific primers to detect sgRNA/Cas9 on T-DNA through PCR and gel electrophoresis (Fig. 3). Among two *E4* target sites, 3 of 12 T_1 homozygous *e4* mutants at the *E4*-SG2 site were "transgene-free", and no homozygous *e4* mutants at the *E4*-SG1 site were detected. Only 2 of 35 T_2 homozygous *e4* mutants at the *E4*-SG1 site did not contain T-DNA, and 7 of 54 T_2 homozygous *e4* mutants at the *E4*-SG2 site did not contain T-DNA.

3.3. Early maturation is promoted by e4 mutants plants

Our T_2 generation constituted the offspring of T_1 plants cultivated under both the LD and SD conditions. It was comprised of homozygous *e4* mutants with two editing types: a 61-bp deletion (*e4-1*) and a 7-bp deletion (*e4-2*) (Fig. 4). Sequencing analysis revealed that homozygosity was stably passed from the T_1 to T_2 generation. Next, the flowering and maturation times of the homozygous *e4* mutants were compared with the WT. The VE, R1, and R7 phases were recorded and analyzed in all plants under both SD and LD conditions.

Under the SD conditions, no significant differences were observed in the average flowering time between *e4*-1, *e4*-2, and WT, which were 22, 21.1, and 21.9 DAE, respectively (Fig. 4e). The average maturity time of WT, *e4-1*, and *e4-2* were 66.8, 63.5, and 63.2 DAE, respectively (Fig. 4f), indicating an earlier maturation in the mutants.

Under the LD conditions, there were no significant differences in flowering times between the *e4-1* mutant, which flowered at 43.8 DAE, and WT, which flowered at 45.9 DAE. However, the *e4-2* mutant exhibited slightly earlier flowering than the WT, with an average time of 42.5 DAE (Fig. 4a). The average maturity times of the *e4-1*, *e4-2*, and WT plants were 138.4, 136.2, and 149.3 DAE, respectively (Fig. 4c). These results demonstrated the early maturation exhibited by the *e4* mutants under both SD and LD conditions. Under ND condition, the mutants showed significantly reduced plant height and node number compared to the WT. However, there were no significant differences in the number of pods and seeds per plant. (Fig. S2).

3.4. Flowering-related gene expression in homozygous e4 mutants

In this study, we examined the expression levels of flowering-related genes *GmFT2a*, *GmFT5a*, *GmFT1a*, and *GmFT4* in WT and T_2 homozygous *e4* mutants grown under LD and SD conditions. Under the SD conditions, no significant changes were observed in the expression levels of any of these genes. However, under the LD conditions, there was a significant decrease in the expression level of *GmFT1a*, while no significant changes were observed in *GmFT2a*, *GmFT5a*, or *GmFT4* (Fig. 5). This down-regulation may play a role in promoting *e4* mutant maturity under LD conditions.

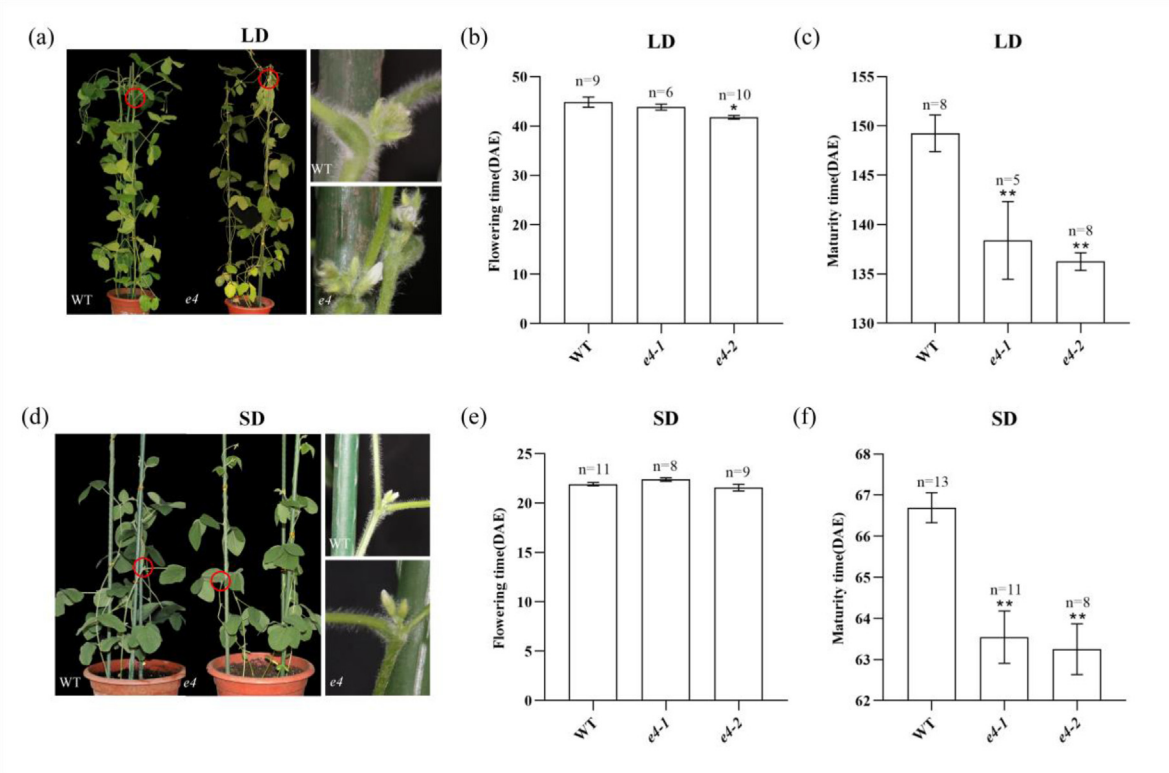

Fig. 4. Homozygous *e4* mutants flowering time under LD and SD conditions. (a) Phenotypes of WT and homozygous *e4* mutants under LD conditions. (b, c) Flowering and maturity time of WT and homozygous *e4* mutants under LD conditions. (d) Phenotypes of WT and homozygous *e4* mutants under SD conditions. (e, f) Flowering and maturity time of WT and homozygous *e4* mutants under SD conditions. n indicated exact number of plants identified. *, homozygous *e4* mutants exhibited highly significant early flowering ($p < 0.05$). DAE, days after emergence. Flowering time was displayed as the mean values ± SE.

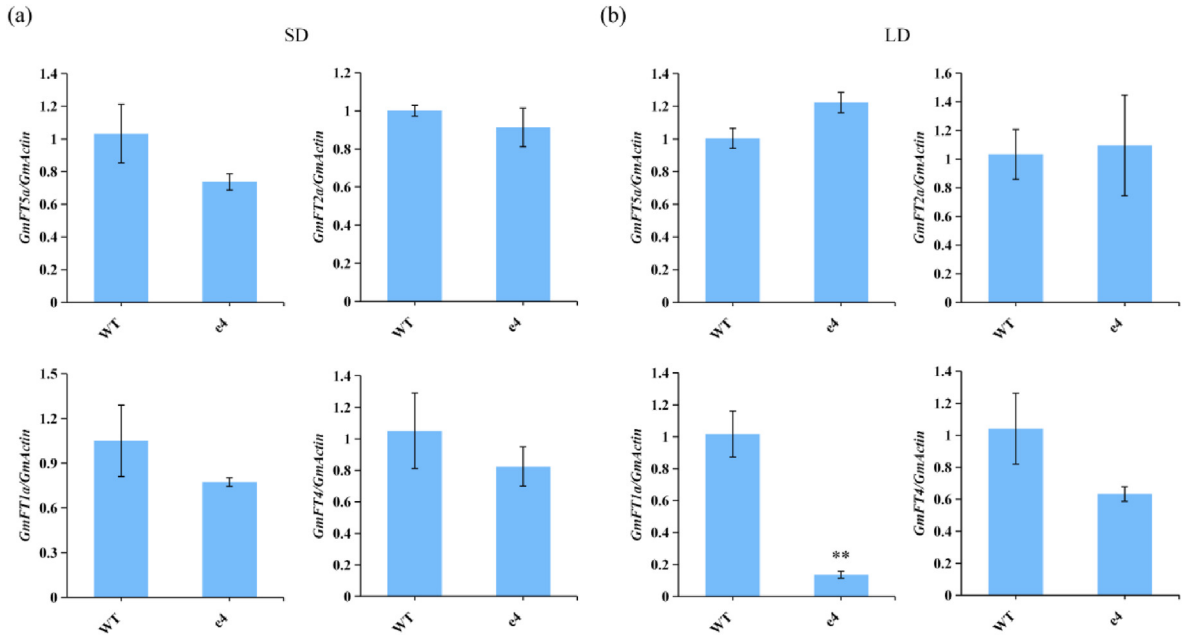

Fig. 5. Expression patterns of flowering-related genes. (a) Expression patterns of flowering-related genes in WT plants and *e4* mutants under SD conditions. (b) Expression patterns of flowering-related genes in WT plants and *e4* mutants under LD conditions. RNA was extracted from trifoliate leaves and the shoot apex at 30 (LD) and 15 DAE (SD). Relative transcription levels were quantified through qRT-PCR and normalized to *GmAction* expression. **, $P < 0.01$. Average values ± SE for three replicates were displayed.

4. Discussion

As a short-day crop, soybean is highly sensitive to photoperiod. Achieving adequate crop yield under LD conditions necessitates a reduction in photoperiod sensitivity and shortened flowering and maturity time. On the contrary, under SD conditions, the flowering and

maturation periods must be increased for optimal performance. In previous studies, the *E1* gene was found to have a significant impact on the nutritional growth stage of various soybean lines, while the *E4* gene played a crucial role in regulating photoperiod responses during the reproductive growth stage (Wang et al., 2008). Another study reported the ability of the *E4* allele to delay flowering by 1–6 days and maturity by 8–20 days (Saindon et al., 1989). An analysis of the near-isogenic line 'Harosoy' revealed that *E4* delays the entire ripening period by lengthening the time from flowering to maturity, but not from emergence to flowering (Ning et al., 2008). The impact of *E4* alleles on flowering is limited to high-latitude regions (Tsubokura et al., 2014; Lu et al., 2015). Our study found that the *e4-1* mutant flowered 2.1 days earlier than the wild type, showing no significant difference, while the *e4-2* mutant flowered 3.4 days earlier than wild type, showing a significant difference statistically. This statistical difference might be due to the difference in sample sizes. The *e4* mutants flowered 2–3 days earlier and matured 11 days earlier under LD conditions. Although no significant differences were recorded in the nutritional growth period, the reproductive growth period was notably shortened, indicating that the mutant plants could be cultivated in higher latitudes.

We then examined the expression levels of several genes associated with flowering and maturity. Our results demonstrated that the expressions of *GmFT2a* and *GmFT5a* remained stable, while *GmFT1a* showed a significant decrease under the LD conditions. Previous studies have documented the regulatory roles of *GmFT2a*, *GmFT5a*, and *GmFT4* flowering, and indicated that the overexpression of *GmFT1a* delays both flowering and maturation time (Zhai et al., 2014; Liu et al., 2018). Therefore, we speculate that *e4* mutants may influence maturation by regulating downstream *GmFT1a*.

Jack is a prominent soybean variety widely cultivated throughout the Midwest of the United States. Its maturity group is MGII (relative maturity 2.9), making it suitable for planting in regions between 40 °N and 42 °N (Nickell et al., 1990). In China, MGII varieties are mainly cultivated between 40 °N and 44 °N in the northeast, consistent with planting regions in the United States (Song et al., 2023). Soybean originated in the Huang-Huai-Hai region, an area known for its rich genetic diversity. However, modern varieties adapted to northern part of northeast China exhibit relatively poor genetic diversity and are typically categorized into the MGI-MG0000 (Li et al., 2008). The *e4* mutants we generated through CRISPR/Cas9 technology display enhanced maturation under LD conditions, indicating the feasibility of modiying elite varieties to thrive in new regions.

Taken together, our results highlight the potential of the CRISPR/Cas9 system to improve the agronomic traits of soybean reproduction by manipulating the *E4* gene, thereby expanding soybean adaptability. Moreover, this study provides a reference for the future use of CRISPR/Cas9 technology in *E* gene knockouts.

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Data availability statement

The data presented in this study are available on request from the corresponding authors.

CRediT authorship contribution statement

Shuiqing Wu: Writing – original draft, Investigation. **Li Chen:** Writing – original draft, Resources. **Mengwei Guo:** Investigation. **Yupeng Cai:** Methodology. **Yang Gao:** Funding acquisition, Conceptualization. **Shan Yuan:** Methodology. **Shi Sun:** Resources. **Yuxian Zhang:** Project administration. **Wensheng Hou:** Writing – review & editing, Funding acquisition, Conceptualization. **Tianfu Han:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following personal relationships which may be considered as potential competing interests: Yang Gao is employed by State Key Laboratory of Crop Germplasm Innovation and Molecular Breeding, Syngenta Biotechnology (China) Co., Ltd.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocsci.2024.05.001.

References

Bernard, R.L., 1971. Two major genes for time of flowering and maturity in soybeans. Crop Sci. 11, 242–244. https://doi.org/10.2135/cropsci1971.0011183x001100020022x.

Bonato, E.R., Vello, N.A., 1999. *E6*, a dominant gene conditioning early flowering and maturity in soybeans. Genet. Mol. Biol. 22, 229–232. https://doi.org/10.1590/s1415-47571999000200016.

Buzzell, R.I., 1971. Inheritance of a soybean flowering response to fluorescent-daylength conditions. Can. J. Genet. Cytol. 13, 703–707. https://doi.org/10.1139/g71-100.

Buzzell, R.I., Voldeng, H.D., 1980. Inheritance of insensitivity to long daylength. Soybean Genet. Newsl. (U.S. Dep. Agric. Agric. Res. Serv.) 7, 26–29.

Cai, Y., Chen, L., Liu, X., Guo, C., Sun, S., Wu, C., Jiang, B., Han, T., Hou, W., 2018a. CRISPR/Cas9-mediated targeted mutagenesis of *GmFT2a* delays flowering time in soyabean. Plant Biotechnol. J. 16, 176–185. https://doi.org/10.1111/pbi.12758.

Cai, Y., Chen, L., Sun, S., Wu, C., Yao, W., Jiang, B., Han, T., Hou, W., 2018b. CRISPR/Cas9-mediated deletion of large genomic fragments in soybean. Int. J. Mol. Sci. 19, 3835. https://doi.org/10.3390/ijms19123835.

Cai, Y., Wang, L., Chen, L., Wu, T., Liu, L., Sun, S., Wu, C., Yao, W., Jiang, B., Yuan, S., et al., 2020a. Mutagenesis of *GmFT2a* and *GmFT5a* mediated by CRISPR/Cas9 contributes for expanding the regional adaptability of soybean. Plant Biotechnol. J. 18, 298–309. https://doi.org/10.1111/pbi.13199.

Cai, Y., Chen, L., Zhang, Y., Yuan, S., Su, Q., Sun, S., Wu, C., Yao, W., Han, T., Hou, W., 2020b. Target base editing in soybean using a modified CRISPR/Cas9 system. Plant Biotechnol. J. 18, 1996–1998. https://doi.org/10.1016/j.molp.2016.11.013.

Chen, L., Cai, Y., Liu, X., Yao, W., Guo, C., Sun, S., Wu, C., Jiang, B., Han, T., Hou, W., 2018. Improvement of soybean *Agrobacterium*-mediated transformation efficiency by adding glutamine and asparagine into the culture media. Int. J. Mol. Sci. 19, 3039. https://doi.org/10.3390/ijms19103039.

Chen, L., Cai, Y., Qu, M., Wang, L., Sun, H., Jiang, B., Wu, T., Liu, L., Sun, S., Wu, C., et al., 2020. Soybean adaption to high-latitude regions is associated with natural variations of *GmFT2b*, an ortholog of *FLOWERING LOCUS T*. Plant Cell Environ. 43, 934–944. https://doi.org/10.1111/pce.13695.

Cober, E.R., Voldeng, H.D., 2001. A new soybean maturity and photoperiod-sensitivity locus linked to *E1* and *T*. Crop Sci. 41, 698–701. https://doi.org/10.2135/cropsci2001.413698x.

Cober, E.R., Molnar, S.J., Voldeng, H.D., 2010. A new locus for early maturity in soybean. Crop Sci. 50, 524–527. https://doi.org/10.2135/cropsci2009.04.0174.

Fang, C., Liu, J., Zhang, T., Su, T., Li, S., Cheng, Q., Kong, L., Li, X., Bu, T., Li, H., et al., 2021. A recent retrotransposon insertion of *J* caused *E6* locus facilitating soybean adaptation into low latitude. J. Integr. Plant Biol. 63, 995–1003. https://doi.org/10.1111/jipb.13034.

Fehr, W.R., Caviness, C.E., Burmood, D.T., Pennington, J.S., 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11, 929. https://doi.org/10.2135/cropsci1971.0011183x001100060051x.

Han, J., Guo, B., Guo, Y., Zhang, B., Wang, X., Qiu, L.J., 2019. Creation of early flowering germplasm of soybean by CRISPR/Cas9 technology. Front. Plant Sci. 10, 1446. https://doi.org/10.3389/fpls.2019.01446.

Jiang, B., Nan, H., Gao, Y., Tang, L., Yue, Y., Lu, S., Ma, L., Cao, D., Sun, S., Wang, J., et al., 2014. Allelic combinations of soybean maturity loci *E1, E2, E3* and *E4* result in diversity of maturity and adaptation to different latitudes. PLoS One 9, e106042. https://doi.org/10.1371/journal.pone.0106042.

Kong, F., Liu, B., Xia, Z., Sato, S., Kim, B.M., Watanabe, S., Yamada, T., Tabata, S., Kanazawa, A., Harada, K., Abe, J., 2010. Two coordinately regulated homologs of *FLOWERING LOCUS T* are involved in the control of photoperiodic flowering in soybean. Plant Physiol. 154, 1220–1231. https://doi.org/10.1104/pp.110.160796.

Kong, F., Nan, H., Cao, D., Li, Y., Wu, F., Wang, J., Lu, S., Yuan, X., Cober, E.R., Abe, J., Liu, B., 2014. A new dominant gene *E9* conditions early flowering and maturity in soybean. Crop Sci. 54, 2529–2535. https://doi.org/10.2135/cropsci2014.03.0228.

Li, Y., Guan, R., Liu, Z., Ma, Y., Wang, L., Li, L., Lin, F., Luan, W., Chen, P., Yan, Z., et al., 2008. Genetic structure and diversity of cultivated soybean (*Glycine max* (L.) Merr.) landraces in China. Theor. Appl. Genet. 117, 857–871. https://doi.org/10.1007/s00122-008-0825-0.

Lin, X., Dong, L., Tang, Y., Li, H., Cheng, Q., Li, H., Zhang, T., Ma, L., Xiang, H., Chen, L., et al., 2022. Novel and multifaceted regulations of photoperiodic flowering by

phytochrome A in soybean. Proc. Nat. Acad. Sci. USA 119, e2208708119. https://doi.org/10.3410/f.742348246.793595788.

Liu, B., Kanazawa, A., Matsumura, H., Takahashi, R., Harada, K., Abe, J., 2008. Genetic redundancy in soybean photoresponses associated with duplication of the *Phytochrome A* gene. Genetics 180, 995–1007. https://doi.org/10.1534/genetics.108.092742.

Liu, W., Jiang, B., Ma, L., Zhang, S., Zhai, H., Xu, X., Hou, W., Xia, Z., Wu, C., Sun, S., et al., 2018. Functional diversification of *Flowering Locus T* homologs in soybean: *GmFT1a* and *GmFT2a/5a* have opposite roles in controlling flowering and maturation. New Phytol. 217, 1335–1345. https://doi.org/10.1111/nph.14884.

Liu, L., Song, W., Wang, L., Sun, X., Qi, Y., Wu, T., Sun, S., Jiang, B., Wu, C., Hou, W., et al., 2020. Allele combinations of maturity genes *E1-E4* affect adaptation of soybean to diverse geographic regions and farming systems in China. PLoS One 15, e0235397. https://doi.org/10.1371/journal.pone.0235397.

Lu, S., Li, Y., Wang, J., Srinives, P., Nan, H., Cao, D., Wang, Y., Li, J., Li, X., Fang, C., et al., 2015. QTL mapping for flowering time in different latitute in soybean. Euphytica 206, 725–736. https://doi.org/10.1007/s10681-015-1501-5.

Lu, S., Zhao, X., Hu, Y., Liu, S., Nan, H., Li, X., Fang, C., Cao, D., Shi, X., Kong, L., et al., 2017. Natural variation at the soybean *J* locus improves adaptation to the tropics and enhances yield. Nat. Genet. 49, 773–779. https://doi.org/10.1038/ng.3819.

Nickell, C.D., Noel, G.R., Thomas, D.J., Waller, R., 1990. Registration of 'Jack' soybean. Crop Sci. 30, 426–427. https://doi.org/10.2135/cropsci1990.0011183x003000060059x.

Ning, H., Li, Y., Liu, Z., Chang, R.Z., Guan, R.X., Luo, S.P., Qiu, L.J., 2008. Deducing maturity genotype of the Chinese soybean varieties. Acta Agron. Sin. 34, 382–387. https://doi.org/10.3724/sp.j.1006.2008.00382.

Ray, J.D., Hinson, K., Jeb, M., Malo, M.F., 1995. Genetic control of a long-juvenile trait in soybean. Crop Sci. 35, 1001–1006. https://doi.org/10.2135/cropsci1995.0011183x003500040012x.

Saindon, G., Voldeng, H.D., Beversdorf, W.D., Buzzell, R.I., 1989. Genetic control of long daylength response in soybean. Crop Sci. 29, 1436–1439. https://doi.org/10.2135/cropsci1989.0011183x002900060021x.

Samanfar, B., Molnar, S.J., Charette, M., Schoenrock, A., Dehne, F., Golshani, A., Belzile, F., Cober, E.R., 2016. Mapping and identification of a potential candidate gene for a novel maturity locus, *E10*, in soybean. Theor. Appl. Genet. 130, 377–390. https://doi.org/10.1007/s00122-016-2819-7.

Song, W., Liu, L., Sun, S., Wu, T., Zeng, H., Tian, S., Sun, B., Li, W., Liu, L., Wang, S., et al., 2023. Precise classification and regional delineation of maturity groups in soybean cultivars across China. Eur. J. Agron. 151, 126982. https://doi.org/10.2139/ssrn.4476149.

Sun, H., Jia, Z., Cao, D., Jiang, B., Wu, C., Hou, W., Liu, Y., Fei, Z., Zhao, D., Han, T., 2011. *GmFT2a*, a soybean homolog of *FLOWERING LOCUS T*, is involved in flowering transition and maintenance. PLoS One 6, e29238. https://doi.org/10.1371/journal.pone.0029238.

Tsubokura, Y., Matsumura, H., Xu, M., Liu, B., Nakashima, H., Anai, T., Kong, F., Yuan, X., Kanamori, H., Katayose, Y., et al., 2013. Genetic variation in soybean at the maturity locus *E4* is involved in adaptation to long days at high latitudes. Agronomy 3, 117–134. https://doi.org/10.3390/agronomy3010117.

Tsubokura, Y., Watanabe, S., Xia, Z., Kanamori, H., Yamagata, H., Kaga, A., Katayose, Y., Abe, J., Ishimoto, M., Harada, K., 2014. Natural variation in the genes responsible for maturity loci *E1, E2, E3* and *E4* in soybean. Ann. Bot. 113, 429–441. https://doi.org/10.1093/aob/mct269.

Wan, Z., Liu, Y., Guo, D., Fan, R., Liu, Y., Xu, K., Zhu, J., Quan, L., Lu, W., Bai, X., Zhai, H., 2022. CRISPR/Cas9-mediated targeted mutation of the *E1* decreases photoperiod sensitivity, alters stem growth habits, and decreases branch number in soybean. Front. Plant Sci. 13, 1066820. https://doi.org/10.3389/fpls.2022.1066820.

Wang, Y., Wu, C.X., Zhang, X.M., Wang, Y.P., Han, T.F., 2008. Effects of soybean major maturity genes under different photoperiods. Acta Agron. Sin. 34, 1160–1168. https://doi.org/10.3724/sp.j.1006.2008.01160.

Watanabe, S., Hideshima, R., Xia, Z., Tsubokura, Y., Sato, S., Nakamoto, Y., Yamanaka, N., Takahashi, R., Ishimoto, M., Anai, T., et al., 2009. Map-based cloning of the gene associated with the soybean maturity locus *E3*. Genetics 182, 1251–1262. https://doi.org/10.1534/genetics.108.098772.

Watanabe, S., Xia, Z., Hideshima, R., Tsubokura, Y., Sato, S., Yamanaka, N., Takahashi, R., Anai, T., Tabata, S., Kitamura, K., Harada, K., 2011. A map-based cloning strategy employing a residual heterozygous line reveals that the *GIGANTEA* gene is involved in soybean maturity and flowering. Genetics 188, 395–407. https://doi.org/10.1534/genetics.110.125062.

Xia, Z., Watanabe, S., Yamada, T., Tsubokura, Y., Nakashima, H., Zhai, H., Anai, T., Sato, S., Yamazaki, T., Lü, S., et al., 2012. Positional cloning and characterization reveal the molecular basis for soybean maturity locus *E1* that regulates photoperiodic flowering. Proc. Nat. Acad. Sci. USA 109, E2155–E2164. https://doi.org/10.1073/pnas.1117982109.

Xu, M., Yamagishi, N., Zhao, C., Takeshima, R., Kasai, M., Watanabe, S., Kanazawa, A., Yoshikawa, N., Liu, B., Yamada, T., Abe, J., 2015. The soybean-specific maturity gene *E1* family of floral repressors controls night-break responses through down-regulation of *FLOWERING LOCUS T* orthologs. Plant Physiol. 168, 1735–1746. https://doi.org/10.1104/pp.15.00763.

Yue, Y., Liu, N., Jiang, B., Li, M., Wang, H., Jiang, Z., Pan, H., Xia, Q., Ma, Q., Han, T., Nian, H., 2017. A single nucleotide deletion in *J* encoding GmELF3 confers long juvenility and is associated with adaption of tropic soybean. Mol. Plant 10, 656–658. https://doi.org/10.1016/j.molp.2016.12.004.

Zhai, H., Lü, S., Liang, S., Wu, H., Zhang, X., Liu, B., Kong, F., Yuan, X., Li, J., Xia, Z., 2014. *GmFT4*, a homolog of *FLOWERING LOCUS T*, is positively regulated by *E1* and functions as a flowering repressor in soybean. PLoS One 9, e89030. https://doi.org/10.1371/journal.pone.0089030.

Zhao, C., Takeshima, R., Zhu, J., Xu, M., Sato, M., Watanabe, S., Kanazawa, A., Liu, B., Kong, F., Yamada, T., Abe, J., 2016. A recessive allele for delayed flowering at the soybean maturity locus *E9* is a leaky allele of *FT2a*, a *FLOWERING LOCUS T* ortholog. BMC Plant Biol. 16, 20. https://doi.org/10.1186/s12870-016-0704-9.

Zhao, F., Lyu, X., Ji, R., Liu, J., Zhao, T., Li, H., Liu, B., Pei, Y., 2022. CRISPR/Cas9-engineered mutation to identify the roles of phytochromes in regulating photomorphogenesis and flowering time in soybean. Crops J 10, 1654–1664. https://doi.org/10.1016/j.cj.2022.03.008.