



GmEID1 modulates light signaling through the Evening Complex to control flowering time and yield in soybean

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Soybean (*Glycine max*) morphogenesis and flowering time are accurately regulated by photoperiod, which determine the yield potential and limit soybean cultivars to a narrow latitudinal range. The *E3* and *E4* genes, which encode phytochrome A photoreceptors in soybean, promote the expression of the legume-specific flowering repressor *E1* to delay floral transition under long-day (LD) conditions. However, the underlying molecular mechanism remains unclear. Here, we show that the diurnal expression pattern of *GmEID1* is opposite to that of *E1* and targeted mutations in the *GmEID1* gene delay soybean flowering regardless of daylength. *GmEID1* interacts with J, a key component of circadian Evening Complex (EC), to inhibit *E1* transcription. Photoactivated *E3/E4* interacts with *GmEID1* to inhibit *GmEID1*-J interaction, promoting J degradation resulting in a negative correlation between daylength and the level of J protein. Notably, targeted mutations in *GmEID1* improved soybean adaptability by enhancing yield per plant up to 55.3% compared to WT in field trials performed in a broad latitudinal span of more than 24°. Together, this study reveals a unique mechanism in which *E3/E4*-*GmEID1*-EC module controls flowering time and provides an effective strategy to improve soybean adaptability and production for molecular breeding.

phytochrome A | flowering | *GmEID1* | circadian Evening Complex | yield

Soybean (*Glycine max* L.) is one of the most economically important legume crops that provides plant oil and protein to humans and livestock around the world. As a facultative short-day (SD) plant, soybean flowers earlier under SD conditions than under long-day (LD) conditions (1). This photoperiodic response significantly limits the yield potential of soybean cultivars at different latitudes (2). Molecular breeding by modulating flowering time and reducing sensitivity to daylength are effective ways to improve the adaptability and production of soybean (3).

Garner and Allard discovered photoperiodism by comparing the flowering time of plants (including soybean) grown under SD or LD conditions in 1920 (4). To date, more than a dozen genes/loci related to flowering time and maturity have been identified in soybean, including *E1* to *E11*, *J*, *TOF5*, *TOF11*, *TOF12*, *TOF16*, and *TOF18*. Among them, *E1* encodes a legume-specific flowering repressor that inhibits flowering under LD conditions (5–7); *E2* is homologous to *Arabidopsis* *GIGANTEA* (8); *E3* and *E4* encode phytochrome A homologs, named *GmPHYA3* and *GmPHYA2*, respectively (9, 10); *E6* and *J*, renowned for “long-juvenile” gene, encode a homolog of EARLY FLOWERING 3, named *GmELF3a* (11–13), a key component of the circadian Evening Complex (EC) consisting of *ELF3*, *ELF4*, and *LUX ARRHYTHMO* (*LUX*) (14); *E9* encodes a homolog of *FLOWERING LOCUS T*, named *GmFT2a* (15, 16); and *TOF5* encodes a homolog of *Arabidopsis* *FRUITFULL* (*FUL*) (17, 18). *TOF11* and *TOF12* encode homologs of *Arabidopsis* *PSEUDO-RESPONSE REGULATOR 3*, named *GmPRR3a* and *GmPRR3b*, respectively (19–21). *TOF16* encodes a homolog of *LATE ELONGATED HYPOCOTYL* (*LHY*) (22). *TOF18* encodes a homolog of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1/AGL20*) (23). It should be noted that *E1*, as the central flowering repressor in the photoperiodic flowering pathway in soybean, expresses at a high level under LD conditions to inhibit *GmFT2a* and *GmFT5a* transcription (5, 24, 25). *J*, as one of the EC components, represses *E1* transcription by directly binding the *E1* promoter (11–13). In cultivars with loss-of-function *j* alleles, *E1* expression is released from the inhibition of EC, allowing *E1* to delay flowering and maturation and increase yield by 30 to 50% in low latitudinal regions (12). Light signals are perceived by *E3* and *E4* that indirectly up-regulate *E1* expression under LD conditions (12, 26). However, the factors that link the perception of light or photoperiod by *E3* and *E4* to the transcriptional upregulation of *E1* remain largely unknown.

The *EID1* gene (*EMPFINDLICHER IM DUNNKELOTEN LICHT 1*) encodes an F-box protein that functions as a negative regulator in the light signaling cascade downstream

Significance

The flowering time and architecture of soybean is extremely sensitive to photoperiod, which limits the suitable planting area of a certain soybean cultivar to a small latitudinal span. It is known that light signaling through phytochrome A (*E3/E4*) modulates the activity of J, a key component of circadian Evening Complex (EC), to control photoperiodic flowering. However, the molecular mechanism remains unclear. Here, we revealed that *GmEID1* acts as a bridge to link the light signals perceived by *E3/E4* to the activity of EC. In addition to this unique insight into flowering time mechanisms, our results suggest that *GmEID1* is a potential target for adjustment of soybean flowering time to improve adaptation and yield, via gene editing and classical breeding.

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of the photoreceptor PHYA in *Arabidopsis* (27–29). Meanwhile, a domesticated gene (*Solyc09g075080*) homologous to *EID1* is responsible for the deceleration of the circadian clock in cultivated tomato (30–32). In this study, we identified an orthologous gene for *EID1* in soybean (named *GmEID1*) that regulates flowering time by physically interacting with and stabilizing J protein. Furthermore, E3 and E4 interact with GmEID1 in a light-dependent manner to interfere with the GmEID1–J interaction, which reduces J protein abundance under LD conditions. The CRISPR-Cas9 engineered mutations in the *GmEID1* gene dramatically increased yield in field trials, providing a promising approach to substantially improve soybean adaptability and production.

Results

Identification of *GmEID1* as a Flowering Regulator. The *E1* gene expression pattern is characterized by a robust rhythmic fluctuation with a low level during the day except for a slight hump at ZT4, peaking at dusk and subsequently decreasing during the night under LD conditions (6, 12, 25). We surmised that the gene upstream of *E1* may be characterized with an opposite or consistent expression pattern compared to *E1*. Therefore, we screened flowering candidate genes exhibiting such expression patterns

by transcriptome sequencing (RNA-seq) analysis within an LD photoperiodic cycle (Datasets S1 and S2) (33). Bioinformatic analysis identified four opposite-pattern genes and ten consistent-pattern genes compared to *E1* (SI Appendix, Fig. S1). We noticed that one of the opposite-pattern genes, named *GmEID1* hereafter, is orthologous to *Arabidopsis EID1* (*AtEID1*) that encodes an F-box protein involved in the PHYA-mediated light signaling pathway (27) (Fig. 1A and SI Appendix, Fig. S1). We found five homologous proteins for *EID1* in soybean, among which GmEID1 is mostly conserved with *AtEID1* (SI Appendix, Figs. S2 and S3). Tissue-specific expression analysis showed that the *GmEID1* gene is highly expressed in above-ground tissues, including leaves and shoot tips (SI Appendix, Fig. S4A), which is similar to the *J* gene (18). The subcellular localization experiment demonstrated that GmEID1 is localized in the nucleus (SI Appendix, Fig. S4B), which is reminiscent to that of *AtEID1* (29), suggesting that GmEID1 may also be involved in the GmPHYA-mediated light signaling pathway in soybean.

To test this possibility, we generated mutants engineered with CRISPR/Cas9 in two genetic backgrounds (*Gmeid1-1* and *Gmeid1-2* in the Tianlong1 (TL1) background, *Gmeid1-3* and *Gmeid1-4* in the Williams82 (W82) background) (SI Appendix, Fig. S5 A and B). Meanwhile, we made the *35S::YFP-GmEID1*

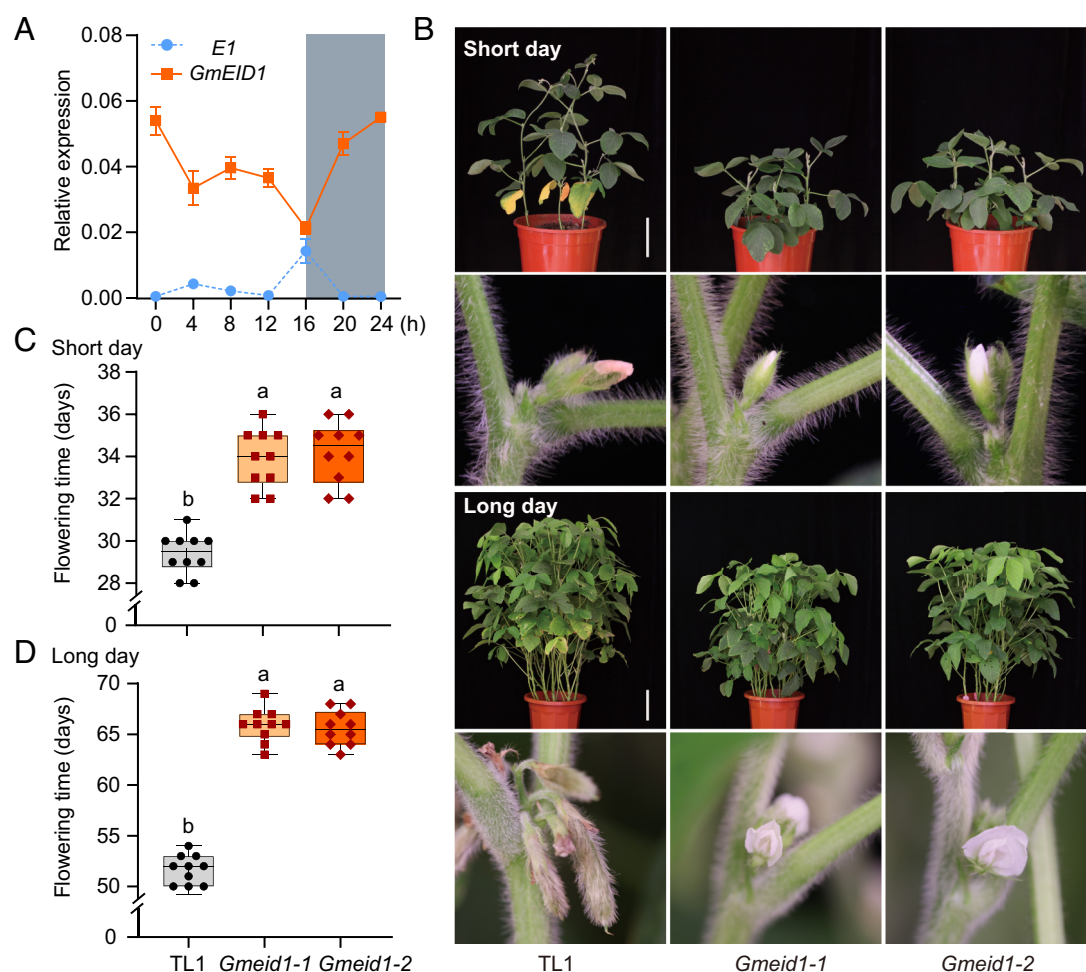


Fig. 1. *GmEID1* is a flowering enhancer. (A) Dynamic mRNA levels of *E1* and *GmEID1* in wild-type TL1 plants grown under LD conditions (16 h light/8 h dark). New fully expanded unifoliate leaves were collected for qRT-PCR analysis. Data are mean \pm SD of three biological replicates. The relative expression level of each indicated gene was calculated using the *GmActin* gene as an internal control. (B) Photos of wild-type TL1 and *Gmeid1* mutant lines grown under SD conditions (12 h light/12 h dark, upper two panels) or LD conditions (lower two panels) in phytotrons. (Scale bar, 10 cm.) (C and D) Flowering time of indicated lines grown under SD conditions (C) or LD conditions (D) as in B. Mean values \pm SD ($n > 8$) are shown. The lowercase letters above the dots indicate significant differences ($P < 0.01$, ANOVA with Tukey's post-test).

and *35S::GmEID1-YFP* overexpression constructs and obtained multiple transgenic lines in the TL1 background (SI Appendix, Fig. S5 C–E). Phenotypic analysis demonstrated that knockout or overexpression of the *GmEID1* gene resulted in significantly later or early flowering phenotype, respectively, under both LD and SD conditions (Fig. 1 B–D and SI Appendix, Fig. S6 A–F). The above results demonstrate that the *GmEID1* gene functions as a photoperiod-independent flowering regulator in soybean.

GmEID1 Inhibits E1 Transcription. To gain insight into how GmEID1 accelerates flowering, we compared the diurnal transcript levels of key flowering genes in wild-type TL1 and *Gmeid1* mutants grown under LD or SD conditions. The qRT-PCR results demonstrated that the mRNA levels of *GmFT2a* and *GmFT5a* were much lower in the *Gmeid1* mutants (Fig. 2 A–D) but increased in the *GmEID1* overexpression lines compared to TL1 (SI Appendix, Fig. S7 A and B). Consistently, the *E1* mRNA level was significantly up-regulated in the *Gmeid1* mutants (Fig. 2 E and F) but down-regulated in the *GmEID1* overexpression lines (SI Appendix, Fig. S7 C). Interestingly, the expression levels of *J*, *GmCCA1a*, and *GmPRR3b*, which are circadian clock component genes upstream of *E1* (19), did not show significant changes in the *Gmeid1* mutants (SI Appendix, Fig. S8 A and B) and *GmEID1* overexpression lines at most of the time points tested under diurnal conditions (SI Appendix, Fig. S7 D–F). Taken together, the above results suggest that the *GmEID1* gene promotes flowering through inhibiting *E1* expression in soybean.

GmEID1 Interacts with J to Promote Flowering. Given that GmEID1 and J behave similarly in the aspects of repressing *E1* expression and promoting flowering, together with the fact that *J* transcription level does not change significantly in both *Gmeid1* mutants and *GmEID1* overexpression lines (SI Appendix, Figs. S7 D–F and S8 A and B), we hypothesized that GmEID1 may function by physically interacting with J. Consistent with this hypothesis, the

β -galactosidase activity assay in yeast demonstrated that GmEID1 interacted not only with J/GmELF3a (Fig. 3A), but also with the other two ELF3 coorthologs, GmELF3b-1 and GmELF3b-2 (12). Furthermore, other potential EC components including GmELF4a and GmELF4b, but not GmLUX1 and GmLUX2, were also shown detectable interaction with GmEID1 (Fig. 3A). Physical associations between GmEID1 and GmELF3s were further confirmed by coimmunoprecipitation (Co-IP) assays and dual-luciferase assays in tobacco leaves (Fig. 3B and SI Appendix, Fig. S9). Taken together, these results suggested that GmEID1 may affect the activity of the EC by interacting with GmELF3s and GmELF4s to modulate flowering time.

GmEID1 Enhances the Abundance of J Protein. Considering that GmEID1 is an F-box protein that is supposed to function as an E3 ligase destabilizing target proteins through the ubiquitin pathway, we tested whether GmEID1 affects the stability of J protein through the root-induced callus expression (RICE) system in soybean (19, 34, 35). Briefly, the *35S::J-3xFlag* construct was transformed into hairy root which was consequently induced into uniform callus by tissue culture. Multiple independent transgenic callus lines in the wild-type W82 background or in the *Gmeid1-4* mutant background were used to compare the abundance of J protein. Interestingly, the western blot results showed that the levels of the J-Flag protein were prone to be higher in the W82 background than those in the *Gmeid1-4* mutant background (SI Appendix, Fig. S10A), suggesting that GmEID1 is positively correlated with the abundance of J protein. In particular, the abundance of the J-Flag protein was positively correlated with its transgenic mRNA level in the W82 background, but not in the *Gmeid1-4* mutant background (SI Appendix, Fig. S10B). Consistent with this, the overall expression levels of *E1* were higher in the *Gmeid1-4* mutant background than those in the wild-type W82 background (SI Appendix, Fig. S10C). Next, we tested how GmEID1 affects J protein abundance in a time-course manner

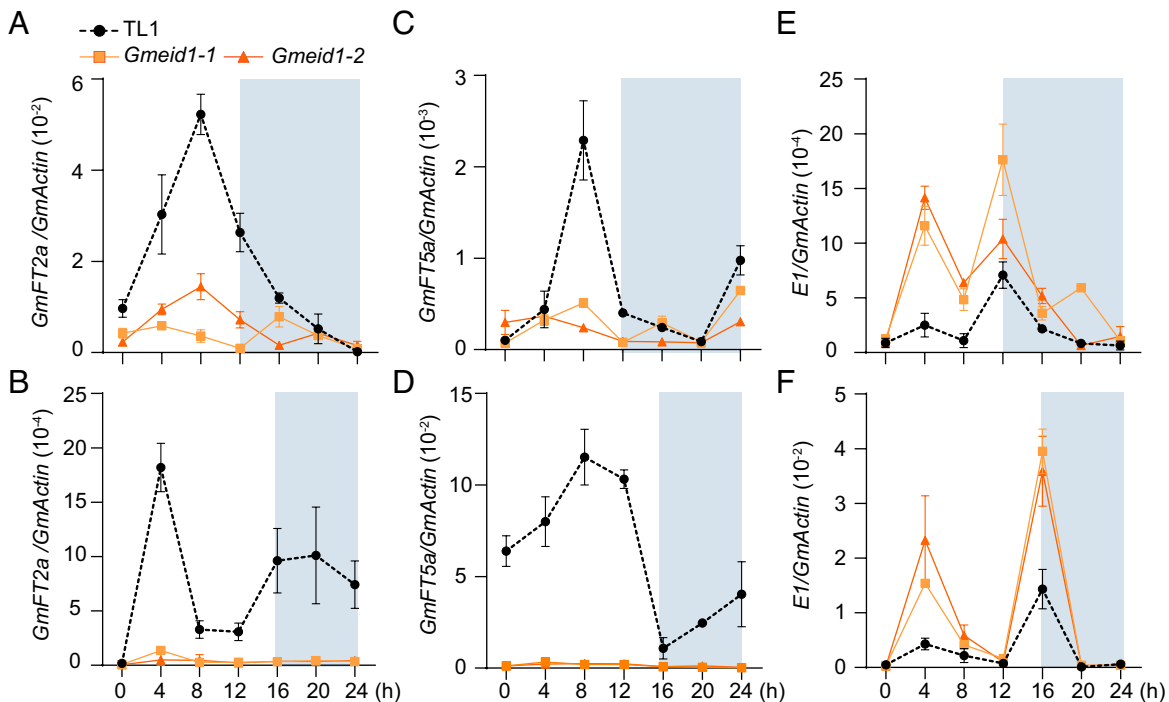


Fig. 2. Temporal expressions of flowering time-associated genes in the indicated lines. (A–F) Diurnal variation in the transcript levels of *GmFT2a*, *GmFT5a*, and *E1* in the wild-type TL1 and *Gmeid1* mutants under SD conditions (A, C, and E, respectively) and LD conditions (B, D, and F, respectively). Second trifoliate leaves of 20-d-old plants were collected for qRT-PCR analysis. Mean values \pm SD (n = 3) are shown. *GmActin* was used as an internal control.

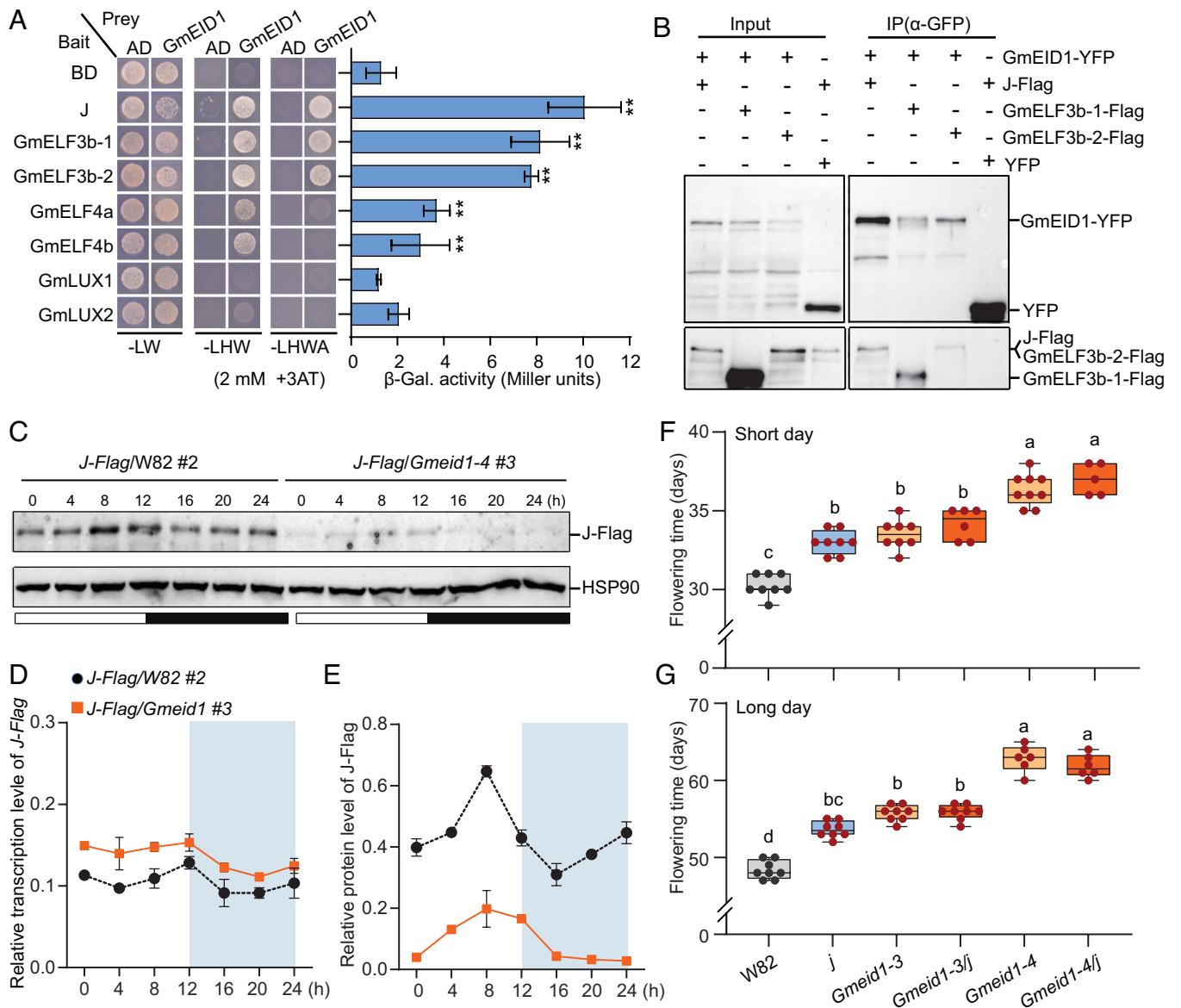


Fig. 3. GmEID1 interacts with EC and influences the abundance of J protein to regulate flowering time. (A) The interaction between GmEID1 and each EC component (J, GmELF3b-1, GmELF3b-2, GmELF4a, GmELF4b, GmLUX1, or GmLUX2) in yeast. For auxotrophic analysis, yeast cells transformed with the indicated genes were selected on -LW (lacking Leu and Trp), -LWH (lacking Leu, Trp, and His), and -LHWA (lacking Leu, Trp, His, and Ade) media. β -galactosidase assays show the interaction strength of GmEID1 with each EC component. Data are means \pm SD ($n = 3$) with significant differences determined by two-tailed Student's t test (* $P < 0.05$, ** $P < 0.01$). (B) Coimmunoprecipitation (Co-IP) showing the interaction of GmEID1 with J, GmELF3b-1, or GmELF3b-2 in tobacco leaves. The YFP protein was used as a negative control. (C) Immunoblot showing the fluctuation of J-Flag protein levels in the wild-type W82 background or the *Gmeid1-4* mutant background under diurnal conditions. The membrane was probed by the anti-Flag antibody, stripped, and then probed by the anti-HSP90 antibody. (D and E) Quantitative assays of *J-Flag* transcript levels relative to *GmActin* (D) and J-Flag protein levels relative to HSP90 (E) in samples as in C. (F and G) Flowering time of the indicated lines grown under SD conditions (F) and LD conditions (G). Data are means \pm SD ($n \geq 5$). The lowercase letters above the dots indicate significant differences ($P < 0.01$, ANOVA with Tukey's post-test).

under diurnal conditions. We selected two representative callus lines expressing similar levels of transgenic *J-Flag* mRNA in the wild-type W82 background (*J-Flag/W82* #2) and in the *Gmeid1* mutant background (*J-Flag/Gmeid1* #3) (Fig. 3 C and D). The immunoblot results demonstrated that the levels of the J-Flag protein were constitutively lower in the *Gmeid1* mutant than those in the wild-type W82 (Fig. 3 C and E). To exclude the influence of different genetic backgrounds, we tested the abundance of J protein in the callus of the *Gmeid1-1* mutant in the TL1 cultivar background, and the result further supported that GmEID1 enhances the accumulation of J protein (SI Appendix, Fig. S11 A and B). Consistent with the above observations, *E1* transcriptional levels were significantly higher in the *Gmeid1* mutant callus than those in wild-type callus (SI Appendix, Fig. S11C). Meanwhile,

E1 protein levels increased significantly in the *Gmeid1* mutant compared to wild type, especially after dusk (SI Appendix, Fig. S11 D and E).

To test the genetic relationship between *GmEID1* and *J*, we used the *Gmeid1-3* and *Gmeid1-4* mutants to cross with the *j* mutant, which are all in the W82 background (SI Appendix, Fig. S12 A and B). Molecular analysis showed that *Gmeid1-4* is likely a null mutant due to a frameshift mutation, while *Gmeid1-3* is possibly a weak mutant expressing an incomplete GmEID1 protein missing 17 amino acids (SI Appendix, Fig. S5 A and B). Consistently, the *Gmeid1-4* mutant showed a more severely late flowering phenotype than that of the *Gmeid1-3* mutant (SI Appendix, Fig. S6 E and F). The flowering time of the *j* mutant was similar to that of the *Gmeid1-3* mutant, but earlier than that of the *Gmeid1-4* mutant

under both SD and LD conditions (Fig. 3 *F* and *G*), suggesting that GmEID1 stabilizes not only J but also other J-like proteins, including GmELF3b-1 and GmELF3b-2 (Fig. 3 *A* and *B* and *SI Appendix, Fig. S9*). Furthermore, the flowering time of *Gmeid1-3lj* and *Gmeid1-4lj* was similar to that of *Gmeid1-3* and *Gmeid1-4*, respectively (Fig. 3 *F* and *G*), supporting that *GmEID1* and *J* function in a same genetic pathway.

Light-Dependent Interaction between E3/E4 and GmEID1 Interferes with the GmEID1-J Interaction. It has been documented that J mediates the regulation of *E1* expression through a GmPHYA (E3 and E4) signaling pathway (12). Moreover, we were unable to detect the direct interaction between E3/4 and J (*SI Appendix, Fig. S13*) (36). In this context, we tested whether E3 and E4 may interact directly with GmEID1. Both the β -galactosidase activity assay and the auxotrophic assay showed that the light receptors E3 and E4 were able to interact with GmEID1 in a red or far-red light-dependent manner in yeast cells (Fig. 4*A* and *SI Appendix, Fig. S14A*). The E3/E4-GmEID1 interactions were further confirmed by dual-luciferase and Co-IP assays by transient expression in tobacco leaves (*SI Appendix, Fig. S14 B and C*). Next, we crossed the CRISPR-Cas9-engineered *e3* mutant

which has an early flowering phenotype (37), with the *Gmeid1-1* mutant which has a later flowering phenotype, to obtain the *Gmeid1/e3* double mutant (*SI Appendix, Fig. S15A*). Phenotypic analysis showed that the *Gmeid1/e3* mutant flowered as late as the *Gmeid1* mutant under natural LD conditions (Fig. 4*B*), demonstrating that the *Gmeid1* mutant could completely suppress the early flowering phenotype of the *e3* mutant. Consistent with this, the expression levels of *E1*, *GmFT2a*, and *GmFT5a* in the *Gmeid1/e3* mutant were similar to that in the *Gmeid1-1* mutant (*SI Appendix, Fig. S15B*), confirming that the *GmEID1* gene is epistatic to the *E3* gene in terms of genetic relationship.

Next, we investigated whether E3 may affect the interaction between GmEID1 and J by Co-IP (Fig. 4*C* and *SI Appendix, Fig. S16A*). Briefly, the GmEID1-YFP and J-Flag proteins were coexpressed with or without the E3-Flag protein in tobacco leaves. The GmEID1-J interaction was compared in the presence or absence of E3-Flag in response to white light (WL) or far-red light treatment. We found that the J-Flag was equally coprecipitated by GmEID1-YFP in the absence of E3-Flag regardless of light or dark treatment. However, in the presence of E3-Flag, a much less amount of J-Flag was coprecipitated by GmEID1-YFP upon WL

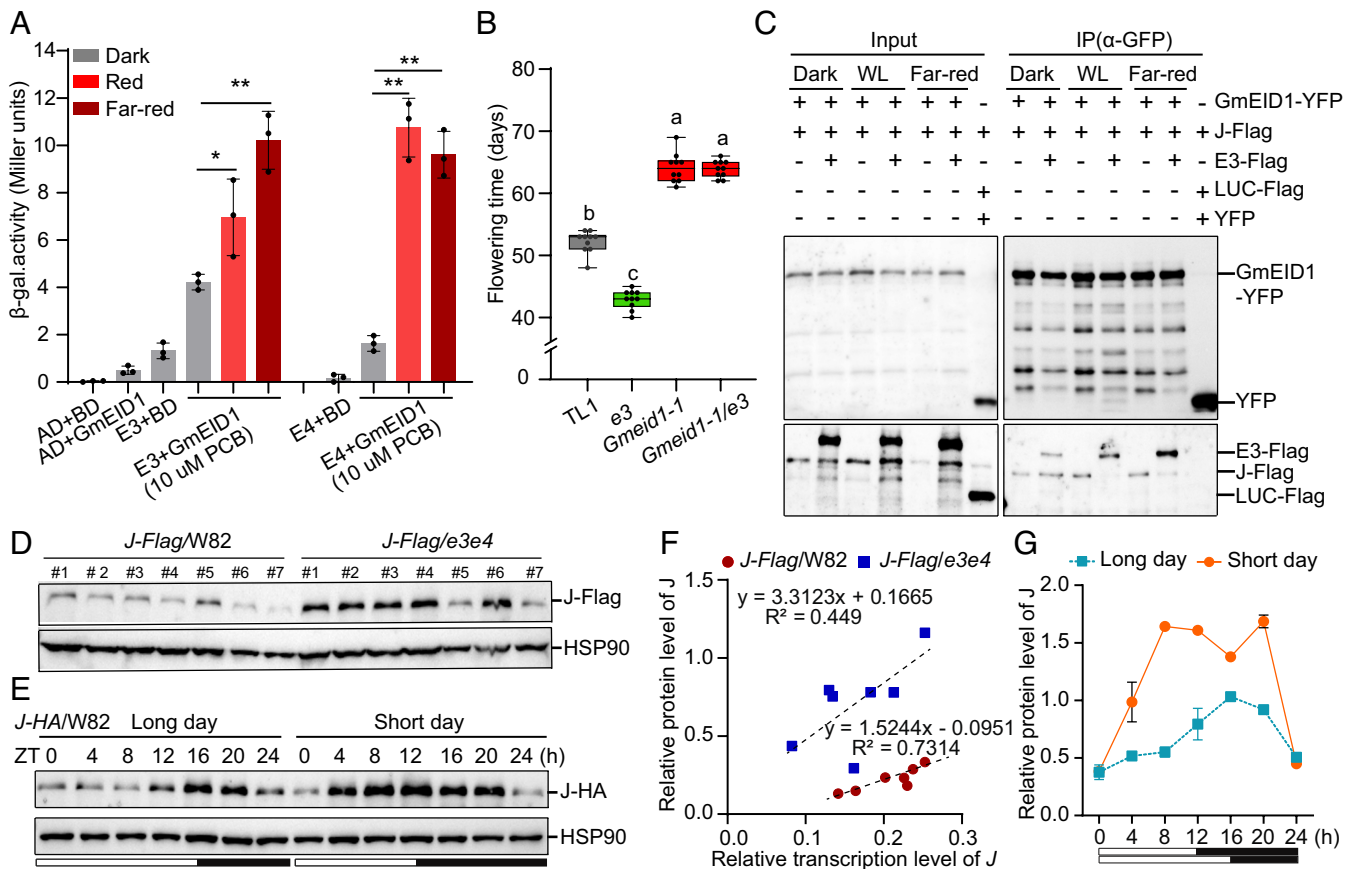


Fig. 4. Photoactivated E3/E4 interacts with GmEID1 to inhibit the GmEID1-J interaction and promotes the degradation of J protein. (A) β -galactosidase assays to compare the interacting strength between GmEID1 and E3/E4 in yeast cells treated with red light ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$), far-red light ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$), or darkness. AD, activation domain; BD, binding domain. PCB, phytylcyanobillin, which is the natural precursor of the phytochrome chromophore. Data are means \pm SD ($n = 3$) with significant differences determined by two-tailed Student's *t* test (* $P < 0.05$, ** $P < 0.01$). (B) Flowering time of indicated lines. Data are means \pm SD ($n = 10$). The lowercase letters above the dots indicate significant differences ($P < 0.01$, ANOVA with Tukey's post-test). (C) Co-IP assay showing the light-dependent attenuation of the GmEID1-J interaction by E3 in tobacco leaves. The *35S::J-Flag35S::YFP-GmEID135S::E3-Flag*, *35S::YFP*, and *35S::LUC-Flag* constructs were combined into tobacco leaves as indicated and incubated at 25°C for 12 h in the dark and then transferred to white light (WL, $80 \mu\text{mol m}^{-2} \text{s}^{-1}$), far-red light ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$) or kept in the dark for 36 h, then the leaves were collected for the Co-IP experiment. (D) Immunoblot to compare the J-Flag protein levels in the indicated root hair callus lines cultured under LD conditions. The *35S::J-Flag* construct was transformed into the hair root of *e3e4* mutant in W82 background (*J-Flag/e3e4*). Multiple transgenic callus lines were harvested at ZT0 for immunoblot using anti-Flag antibody. HSP90 was used as a loading control. (E) Comparison of transgenic J-HA protein levels under LD and SD conditions by immunoblot using HA antibody. The first trifoliate leaves of *J-HA* overexpression line in W82 background were collected at the indicated time points. (F) Scatter plot to compare the correlation between transcript levels and protein levels of transgenic J-Flag in the *e3e4* mutant and wild-type W82 as in D. (G) Quantitative assay of J-HA protein levels relative to HSP90 in samples as in E.

or far-red light treatment compared to that of dark-adapted control (Fig. 4C and *SI Appendix*, Fig. S16A). This mechanism was further confirmed by yeast three-hybrid assay and dual-luciferase assays in plant cells (*SI Appendix*, Fig. S16 B–D). For yeast three-hybrid assay, the interaction between the bait (BD-GmEID1) and the prey (AD-J) was tested in the absence or presence of the third protein bait mate (BM-E3) in response to far-red light treatment. As expected, GmEID1–J interaction was not affected by far-red light in the absence of PCB or E3. However, the extent of GmEID1–J interaction was significantly reduced in the presence of both E3 and PCB when yeast cells were exposed to far-red light (*SI Appendix*, Fig. S16B), demonstrating that E3 could inhibit the GmEID1–J interaction in a far-red light-dependent manner. The above results demonstrate that photoactivated E3/E4 can disrupt the GmEID1–J interaction and suggest that E3/E4 may promote the degradation of J protein.

To test this possibility, we compared the abundance of J-Flag protein in the presence or absence of E3/E4 by RICE system. The western blot results showed that the levels of the J-Flag protein were prone to be higher in the *e3* or *e3e4* mutant background than those in the wild-type TL1 or W82 background, respectively (Fig. 4D and *SI Appendix*, Figs. S17A and S18). Correlation analysis between *J-Flag* mRNA and J-Flag protein levels confirmed that J-Flag proteins were more efficiently accumulated in the *e3e4* or *e3* mutant than those in the wild-type W82 or TL1, respectively (Fig. 4F and *SI Appendix*, Fig. S17B). Given that E3 and E4 mediate photoperiod signals to regulate flowering time, we tested if daylength affects the abundance of J protein using a stable transgenic soybean line overexpressing J-HA protein. The immunoblot results demonstrated that the J-HA protein levels were higher under SD conditions than those under LD conditions (Fig. 4E and G). Intriguingly, the J-HA protein levels gradually increased during the light period, which is likely associated with the gradual decline of *E3/E4* transcripts and proteins during the day (Fig. 4G and *SI Appendix*, Fig. S17 C–F). Taken together, it is conceivable that photoactivated E3 and E4 act as a competitive inhibitor of the GmEID1–J interaction and consequently promote *E1* expression to inhibit flowering in soybean (*SI Appendix*, Fig. S19).

Deactivation of GmEID1 Improved Adaptability and Yield Performance. Given that the natural variations of the *J* gene have been successfully utilized for soybean breeding (11, 12, 38) together with the fact that GmEID1 affects the abundance of J protein, we tested the performance of *Gmeid1* mutants and *GmEID1-OX* lines in field trials in Beijing for two consecutive years in 2020 and 2021. Attractively, besides the later flowering phenotype, the *Gmeid1* mutants exhibited multiple preferential agronomic traits, including more branch number, thicker main stem with more nodes, shorter internode length, and greater biomass and yield per plant compared to those of wild-type TL1 (Fig. 5A and *SI Appendix*, Fig. S20 A–E). Consistently, overexpression of *GmEID1* resulted in opposite phenotypic changes including less branch and node number and lower biomass and yield per plant compared to the absence of *GmEID1* (*SI Appendix*, Fig. S20).

Next, we tested whether target mutations in *GmEID1* could improve soybean performance at different latitudes by field trials in Changchun (44°N), Beijing (39°N), Xuchang (34°N), and Sanya (18°N). As expected, the *Gmeid1* mutants displayed consistent beneficial traits, including delayed flowering time and increased number of nodes, branches, and pods per plant at all planting locations (Fig. 5B and *SI Appendix*, Fig. S21 A–D). Consequently, the yield per plant of the mutant lines increased by at least 15.0% compared to that of wild-type TL1. To be noted, the yield per plant

of *Gmeid1* increased by 55.3% in Xuchang, which is the origin and main planting area (Huang-Huaihai region of China) for the TL1 cultivar, and by 20.6% in Sanya under typical SD conditions of tropic region (Fig. 5C). The yield per plant of TL1 increased in Changchun and Beijing relative to its main planting area, likely due to increased vegetative growth under LD conditions. The above results demonstrated that targeted mutagenesis of the *GmEID1* gene can improve the adaptability of soybean by enhancing yield potential at different latitudinal regions.

Discussion

In summary, we identified a unique flowering-time regulator GmEID1 that could conditionally interact with either J or E3/E4 in soybean in response to light variations. The GmEID1–J interaction enhances the accumulation of the J protein, which directly inhibits the *E1* transcript to accelerate flowering. The light-dependent GmEID1–E3/E4 interaction interferes with the GmEID1–J interaction to reduce J protein level and modulate flowering time. Although the regulatory mechanism of how GmEID1-stabilizing J protein remains to be studied in the future, our findings bridge the gap between light signal perception by E3 and E4 to flowering time regulation in soybean. Importantly, the CRISPR/Cas9-engineered *Gmeid1* mutants generated in this study provide valuable genetic resources and an approach to breed high-yield soybean cultivars characterized with wide adaptability to different latitudes.

Previous investigations have proposed a possible genetic pathway that controls photoperiodic flowering in soybean: Red/far-red light receptors *E3* (*GmPHYA3*) and *E4* (*GmPHYA2*) act upstream of *J*, and *J* directly inhibits *E1* expression to regulate flowering time (12). Consistent with this hypothesis, *E1* transcript levels were significantly reduced by the mutations in *E3* and *E4* (5, 6, 26), but up-regulated in the absence of *J* (12), while the transcript of *J* was not obviously affected by the dysfunction of *E1* (12). However, in contrast to the striking down-regulation of *E1*, the transcript levels of *J* only slightly increased in the absence of *E3* and *E4* (12). Our findings that the photoactivated *E3* and *E4* suppress the accumulation of the J protein by competitive interaction with GmEID1, together with the fact that the J protein tends to accumulate to a higher level under SD conditions than under LD conditions (Fig. 4E and G), at least partially explain how *E3* and *E4* dynamically perceive the photoperiod signals to modulate soybean flowering time. EID1 was first discovered as a negatively acting component in the phyA-dependent light pathway, and the expression of the *eid1* phenotype requires the presence of functional phyA in *Arabidopsis* (32). However, the *Gmeid1/e3* mutant showed the same phenotype as *Gmeid1* (Fig. 4B), indicating a legume-specific function of *GmEID1* in soybean.

Gmeid1 mutants exhibit a range of traits, including delayed flowering time, a thick main stem, short internode length, and increased node number and branch number, under natural field conditions (Fig. 5A and *SI Appendix*, Fig. S20 A–E). These traits are in line with the breeding goal of the “Soybean Green Revolution” (39) and have been shown to increase yield in four different locations compared to that of the wild-type TL1 cultivar (Fig. 5C). The TL1 cultivar is susceptible to lodging under LD conditions, which limits its ability to be grown commercially in northern latitudes. Mutations in *GmEID1* result in a stout main stem and short internodes (*SI Appendix*, Fig. S20 D and E), which can improve the lodging resistant ability and thus expand the adaptive range of TL1 to northern latitudes. On the contrary, short-day conditions lead to early flowering, less vegetative growth, and

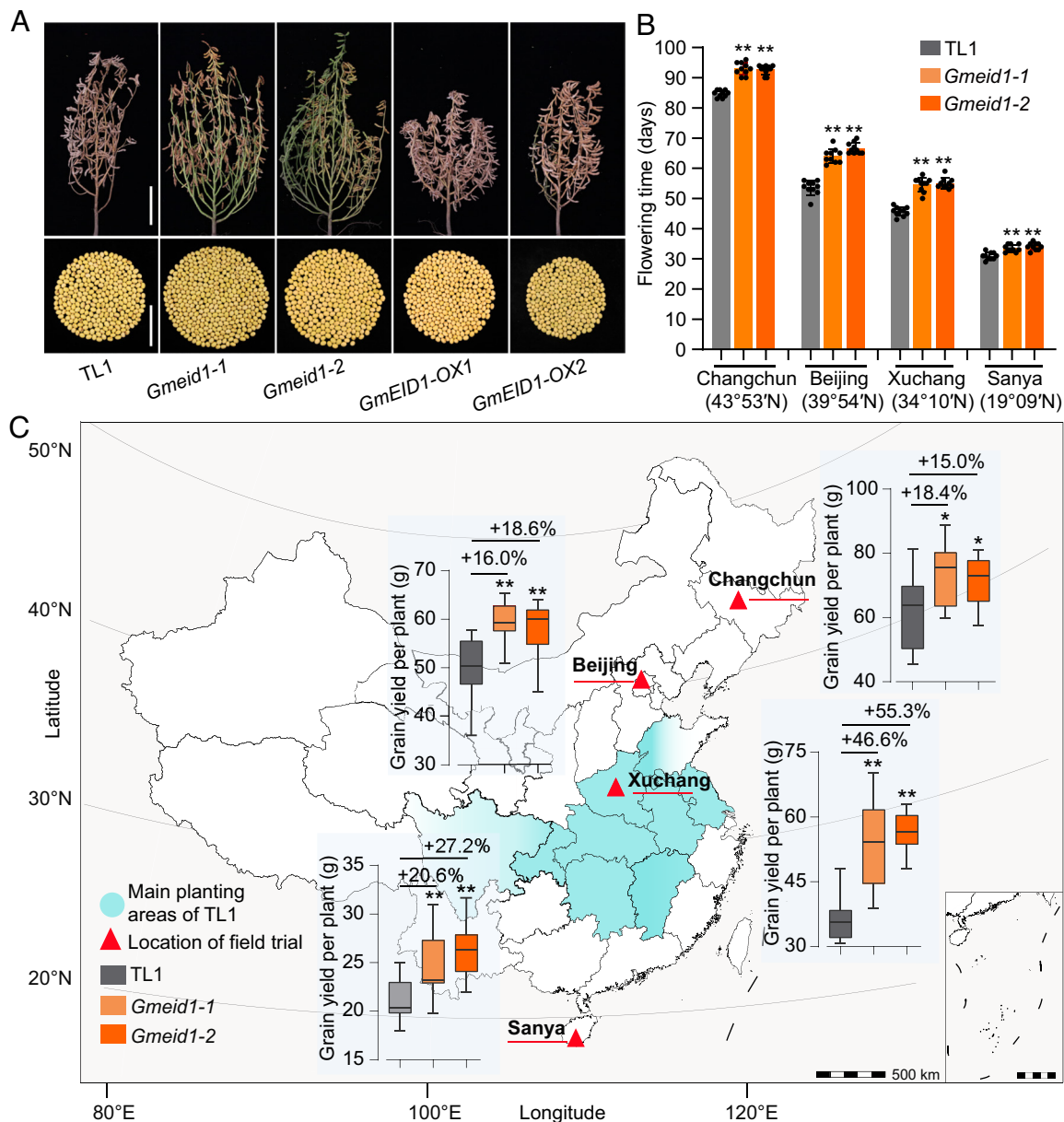


Fig. 5. Disruption of *GmEID1* enhances soybean yield in a broad latitudinal region. (A, Upper) Photographs of wild-type TL1, *Gmeid1* mutants, and *GmEID1* overexpression lines at the R8 stage grown under Beijing field conditions in 2021. (Scale bar, 20 cm.) (Lower) Whole seeds produced by the respective plants in the Upper panel. (Scale bar, 5 cm.) (B) Comparison of flowering time of wild-type TL1 and *Gmeid1* mutants grown in four locations (Changchun, Beijing, Xuchang, and Sanya) in 2021. (C) The geographical distribution of field trials in 2021. The indicated lines were cultivated under a planting density of 67,000 plants/ha in the fields of different latitudes. Wathet blue indicates the main planting areas of the TL1 cultivar. The yield per plant of each line is shown in the bar graph. Data are means \pm SD ($n = 10$) with significant differences determined by two-tailed Student's *t* test (* $P < 0.05$, ** $P < 0.01$).

reduced yield at low latitudes, while dysfunction of *GmEID1* delays flowering time, increases the number of internodes and branches, and enhances yield, thus expanding the adaptability of TL1 to southern regions.

To be noted, the field trials in this study were conducted at a lower planting density (67,000 plants/hectare) than normal. This is due to TL1 being prone to lodging at normal densities (200,000 to 250,000 plants/hectare) at high latitudes (Beijing and Changchun), which often leads to problems with growth uniformity. As such, we investigated the agronomic traits of the *Gmeid1* mutant and TL1 under lower planting densities to avoid any serious morphological differences among individual plants. All tested lines were planted under the same low planting density in different field locations, in order to assess the effect of different latitude/photoperiods on soybean morphology and yield. Indeed, this low planting density

led to a much higher increase in the yield of *Gmeid1* mutant compared to that of TL1 (reaching up to 55.3% in yield per plant in Xuchang). Subsequently, we evaluated the yield performance further under its normal planting density (200,000 plants/hectare) in Xuchang. The results showed that the yield per plant of *Gmeid1* mutant (16.8 g/plant) was around 16% higher than that of TL1 (14.5 g/plant) (*SI Appendix, Fig. S20 E*). According to the plot yield level (up to 2,850 kg/hectare) and planting density (200,000 to 250,000 plants/hectare) of TL1 in the main producing area, about 14 g/plant is estimated for the locally grown TL1, which is in agreement with our result for TL1 under similar planting density in this study.

The *GmEID1* gene has been neither recovered in flowering time Quantitative Trait Locus (QTLs) (33) (<https://www.soybase.org/>) and nor identified in previous screens for flowering-time mutants

in soybean, which is likely due to the existence of few allelic variations associated with flowering time in natural populations. Therefore, saturation mutagenesis at *GmEID1* by target genome editing is worth to explore in future to precisely manipulating flowering time and other agronomic traits in soybean. In conclusion, this study not only outlines a fundamental difference in EID1 function between model plant *Arabidopsis* and soybean, but also provides an important perspective on photoperiodic flowering, latitudinal adaptation, and high yield breeding of soybean which is the key crop for sustainable consumption of plant protein and oil products in the world.

Materials and Methods

Plant Materials, Growth Conditions, and Phenotyping. The soybean [*Glycine max* (L.) Merr.] Tianlong 1 (TL1) or Williams 82 (W82) cultivar was used as wild-type control. The CRISPR-Cas9-engineered mutants and transgenic lines were generated in the TL1 and W82 background. For the analysis of flowering time, the indicated lines were grown under LD conditions (16 h light/8 h dark, 26 °C) or SD conditions (12 h light/12 h dark, 26 °C) in controlled growth chambers. TL1 is a commercial cultivar raised by the Oil Crops Research Institute of Chinese Academy of Agricultural Sciences in 2008 (approval number: State-approved bean 2008023). TL1 is a relatively high-yield variety (up to 2,850 kg/hectare) suitable for spring planting in the Yangtze River Basin in China, which has been used as the control of field trials in the Yangtze River Basin for national soybean variety certification since 2017. For field trials, the indicated lines were grown under natural conditions on the farmlands of Beijing, Changchun, and Xuchang in the summer and Sanya in the winter with a plant spacing of 30 cm and a row spacing of 50 cm in a 3 × 2.5-m plot (50 plants/plot, about 67,000 plants/hectare). To compare with the locally grown TL1 under normal planting density (200,000 to 250,000 plants/hectare) in the main producing area, the seeds of each line were sown with a plant spacing of 10 cm and a row spacing of 50 cm in a 3 × 2.5-m plot (150 plants/plot, about 200,000 plants/hectare). At least ten plants inside the plot were randomly selected for phenotypic analysis. All field experiments were performed in three independent plots. The flowering time was recorded at the R1 stage (days from emergence to the first open flower appeared at any node on the main stem). Other agronomic traits were recorded at harvest.

Yeast Three-Hybrid Experiments. The *pBridge* vector expressing both the bait-BD fusion protein and the bait mate protein and the *pGADT7* vector expressing the prey-AD fusion protein were constructed for yeast three-hybrid assay. The CDS of *GmEID1* was fused with BD to construct the *pBridge-GmEID1* vector. The CDS of *E3* was inserted into the *pBridge-GmEID1* vector to generate the *pBridge-GmEID1-E3* vector. The CDS of *J* was fused with AD to make the *pGADT7-J* vector. The yeast strain AH109 was transformed with the indicated vector combinations (SI Appendix, Fig. S16B). The individual colony was selected and cultured in a 10-mL centrifuge tube containing 4 mL SD medium (–Leu/–Met/–Trp/+Asp) at 28 °C, 180 rpm in the dark until OD₆₀₀ = 0.1. An aliquot of 2 mL yeast culture was divided into 8 mL YPDA culture solution with or without 10 mM PCB in a 50-mL centrifuge tube and cultured at 25 °C, 180 rpm under far-red

light (30 μmol m⁻² s⁻¹) or dark conditions until OD₆₀₀ = 0.5 to 0.8 prior to the β-galactosidase assay.

Coimmunoprecipitation Assays (Co-IP). After infiltration with the indicated constructs, the tobacco plants were incubated in the dark at 25 °C for 12 h, and then transferred to light growth conditions for an additional 36 h. Samples were harvested and ground in lysis buffer (1 mM MgCl₂, 10 mM EDTA [pH 8.0], 1 mM PMSF, 5 mM DTT, Roche protease inhibitor cocktail). The extracts were centrifuged at 14,000 rpm at 4 °C for 30 min. The supernatant was incubated with 20 μL anti-GFP Trap Agarose (Chromotek, catalog number gta-20) at 4 °C for 2 h and then washed 3 times with lysis buffer. The samples were boiled in SDS-PAGE sample buffer, and the supernatant was analyzed by immunoblot probed with anti-GFP or anti-Flag antibody. To test the effect of E3 on the GmEID1–J interaction, 35S::J-Flag, 35S::GmEID1-YFP, and 35S::E3-Flag constructs were cotransferred into tobacco leaves and incubated at 25 °C for 12 h in the dark and then transferred to white light (80 μmol m⁻² s⁻¹), far-red light (30 μmol m⁻² s⁻¹) or kept in the dark for an additional 36 h prior to Co-IP analysis.

Details of RNA sequencing and data analysis, gene expression analysis, plasmid construct, plant transformation, subcellular localization in protoplasts, yeast two-hybrid experiments, dual-luciferase assay, in-vitro pull-down assay, RICE system to investigate J protein levels, immunoblot assay, statistical analysis, and primers and accession numbers are provided in SI Appendix, Materials and Methods.

Data, Materials, and Software Availability. All study data are included in the article and/or SI Appendix. Gene sequences were obtained from the Phytozome database (<https://phytozome-next.jgi.doe.gov/>) by selecting the reference genome *Glycine max* Wm82.a2.v1 with accession number ACUP01000000 (40). The genes used in this study and their respective identifiers are as follows: GmEID1 (Glyma.03G214300), E3 (Glyma.19G224200), E4 (Glyma.20G090000), J (Glyma.04G050200), GmELF3b-1 (Glyma.14G091900), GmELF3b-2 (Glyma.17G231600), GmELF4a (Glyma.11G229700), GmELF4b (Glyma.18G027500), GmLUX1 (Glyma.12G060200), GmLUX2 (Glyma.11G136600), E1 (Glyma.06G207800), GmFT2a (Glyma.16G150700), GmFT5a (Glyma.16G044100), GmCCA1a (Glyma.07G048500), GmPRR3b (Glyma.12G073900), and GmActin (Glyma.18G290800).

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