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# Soybean LEAFY COTYLEDON 1: A Key Target for Genetic Enhancement of Oil Biosynthesis

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**Abstract:** Soybean is an important oilseed crop that is used as a feed for livestock and has several industrial uses. Lipid biosynthesis and accumulation primarily occur during seed development in plants. This process is regulated by several transcription factors and interconnected biochemical pathways. This study investigated the role of glycine max LEAFY COTYLEDON 1 (*GmLEC1*) in soybean seed development and the accumulation of storage reserves. The overexpression of GmLEC1 significantly increased the amount of triacylglycerol (TAG) in transgenic Arabidopsis seeds compared to the wild-type and an *atlec1* mutant. Similarly, the high expression of GmLEC1 led to a 12% increase in TAG content in transgenic soybean hairy roots compared to the control. GmLEC1 also altered the fatty acid composition in transgenic Arabidopsis seeds and soybean hairy roots. Additionally, the overexpression of GmLEC1 resulted in a reduction in starch accumulation in seeds and vegetative tissues, as well as changes in cotyledon and seed morphology. The cotyledons of the *atlec1* mutant displayed abnormal trichome development, and the seeds were smaller and less tolerant to desiccation. A complementation assay in Arabidopsis restored normal cotyledon phenotype and seed size. The main downstream targets of *LEC1* are *GL2* and *WRI1*, which were found to participate in fatty acid biosynthesis and trichome formation through the regulation of phytohormones and various transcription factors involved in seed development and maturation. The findings of this study suggest that *GmLEC1* controls seed development and regulates the accumulation of seed storage compounds. Furthermore, these results demonstrate that *GmLEC1* could be a reliable target for the genetic improvement of oil biosynthesis in soybean.

**Keywords:** soybean; LEAFY COTYLEDON 1; seed development; seed oil content; TAG composition



**Citation:** Manan, S.; Alabbosh, K.F.; Al-Andal, A.; Ahmad, W.; Khan, K.A.; Zhao, J. Soybean LEAFY COTYLEDON 1: A Key Target for Genetic Enhancement of Oil Biosynthesis. *Agronomy* **2023**, *13*, 2810. https://doi.org/10.3390/agronomy13112810

Academic Editor: Monica Boscaiu

Received: 15 September 2023
Revised: 6 October 2023
Accepted: 9 October 2023
Published: 13 November 2023



## 1. Introduction

The transcription factor LEAFY COTYLEDON 1 (LEC1), which belongs to the nuclear factor (NF-Y) family and encodes the HAP3 subunit of the CCAAT binding transcription factor, is a crucial regulator of embryogenesis and post-embryonic developments [1]. LEC1 and its closely related sequence *AtLEC1-LIKE* (*L1L*) are primarily expressed in developing embryos and seedlings but not in Arabidopsis leaves [2]. They interact with other transcription factors, such as BASIC LEUCINE ZIPPERS (bZIPs), LEAFY COTYLEDON (LEC2), and ABSCISIC ACID INSENSITIVE 3 (ABI3), and can also modify chromatin accessibility

and facilitate active histone modifications. LEC1 plays a key role in the long-term reprogramming of the embryonic chromatin state and the transition from embryonic state to a flowering plant [3]. It globally regulates trichome development and the accumulation of seed storage compounds [4]. Its main targets include genes controlling essential metabolic pathways like glycolysis, as well as chain elongation, condensation, and desaturation of glycerolipid biosynthesis [5]. Loss-of-function mutations in *atlec1* lead to the premature development of vascular tissues, desiccation-intolerant embryos, underdeveloped mesophyll tissues, trichome-containing cotyledons, and defects in the accumulation of seed storage compounds [6]. Conversely, the overexpression of LEC1 prolongs the embryonic state and results in unexpanded cotyledons after germination. In Arabidopsis, LEC1 induces embryonic characteristics in true leaves, which lack trichomes and have a high content of stored lipids in overexpressed seeds [1]. Experiments have shown that seed-specific over-expression of maize *LEC1* (*ZmLEC1*) increased seed oil content by 48% but negatively impacted seed germination and plant growth in field conditions [7]. Similarly, a 2–3 fold increment in the seed-specific mRNA levels of *BnLEC1* or *BnL1L* improved seed oil quantity up to 20% in field trials without disturbing crop yield and nutritional quality [8]. Previous studies have reported the interaction between LEC1 and LEC2 in coordinating lipid metabolism and seedling growth [9]. GmLEC2 has been identified as a key regulator of seed storage substances biosynthesis soybean plant development [3]. It targets genes involved in lipid and protein biosynthesis pathways, which control seed development, maturation, and seedling growth. In cassava, expression analysis has shown that MeLEC1 and MeLEC2 are significantly more active in somatic embryogenic tissues compared to differentiated mature tissues. The upregulation of *MeLEC* genes during the early stages of somatic embryogenesis suggests their role in the transformation from a somatic to an embryonic state and the acquisition of competence for somatic embryogenesis development [10]. Additionally, LEC1 has been found to directly activate WRINKLED 1 (WRI1), which specifically targets BIOTIN CARBOXYL CARRIER PROTEIN 2 (BCCP2) and provides necessary components for lipid biosynthesis [11]. LEC1 is also involved in the regulation of ABI3, another important component in the complex pathways of seed filling and maturation [12]. These findings support the multifunctional role of LEC1 during plant development, particularly in seed development.

Soybean is a highly valuable oilseed crop that is cultivated worldwide for its exceptional contributions to protein production and high-quality oils derived from its seeds. The significance of soybean oil lies in its widespread use as a cooking oil globally, thanks to its exceptional nutritional value and low levels of saturated fat. Additionally, the proteins extracted from soybeans are in high demand due to their well-balanced amino acid profile. These proteins are essential in various products, including sports nutritional supplements, infant formulas, and meat alternatives. Because of these multifaceted benefits, soybean is a valuable crop with immense potential across multiple industries [13]. However, despite its importance, the molecular basis of seed storage compound synthesis and accumulation in soybean is not fully understood.

The current study was designed to investigate the role of soybean LEC1 (GmLEC1) in the development of plants and seeds, with a particular emphasis on lipid accumulation in mature seeds. GmLEC1 was overexpressed in Arabidopsis seeds and soybean hairy roots to analyze their fatty acid composition. The impact of LEC1 overexpression and mutation on embryonic development and seed content was investigated. The results revealed that LEC1 plays a crucial role in embryonic development and controls lipid biosynthesis and storage in seeds. These findings provide new insights into the role of LEC1 in soybean and can help scientists to develop customized crops with enhanced nutritional values. Furthermore, this study highlights the importance of LEC1 in regulating seed development and oil biosynthesis.

## 2. Materials and Methods

### *2.1. Gene Identification and Cloning*

The search for *GmLEC1* genes was carried out by analyzing nucleotide sequences obtained using Arabidopsis LEAFY COTYLEDON1 (AtLEC1) as a query in a BLASTN analysis. The Soybean Genome Database (www.phyozome.net accessed on 14 September 2023) was consulted to compare the acquired sequences to homologous sequences in the genome and to analyze their similarity and coverage. We collected homologous sequences of characterized *LEC1* genes from different plant species (Supplementary Data S1, Accession number) for additional analysis. Phylogenetic analysis was performed by aligning the deduced amino acids of LEC1 protein regions across species and importing the alignment into Mega6. The tree was generated by applying the neighbor-joining method, and pairwise distance was estimated using a p-distance model. Any missing data, alignment gaps, or ambiguous bases were acceptable at any position.

The open reading frame of GmLEC1 (Glyma.07G268100.1) (Supplementary Figure S2) was amplified with the cDNA synthesized from soybean developing seeds using forward and reverse primers (Supplementary Table S1). After amplification, cDNA was cloned into the vector pB2GW7 by GATEWAY cloning and sequenced for further experiments.

### *2.2. Real-Time PCR Analysis*

Total RNA was isolated from different soybean tissues and Arabidopsis leaves following the instructions provided with the RNA isolation kit (Biotech, Beijing, China). The qRT-PCR was performed according to the details provided by Manan et al. [3]. Soybean seed was classified into six stages according to their weight: S1: Stage 1 (30–60 mg), S2: Stage 2 (70–90 mg), S3: Stage 3 (100–150 mg), S4: Stage 4 (200–250 mg), S5: Stage 5 (250–350 mg), and S6: Stage 6 (350–450 mg).

### *2.3. Production of Transgenic Lines*

The GmLEC1 gene was inserted into a binary vector and then introduced to *Agrobacterium tumefactions* GV3101 through electro-transformation. Both mutant (*lec1-1*) and wild-type (Columbia-0) *Arabidopsis thaliana* plants were grown in regulated growth chambers with a photoperiod of 16/8 h of light and dark at 22 °C. They were transformed using the floral dip approach. Transgenic lines resistant to BASTA were confirmed and examined for their TAG contents and fatty acid composition. Additionally, the binary vector cassette was transformed into *Agrobacterium rhizogenes* K599 for hairy root transformation. The transformed bacteria were used to infect soybean cotyledons, resulting in the growth of hairy roots, which were selected on a medium containing phosphinothricin tripeptide, as previously described [3]. The selected hairy roots were then analyzed for gene expression and used for oil extraction.

### *2.4. Lipid Extraction and Fatty Acid Analysis*

The fatty acid levels and composition in TAGs, obtained from either seed-derived sources or purified through TLC after a comprehensive lipid profile analysis of root hairs, were assessed using an Agilent 7890A (Agilent Technologies, Santa Clara, CA, USA) gas chromatography system equipped with a flame ionization detector, following the previously provided parameters [14]. The oil quantity was determined by evaluating the flame ionization detector response of the different components in the sample relative to the internal standard, 17:0 methyl ester from the triheptadecanoin.

### *2.5. Starch Quantification*

The 4-week-old Arabidopsis leaves were stained with iodine, following a previously reported protocol, with some modifications [15]. The leaves were boiled in methanol at 85 °C for 5 min to extract chlorophyll. They were then incubated in an iodine solution for 10 min and photographed using a digital camera to show iodine-stained starch.

To determine the amount of starch in the seeds, we followed the method outlined by Séne et al. [12]. Briefly, a 0.05 g seed sample was agitated twice in 2 mL of 70% acetone to remove lipids and then centrifuged at 1600 g for 15 min. The resulting dry pellet was dissolved in 1 mL $H_2O$ and solubilized in 4 mL NaOH while continuously stirring at room temperature for an hour. We neutralized the mixture with 0.25 mL of HCl and added iodine solution (4 g potassium iodide + 1.27 g iodine/100 mL $H_2O$) to the tubes. After the color developed, we measured the absorbance at 660 nm using a spectrophotometer (MAPADA, Shanghai, China).

## 3. Results

### *3.1. Gene Identification and Expression in Soybean Plant Tissues*

The LEC1 gene in soybean was identified by using the Arabidopsis LEC1 protein sequence. The GmLEC1 protein sequence was retrieved from the Phytozome after performing BLAST analysis. A phylogenetic tree was constructed using homologues of GmLEC1 from Arabidopsis, maize, brassica, and other species (Figure 1A). To determine which tissue in soybean plants expressed GmLEC1 the most, qRT-PCR was performed on eight different tissues, including pod, seed, flower, leaf, root, shoot apical meristem, stem, and nodule. The results showed that GmLEC1 was highly expressed in soybean seeds, followed by pods (Figure 1B). This expression pattern was consistent with the predicted expression pattern provided by Phytozome (Supplementary Figure S1).

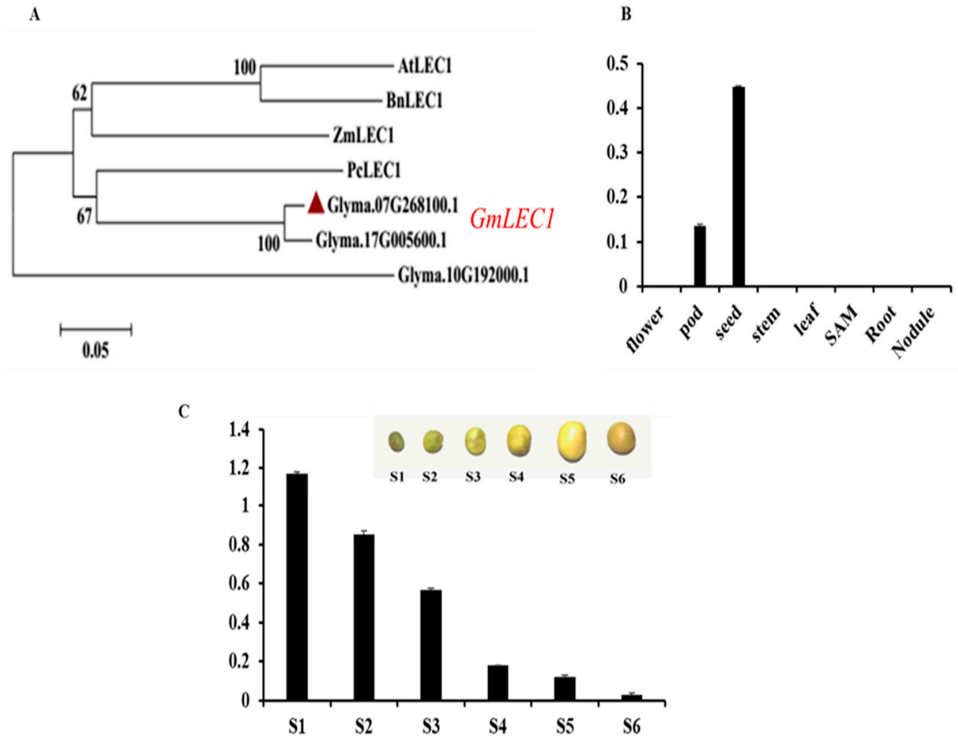


**Figure 1.** (**A**) Phylogenetic relationship between GmLEC1 and LEC1 homologs from other crops. Red triangle represent the GmLEC1 used in this study (**B**) Tissue-specific expression of GmLEC1 (SAM: Shoot apical meristem). (**C**) Expression of GmLEC1 in soybean seeds at various developmental stages. Developmental stages were classified according to seed weight.

To further investigate the expression of GmLEC1 during seed development in soybean, the transcript levels were analyzed at different stages of seed development. Seed weight was used as a reference to classify the stages. The findings revealed elevated expression of GmLEC1 during the initial stages (S1–S3) of development (Figure 1C). These results suggest that GmLEC1 primarily plays a role in embryogenesis and the synthesis of seed contents during early seed development.

### 3.2. Plant Development

An Arabidopsis mutant complementation assay was conducted to investigate the role of the GmLEC1 gene in plant morphology and composition. Homozygous single mutants of atlec1 were transfected with A. tumefaciens harboring pB2GW7-GmLEC1. Three generations of transgenic plants were screened for gene expression and selected for further experiments.

Previous studies have suggested that LEAFY genes play a role in seed development and the transition from the embryonic to the vegetative phase [16–18]. To examine the impact of GmLEC1 on cotyledon structure and appearance, mature green seeds of wild-type, *atlec1*, and *GmLEC1/atlec1* were germinated on a sterilized MS medium. After four days, the shape and structure of cotyledons were observed under the microscope (Figure 2A). The analysis revealed that *atlec1* mutant cotyledons had trichomes, which are characteristics of vegetative leaves. However, the normal cotyledon characteristics were restored when *GmLEC1* was expressed in the mutant seeds. In addition to regulating cotyledon morphology, LEC1 also plays a role in seed shape and contents. Dry mature seeds of *atlec1* were shrunken and desiccation-intolerant, but the expression of *GmLEC1/atlec1* restored healthy seeds (Figure 2B)

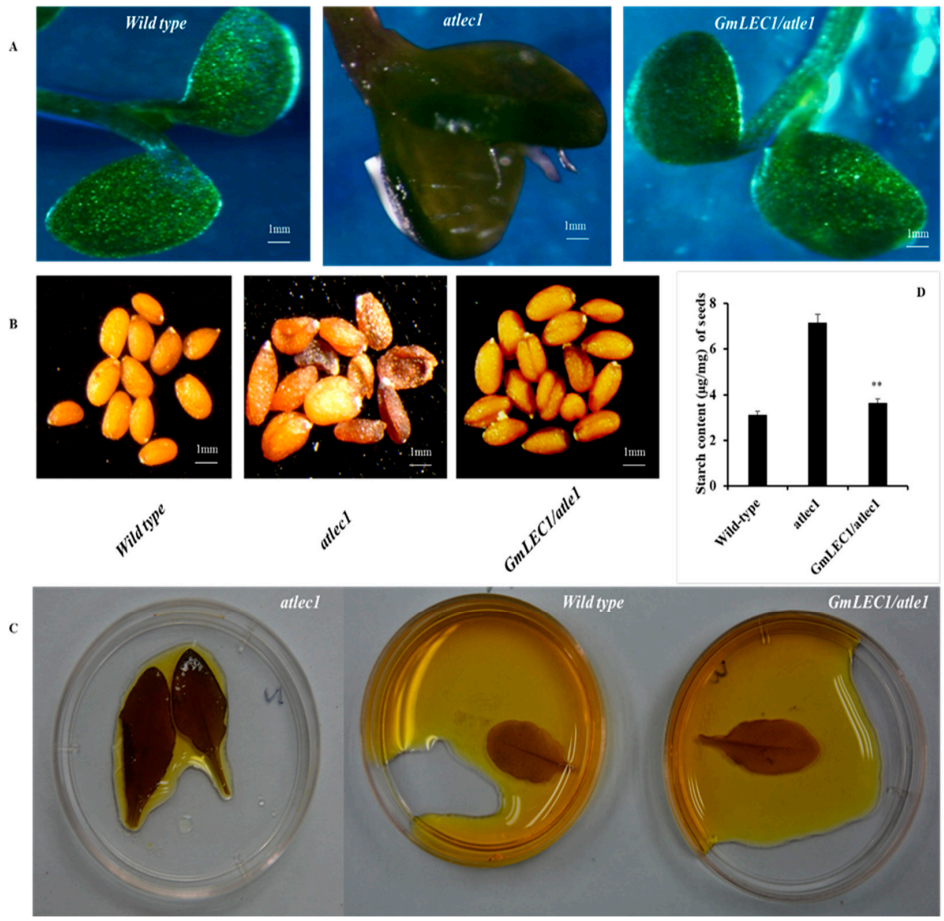


**Figure 2.** Comparison of plant developmental characteristics of atlec1, wild-type, and GmLEC1/atlec1 transgenic plants. (**A**) Four-day-old cotyledons of atlec1 have trichomes on the upper surface, while wild-type and GmLEC1/atlec1 complemented cotyledons showed true cotyledon morphology with no trichrome. (**B**) Mature atlec1 mutant seeds have vague characteristics, such as being shrunken and darker in color compared to the wild type. (**C**) Iodine staining of starch granules in four-week-old leaves of atec1, wild-type, and GmLEC1/atlec1 transgenic plants. The dark color of the atlec1 leaves indicates more accumulated starch in the leaves. (**D**) Content of starch in mature dry seeds. ** $p < 0.01$ by Student's *t*-test ($n = 3$).

During seed development, there is competition between lipid and starch biosynthesis for carbon resources [19]. To investigate the role of GmLEC1 in starch metabolism and its accumulation in the seed and vegetative tissues, the starch content was analyzed in seeds and leaves of mutant, wild-type, and transgenic plants. The leaves from four-week-old plants were incubated in an iodine solution to stain starch granules (Figure 2C). The *atlec1* leaves appeared darker than *GmLEC1/atlec1* and wild-type leaves after iodine staining, indicating a higher starch accumulation. Similarly, the measurement of seed starch content also showed a higher accumulation in *atlec1*, while GmLEC1 expression reduced starch accumulation (Figure 2D). This reduced starch content in *GmLEC1*-expressed seeds may be correlated with a higher TAG content.

### 3.3. Impact on Seed TAG Content and Composition

An analysis of transgenic and control lines was carried out to investigate the impact of the ectopic expression of GmLEC1 on the total TAG content and composition of fatty acid lipid profiling. Total lipids were extracted from 10 mg of mature dry transgenic, wild-type, and mutant seeds and analyzed for total TAG content and TAG composition via GC. The ectopic expression of GmLEC1 altered the composition of TAG compared to wild-type seeds, resulting in a 6.8% decrease in palmitic acid content. Additionally, there were slight reductions of 1.8%, 3%, 3.8%, and 2.8% in the content of 18:0, 18:2, 18:3, and 20:1, respectively. The transgenic seeds had an 18.6% higher oleic acid (18:1) content compared to control seeds (Figure 3A). Furthermore, the expression of GmLEC1 in wild-type (Col-0) plants increased the total TAG content of transgenic seeds by 13% (Figure 3B). In GmLEC1/atlec1 T3 seeds, there was an average increase of 4.5% in total oil content compared to atlec1 seeds (Figure 3D). The palmitic and stearic acid content in GmLEC1/atlec1 seeds increased by 11.7% and 6.5%, respectively. The oleic acid content was 3.7-fold higher in GmLEC1/atlec1 seeds compared to atlec1 seeds. Additionally, the amount of α-linolenic acid (18:3) was 1.7-fold higher in GmLEC1/atlec1 seeds compared to atlec1 mutant seeds. In the atlec1 mutant background, the expression of GmLEC1 resulted in a 9.5% lower eicosenoic acid (20:1) level compared to mutant seeds (Figure 3C).

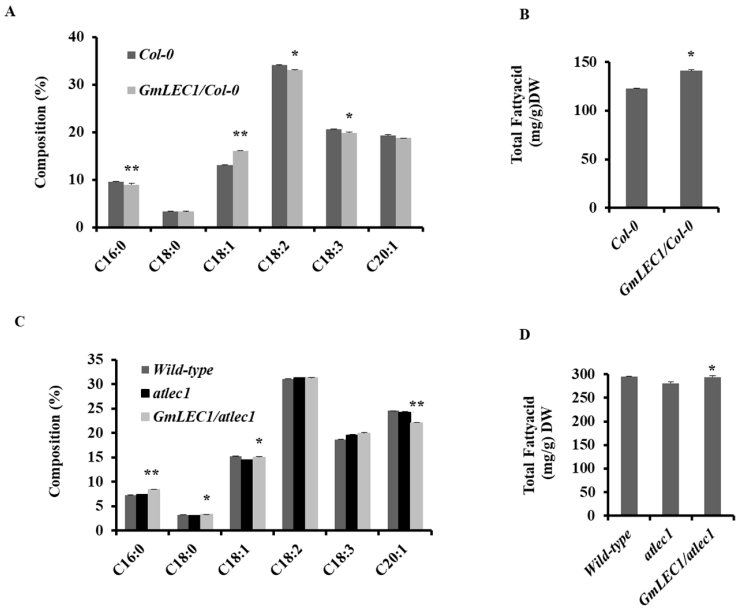


**Figure 3.** Composition of fatty acids (**A**) and total TAG content (**B**) of wild-type and GmLEC1 overexpressed seeds. Composition of fatty acids (**C**) and total TAG content (**D**) of WS-2 (wild-type), atlec1, and genetically complemented GmLEC1/atlec1 seeds. (Palmitic acid:C16:0; Stearic acid:C18:0; Oleic acid:C18:1; Linoleic acid:C18:2; Alpha linolenic acid:C18:3; Eicosenoic acid:C20:1). ** $p < 0.01$ and * $p < 0.05$ by Student's *t*-test ($n = 3$).

### *3.4. Ectopic Expression in Soybean Hairy Roots Induces TAG Storage*

The transcription factor GmLEC1 was introduced into hairy roots derived from soybean cotyledons to better understand its role in oil production and storage, and the results are shown in Figure 4A. Hairy root transformation is commonly used to verify the function of soybean genes due to the low transformation efficiency of soybeans. Additionally, it is a more convenient and efficient method requiring less time [20]. Transgenic hairy roots were first selected based on their growth on the selection medium and confirmed using semi-quantitative RT-PCR and qRT-PCR (Supplementary Figure S3). To evaluate the impact of GmLEC1 overexpression on TAG accumulation, we analyzed at least five independent hairy root lines. Total lipids from control and transgenic hairy roots were extracted and separated using thin-layer chromatography (TLC) on a silica plate. The lipids on the plate were visualized by developing them with sulphuric acid and staining with iodine (Figure 4B). Fatty acid methyl esters from stained TAGs were generated and suspended in hexane for GC analysis. The increased TAG accumulation observed in the GmLEC1 overexpressed roots suggests that GmLEC1 either activated TAG biosynthesis or enhanced TAG accumulation. Analysis of the fatty acid compositions of these TAGs revealed that GmLEC1 promotes the synthesis of TAGs containing high levels of palmitic acid. Compared to control lines, the average content decrease of 18:1, 18:2, and 18:3 in GmLEC1-expressed roots was 12%, 10%, and 22%, respectively (Figure 4C). The GmLEC1-expressing hairy roots had 28% more TAG compared to control lines (Figure 4D).

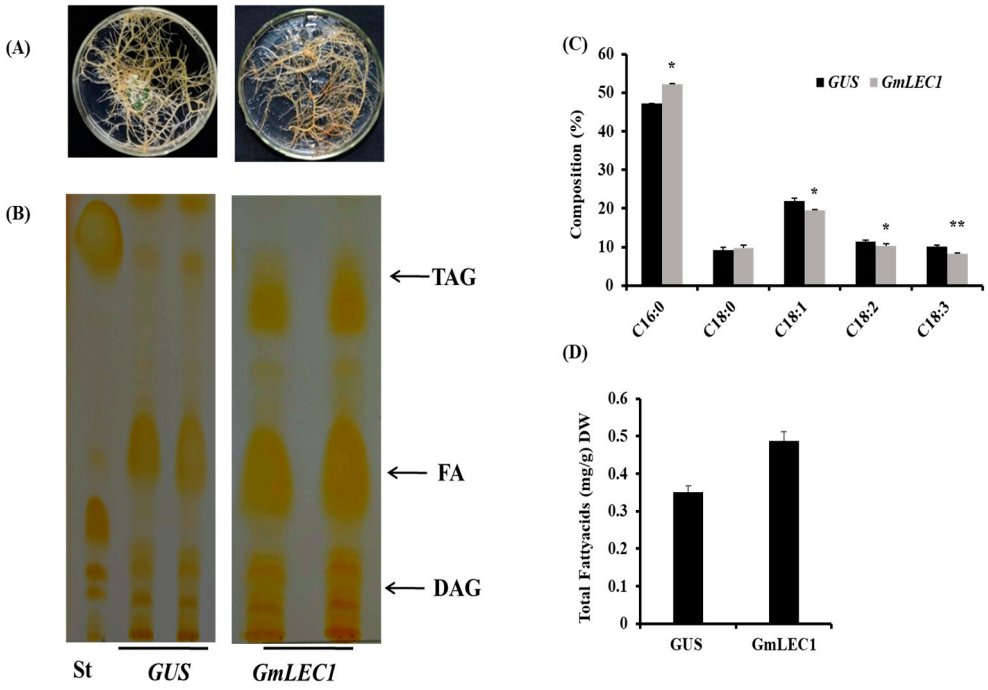


**Figure 4.** GmLEC1 expression in soybean hairy roots. (**A**) Soybean cotyledons were used to produce hairy roots. (**B**) Lipids staining after TLC analysis (St: standard/positive control, GUS: control). (**C**) Composition of TAGs from hairy roots. (**D**) Amount of TAGs. Representative data are from three biological replicates and expressed as the mean SD. ** $p < 0.01$ and * $p < 0.05$ by Student's *t*-test ($n = 3$).

### *3.5. LEC1 Regulates Key Genes of Lipid Biosynthesis in Soybean*

To identify the route through which GmLEC1 induces lipid biosynthesis in soybean hairy roots, we performed qRT-PCR on hairy roots to check the expression of key genes of the lipid biosynthesis pathway (Figure 5). We specifically selected genes known to enhance oil production and TAG storage in seeds, as well as genes that have been shown to inhibit oil biosynthesis. These genes were then analyzed for their expression in transgenic roots. The results showed that GmLEC1 increased the expression of the downstream

target gene GmWRI1 in transgenic hairy roots compared to the control. This elevated expression of GmWRI1 leads to a stable enhancement in seed oil content and alterations in the composition of fatty acids in soybean seeds [21,22]. Additionally, GmLEC1 promotes the expression of various key genes encoding fatty acid biosynthesis enzymes, including BCCP2. Moreover, it modulates the expression of transcription factors like ABI3 and LEC2, which are known to positively regulate seed maturation and lipid accumulation [23]. On the other hand, GmLEC1 downregulates the expression of genes such as GLABRA2 (GL2), MUCILAGE MODIFIED 4 (MUM4), TRANSPARENT TESTA2 (TT2), and other MYB transcription factors that inhibit lipid biosynthesis.

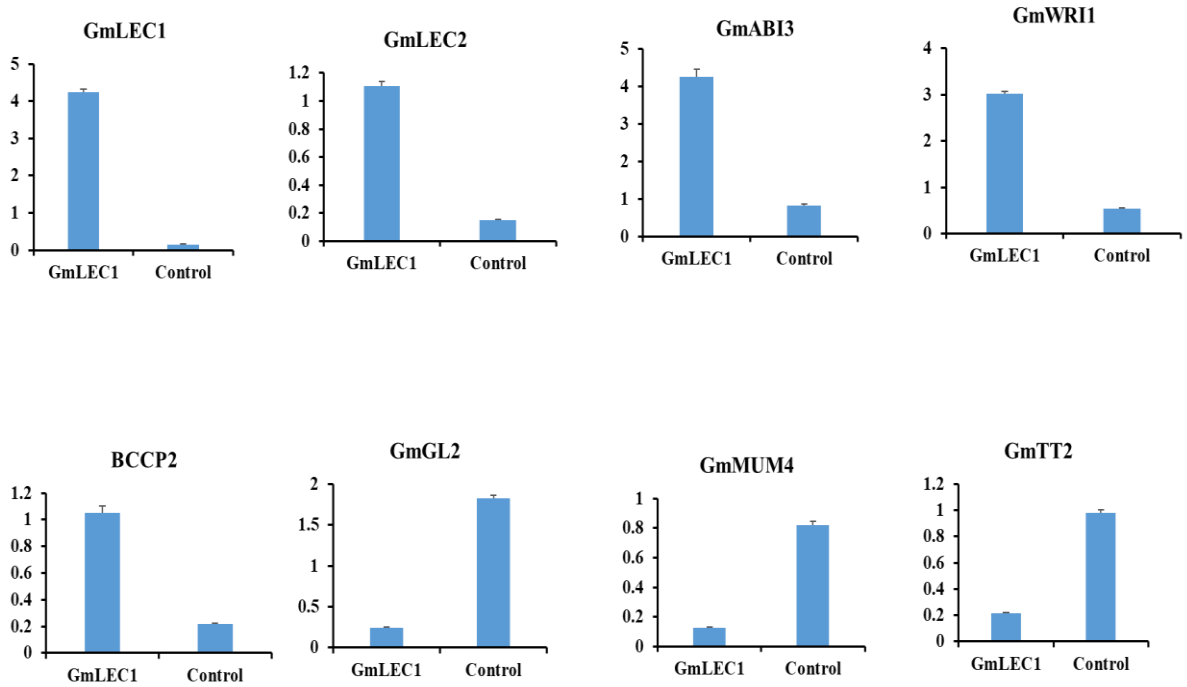


**Figure 5.** Quantitative RT-PCR of a few genes involved in fatty acid/TAG biosynthesis to confirm the role of GmLEC1 in lipid biosynthesis. Representative data are from three biological replicates and expressed as the mean SD. The sequence of all genes is provided in Supplementary Data S2.

## 4. Discussion

Seed development is a complex process in which various seed components are synthesized and accumulated. This process relies on the presence of transcription factors, phytohormones, and environmental factors [24]. Previous studies have shown that LEAFY genes are involved in seed development and maturation [25,26]. During the seedling stage, stored TAG in seeds serves as a source of energy, while transcription factors regulate plant development and hormone production in response to environmental conditions. A mutation in LEC1 disrupts the regulation of genes involved in trichome formation, leading to the development of trichome on cotyledons. In previous research, it was found that LEC1 plays a crucial role in determining cell fate during post-embryonic development and regulates GL2 to prevent trichome formation on cotyledons [6]. In this study, we demonstrate that GmLEC1 is essential for the proper formation of trichome-free cotyledons, distinguishing them from leaves. The expression of GmLEC1 in *atlec1* mutants restored the wild-type cotyledon identity, as cotyledons derived from GmLEC1/atlec1 seeds did not develop trichomes, indicating that GmLEC1 restored the normal function of AtLEC1 in mutants (Figure 2A). Since LEC1 is expressed in both developing and mature seeds, this study also investigated its impact on mature seeds. The atlec*1* mutant seeds are shrunken and smaller in size (Figure 2B). Previous research has suggested that LEC1 may regulate bHLH and MYB transcription factors, which are involved in seed mucilage production and anthocyanin synthesis [1]. In this study, the expression of a MYB transcription factor, TT2, and a

mucilage regulator, MUM4, was reduced in GmLEC1-overexpressing hairy roots (Figure 5), suggesting that GmLEC1 may regulate TT2 and MUM4 during seed development.

Other studies have also reported on the role of LEAFY genes in the development and maturation of seeds [12,27]. As seed filling and the accumulation of storage substances occur during different stages of seed development, this study found a high expression of GmLEC1 in the early stages of seed development, followed by a gradual decrease, which is consistent with a previous study [6] that showed the involvement of LEC1 in embryo development. Additionally, our results support a recent study that found a high expression of MeLEC1 during the seed embryo phase [10]. In several oil crops, the contribution of LEC1 in altering fatty acid composition and regulating the storage of TAG has been reported. Loss-of-function mutations of LEC1 in Arabidopsis have been shown to impair the accumulation of storage protein and lipids, reduce desiccation tolerance, and inhibit germination [28]. On the other hand, ectopic expression of LEC1 activates genes responsible for seed maturation and the regulation of storage and promotes lipid accumulation [29]. A genome-wide analysis comparing *lec1* mutant and wild-type seeds has revealed significant differences in mRNA profiles during the seed maturation phase [30]. Furthermore, genome-wide identification of OsLEC1-binding sites has shown that OsLEC1 binds to genes involved in photo-morphogenesis, photosynthesis, abscisic acid, and gibberellic acid signaling pathways that regulate seed maturation in Arabidopsis [31].

The induced expression of BnLEC1 in transgenic canola seeds caused changes in the fatty acid composition, specifically increasing the levels of 18:1 and decreasing the levels of 20:1 fatty acids [8]. The soybean LEC1 gene was found to increase the amount of 18:1 fatty acid in both soybean seeds and transgenic roots compared to the control (Figures 3 and 4). In contrast, the *GmLEC1* gene reduced the content of 18:3 fatty acids in Arabidopsis seeds and hairy roots, which is an important trait for oil storage (Figure 4). The expression of the *ZmLEC1* gene resulted in a 40% increase in oil production relative to the control, but it also had a negative impact on seed germination and the growth of vegetative parts [7]. However, overexpression of the *GmLEC1* gene did not affect the seed germination rate, but it did increase the production of TAG in the seeds. In fact, GmLEC1 hairy roots contain 12% more TAG compared to the control hairy roots. This increase in lipid accumulation may be due to the downregulation of the *GL2* gene by *GmLEC1*, which is a negative regulator of lipid biosynthesis. Normally, the function of LEC1 is to suppress the activity of GL2, which inhibits trichome formation on cotyledons and activates lipid synthesis and accumulation genes [26]. A previous study found that *gl2* seeds contained 8% more oil content compared to the control, with no effect on seed size [32]. Furthermore, MUM4 is a downstream target of *GL2* and shares similar characteristics with *gl2* [33]. Another study reported an increase in oil content in triple mutants of *myb5*, *tt2*, and *gl2* [34,35]. Additionally, an F-box protein called MYB Interaction Factor 1 (AtMIF1) physically interacts with and degrades MYB5 protein in Arabidopsis, leading to the inactivation of GL2. The overexpression of AtF1M1 was found to increase seed oil biosynthesis without affecting plant growth [36]. These studies highlight the important role of LEC1-induced regulation of GL2 in controlling oil biosynthesis and storage in seeds.

Previous literature has demonstrated that LEC1 directly activates WRI1, which is a key regulator of seed oil biosynthesis [25,26,37]. It has been shown that WRI1 acts as a transcriptional enhancer for genes involved in plant lipid biosynthesis [38]. WRI1 targets the BCCP2 and PKp-b1 enzymes, which provide acyl chains and a glycerol backbone for lipid biosynthesis [11]. The high expression of GmWRI1 has been shown to significantly increase seed oil content and alter the composition of fatty acids in soybean [21,22]. Similarly, overexpression of AtWRI1 in seed-specific protein (*ssp*) mutants has been found to increase TAG content in Arabidopsis seeds [37]. The expression of coconut WRI1 (CoWRI1) has also been shown to increase total fatty acid levels in transgenic Arabidopsis seeds and rice grains, with a specific increase in palmitic and linoleic acid but a decrease in oleic acid [39]. In addition to its role in seed filling and maturation, LEC1 has also been found to regulate ABI3 in complex regulatory pathways [26]. The overexpression of GmABI3 in *atabi3* mutant and wild-type plants has been shown to increase total fatty acid content and alter fatty acid

composition compared to the control seeds [23]. The current study further supports previous findings that GmLEC1 and GmLEC2 activate each other and regulate lipid metabolism and somatic embryo development [3,40]. Overall, this study highlights the crucial role of GmLEC1 in the proper formation of cotyledons without trichomes, distinguishing them from leaves. Additionally, GmLEC1 has been found to regulate genes involved in seed mucilage production and play a role in altering fatty acid composition and TAG storage in various oil crops. These findings underscore the significance of LEC1 in controlling seed development and oil biosynthesis in soybean.

## 5. Conclusions

This study discovered that GmLEC1 interacts with other transcription factors to regulate embryonic and seed development. It regulates genes related to trichome development and phytohormones, and it also stimulates the production of lipids and promotes their accumulation in seeds. The study found that GL2 is the main downstream target of GmLEC1 when it comes to controlling embryo characteristics, and the gene *WRI1* is important for seed filling. Overall, these findings demonstrate that *GmLEC1* activates genes involved in fatty acid biosynthesis genes and suppresses transcription factors such as *GL2*, *MUM4*, *MYB5*, and *TT2* that negatively regulate lipid biosynthesis in the soybean plant. Additionally, GmLEC1 upregulates the expression of transcription factors like LEC2 and ABI3, which enhance lipid biosynthesis and orchestrate plant development. Identifying LEC1 as a key target for genetic enhancement techniques presents new opportunities for improving oil content in soybean through genetic manipulation. Future research should focus on further understanding the complex regulatory network that controls oil biosynthesis.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13112810/s1, Figure S1: Predicted tissue-specific expression of GmLEC1 retrieved from Phytozome, Figure S2: PCR amplification of GmLEC1 using cDNA from soybean seed, Data S1: Protein sequences used to build phylogenetic tree, Data S2: The CDS sequence of all genes used to perform qRT-PCR presented in Figure 5, Table S1: List of primers used in this study.

**Author Contributions:** Conceptualization, S.M. and J.Z.; methodology, S.M. and K.F.A.; software, S.M.; validation, S.M., A.A.-A. and W.A.; formal analysis, S.M.; investigation, S.M. and K.A.K.; resources, J.Z. and K.A.K.; data curation, W.A.; writing—original draft preparation, S.M.; writing—review and editing, K.F.A., A.A.-A., W.A. and K.A.K.; visualization, S.M.; supervision, J.Z.; project administration, J.Z.; funding acquisition, J.Z. and K.A.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Ministry of Science and Technology of China (2018YFD1000601). The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University, Saudi Arabia, for funding this work through the Large Groups Project under grant number RGP2/360/44.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors acknowledge the support of the Unit of Bee Research and Honey Production at King Khalid University, Abha, Saudi Arabia. The authors also thank Zainab from University of Southampton, UK, for language editing and proofreading of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

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