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ARTICLE

Cloning and Characterization of *EuGID1* in *Eucommia ulmoides* Oliver

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ABSTRACT

Gibberellic acid controlled the key developmental processes of the life cycle of landing plants, and regulated the growth and development of plants. In this study, a novel gibberellin receptor gene *EuGID1* was obtained from *Eucommia ulmoides* Oliver. The cDNA of EuGID1 was 1556 bp, and the open reading frame was 1029 bp, which encoded 343 amino acids. *EuGID1* had the homology sequence with the hormone-sensitive lipase family. Amino acid sequence alignment confirmed EuGID1 protein had the highest homology with the GID1 protein of *Manihot esculenta*. EuGID1 was located in the nucleus and cell membrane and had expression in four plant organs. Over-expression of *EuGID1* in transgenic *Arabidopsis* plants promoted plant elongation and increased siliques yield.

KEYWORDS

Eucommia ulmoides Oliver; *EuGID1* gene; overexpression; subcellular localization; heterologous transformation

1 Introduction

Gibberellin (GA) was a tetracyclic diterpenoid compound that controlled key developmental processes in the life cycle of landing plants, including seed germination, flower bud differentiation, leaf extension, rhizome elongation and xylem development [1,2]. When GA was sensed by the receptor, the signal pathway was activated and regulated downstream gene expression [3]. Gibberellin Insensitive Dwarf 1 (*GID1*) gene was a key receptor gene in this pathway. GID1 protein was a nucleocytoplasmic protein, which was a soluble GA-receptor, displayed high affinity for active GA [4]. DELLA protein acted as major growth repressors [5,6], it typically inhibited GA signaling [7–9]. Upon interaction with GA, GID1 underwent a conformational change that increased its affinity for DELLA. It was reported that GA first bound to the receptor protein GID1, then formed a GA-GID1-DELLA complex with DELLA protein, the formation of these stable complex helped F-box protein recognized the DELLA protein, the DELLA protein was further degraded by 26S protease ubiquitination [10,11].

GID1 was a key point in the GA signaling pathway, and *GID1* gene loss-of-function mutants were usually expressed as dwarf plants. Conversely, overexpression of this gene might cause plants to be taller or larger [12,13]. The gibberellin receptor gene was cloned in rice, which was subsequently found in *Arabidopsis* [14], peach tree [15], dasypyrum villosum [16], wheat [17], barley [18], fern [19], tea tree [20] and other plants. There was only one gibberellin receptor in rice, and the gene in the rice mutant was

first cloned and named *OsGID1* by Ueguchi-Tanaka, this mutant showed severe plant dwarfing [21]. In the model plant *Arabidopsis*, it contained three GA receptor genes, namely *AtGID1 a*, *AtGID1 b* and *AtGID1 c*. Studies had found that they were orthologs to the *GID1* gene in rice, and transformation experiments had also confirmed that *AtGID1S* could restore the dwarf phenotype of rice *gidi-1* [22]. El-Sharkawy transformed the *PslGID1* gene in the plum tree into the *Arabidopsis gid1-ac* double mutant, which was found that various growth defects in the mutant were inhibited by the presence of *PslGID1*, which showed that *PslGID1* was similar to *AtGID1* in function, and the higher the transgene level had the more active the growth performance [23]. The *capsicum* gibberellin receptor genes *CaGID1b.1*, *CaGID1b.2* and *CaGID1c* were expressed into the *Arabidopsis* double mutant *gid1a gid1c* by CAO, among which, the effect of *CaGID1b.2* was the most significant [24], the plant height and stem elongation were increased.

Eucommia ulmoides Oliver was a unique forest resource in China, whose wood yield was valued by more and more people [25]. This plant was a deciduous dioecious tree unique to the *Eucommia* genus in the family *Eucommia* [26]. The extracts obtained from different tissues of *E. ulmoides* showed a variety of pharmacological benefits [27–29], which was also the main source of separation and production of gutta-percha. Gene regulation of GA signaling pathway in forest trees had been widely reported [30,31], that was becoming particularly essential to dig out genes that regulated growth and development in woody plants [32,33]. *GID1* gene from *E. ulmoides* was not been reported previously, which might play a crucial role in regulating its growth and development, and also had a guiding significance for its wood yield. This research cloned a GA receptor gene *EuGID1* from *E. ulmoides* and constructed an overexpression vector to reveal its function, which committed to providing a theoretical basis for the study and application of *EuGID1* gene function.

2 Materials and Methods

2.1 Plant Materials

The age of *E. ulmoides* was more than 10 years old, which were planted in the Key Laboratory of Plant Resources Conservation and Germplasm Innovation in Mountainous Region, Guiyang, Guizhou Province, China. The roots, stems, leaves and fruits of *E. ulmoides* were used for total RNA extraction. They were put into liquid nitrogen and stored at −80°C for subsequent RNA extraction. *Nicotiana benthamiana* seeds was provided by Key Laboratory of Plant Resources Conservation, Guiyang, Guizhou Province, China, the *Arabidopsis* seeds were provided by Germplasm Innovation in Mountainous Region, Guiyang, Guizhou Province, China.

2.2 5′-RACE and 3′-RACE Amplifications

The transcriptome and genome annotation database of *E. ulmoides* were compared to screen out homologous gene sequences. The experiment used the mRNA isolated and purified from the delicate leaves of *E. ulmoides* as a template, and the specific primers were synthesized by Sangon Biotech Company (Shanghai, China) (Table 1). Followed the instructions of Invitrogen 5′-RACE kit (Wuhan, China) and Clontech's 3′-RACE kit (Dalian, China) to do the experiment, the amplification procedures used were as follows: pre-denaturation (94°C, 2 min); denaturation (94°C, 30 s), annealing (55°C, 30 s), extension (72°C, 1 min), 35 cycles; total extension (72°C, 10 min), the PCR product was ligated with pMD18T, then the positive clones were sent to Sangon Biotech Company (Shanghai, China) for direct sequencing after transformation.

Table 1: Primer used in this work

Primers	Primer sequences (5′—3′)	Purpose
EuGID1–1	AAATTGGAAATGAGGA	5′RACE
EuGID1–2	AACCACCCTCTTGGATTC	5′RACE
EuGID1–3	GCGTTAACTTCATTACTGCC	5′RACE
EuGID1–4	AGCTACTGTTCTTAGAGAAGGCG	3′RACE
EuGID1–5	CTATTTCTTGCCGAACAACGATCA	3′RACE
EuGID1–6F	ATGGCTGGCAGTAATGAA	ORF amplification
EuGID1–6R	AGTTAAGAGTTGACGAAG	ORF amplification
EuGID1–7F	GCTCTAGAGCCACCATGGCTGGCAGTAATG	Amplification
EuGID1–7R	TGGCTGCAGGAGAGTTGACGAAGCTTTTG	Amplification
EuGID1–8F	TGAGCTCGAGAAGCCACTGA	qPCR
EuGID1–8R	GGCGATCTCCGGTAGTTGAC	qPCR
Actin-F	TTGTTAGCAACTGGGATGATATGG	Reference
Actin-R	CAGGGTGTTCTTCAGGAGCAA	Reference
EuGID1–9F	GTCCTGGGTAACATCCTGCTC	Detection
EuGID1–9R	TCCTCCATTAGGCGATAGAAA	Detection

2.3 Obtaining the Full-Length cDNA Sequence of the Target Gene

Two specific primers were designed, and synthesized by Sangon Biotech Company (Shanghai, China) (Table 1). The first strand of cDNA was used as a template for PCR amplification. The PCR conditions used were as follows: pre-denaturation (94°C, 2 min); denaturation (94°C, 30 s), annealing (55°C, 30 s), extension (72°C, 2 min 30 s), 35 cycles; total extension (72°C, 10 min). The PCR products were analyzed by 1.5% agarose gel electrophoresis. The experiment used the OMEGA kit to recover the agarose gel containing the target DNA (Shanghai, China). TaKaRa's T4 ligase kit was used for ligation (Dalian, China). After transformation, the identification and screening of positive clones were carried out. The PCR procedures for identification of positive clones were as follows: pre-denaturation (94°C, 2 min); denaturation (94°C, 30 s), annealing (55°C, 30 s), extension (72°C, 1 min 40 s), 35 cycles; total extension (72°C, 10 min). After electrophoresis, the positive clones were picked out and sent to Sangon Biotech Company (Shanghai, China) for sequencing.

2.4 Bioinformatics Analysis of the EuGID1 Protein

Mega 6.0 was used for gene homology comparison; DNAMAN software was used to construct evolutionary trees; TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) was used to analyze protein transmembrane structure Domain; Prot Param (http://web.expasy.org/prot-param/) was used to analyze the composition and so on; and Compute pI tool (ExPASy-Compute pI/Mw tool/) was used to calculate the relative molecular weight and theoretical isoelectric point of EuGID1 protein. Prot Scale (ExPASy-ProtScale) was used for hydrophilicity and hydrophobicity analysis; Signal P 4.1 was used for signal peptide analysis; (https://NPS@:SOPM Secondary Structure Prediction (ibcp.fr)/) was used to predict protein secondary structure; and SMART (http://smart.embl-heidelberg.de/) was used to predict the functional domain of EuGID1 protein.

2.5 Construction of the Vector

Amplification primers were designed according to the full-length ORF sequence of *E. ulmoides* gene (Table 1). The PCR conditions used were as follows: 98°C pre-denaturation for 5 min; 98°C denaturation for 10 s, 55°C annealing for 30 s, 68°C extension for 1 min, 35 cycles; total Extension at 68°C for 5 min. The PCR products were analyzed after electrophoresis on a 1.5% agarose gel. The PCAMBIA-super1300 plasmid was subjected to double digestion with XbaI and PstI (provided by Prof. Qinglin Liu, Sichuan Agricultural University), then the PCR product and double digestion product were recovered. The target gene was ligated with the PCAMBIA-super1300 overexpression vector. The PCAMBIA-super1300-35S::EuGID1::EGFP fusion expression vector was transformed into E. coli DH5α competent cells, and then the positive clones were identified by PCR. After the plasmid containing the target gene was extracted, it was transformed into *Agrobacterium* GV3101 competent cells. The detection primers were the same as the amplification primers. The PCR conditions used were as follows: pre-denaturation (94°C, 2 min); denaturation (94°C, 30 s), annealing (55°C, 30 s), extension (72°C, 1 min), 35 cycles; total extension (72°C, 10 min). The positive monoclonal colonies were preserved.

2.6 Subcellular Localization of EuGID1 Protein

Nicotiana benthamiana seeds were sown and cultivated for 1 month under the conditions of light for 12 h, temperature of 25°C and humidity of 60%; 35S::EuGID1::EGFP overexpression vector and 35S-sGFP empty vector were amplified by *Agrobacterium*. The experiment used a 1 mL syringe with a pipette tip to inject the epidermis of *Nicotiana benthamiana* leaves and mark them: plants were cultured in low light for 2 days and observed using a laser confocal microscope (Nikon C2-ER). The excitation wavelength of chloroplast fluorescence signal was as follows: 640 nm, emission wavelength: 675 nm. Green fluorescent protein GFP: excitation light 488 nm, emission light 510 nm.

2.7 Tissue-Specific Expression Analysis of EuGID1

The CTAB method was used to extract RNA from roots, stems and leaves of *E. ulmoides* seedlings and RNA from *E. ulmoides* fruits, with 3 replicates in each tissue. Invitrogen's superscript III first-strand synthesis system was used to synthesize the first strand of cDNA. The fluorescent quantitative PCR primers of *EuGID1* gene were designed and synthesized according to the instrument operation guide (Table 1). Using *EuActin* as the internal standard (Table 1), the 7500 Real-time PCR system (ABI, USA) was used for amplification, and the relative gene expression was calculated according to the software provided by ABI. This experiment was analyzed and compared the specific expression of *EuGID1* gene in different organs and tissues of various *E. ulmoides* plants.

2.8 Genetic Transformation of EuGID1

Arabidopsis seeds were sown and cultivated under the conditions of light for 12 h, temperature of 23°C. The *Agrobacterium* solution containing the overexpression vector was used as the working solution, and the flowering *Arabidopsis* plants were transformed by the floral dip method. The T0 generation seeds were collected and screened on the MS solid medium plate containing hygromycin. The total DNA of leaves was used CTAB Method extraction, and the hygromycin gene-specific primers were used for PCR identification and detection. T2 generation was screened by same method, and the gene-specific primers were used for PCR identification and detection (Table 1).

3 Results

3.1 Obtaining of the Full-Length Sequence of EuGID1 Gene

Based on the data acquired from a previous transcriptomics experiment, the 5′-RACE, 3′-RACE and ORF cloning was used to obtain the full-length sequence of the gene (Fig. 1). After the sequence was spliced and the vector sequence was removed, the comparison was performed on NCBI to further

confirmed the final result. The sequencing result was as follows: the *EuGID1* was named according to its strong sequence similarity to the *Arabidopsis* gene *AtGID1*. The full-length *EuGID1* cDNA was 1556 bp, the open reading frame (ORF) was 1029 bp in length. The 5′untranslated region (UTR) was 84 bp, 3′ untranslated region (UTR) was 443 bp, and there was an obvious tailing signal downstream of its 3′ end (Fig. 2).

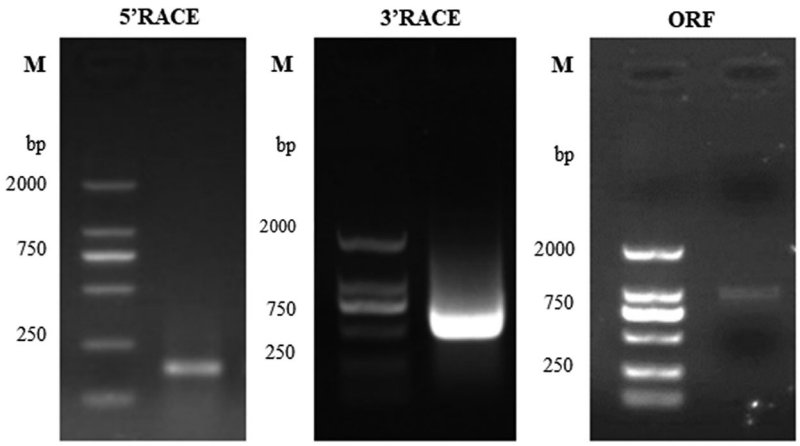

Figure 1: Electrophoresis results of *EuGID1* cloning

3.2 Molecular Characteristics of the EuGID1 Protein

The gene encoded 342 amino acids, and the molecular formula was $C_{1756}H_{2687}N_{485}O_{501}S_8$, Molecular weight was equal to 38.87 kDa, the theoretical isoelectric point was 7.15, the positive and negative charge residues were 41, and the fat coefficient was 86.32. Used NCBI BLAST to search, the protein ID number was XP_012092333.1. The protein sequence encoded by this gene had no transmembrane domain and no signal peptide, whose hydrophilicity was −0.296. The instability coefficient of the encoded protein sequence was 44.33, which was an unstable protein. The gene predicted protein secondary structure: there were more α-helixes and random coils, α-helix (α-helix) was 30.99%, random coil (random coil) was 34.50%, β-turn (β-turn) turn) was less at 10.23%.

3.3 Amino Acid Sequence Alignment of EuGID1 Protein

EuGID1 belonged to the α/β hydrolase superfamily. The protein sequence encoded by this gene contained the conserved sequence of the hormone-sensitive lipase family (HSL), which had 15 key sites for the binding of GID1 to active GA. In addition, GID1 protein bound GA and DELLA protein to a total of 13 functional domains. The amino acid sequence included TWVLIS, LDR, FFHGGSF, HS, IYD, YRR, DGW, GDSSGGNI, GNI, MF, LDGKYF, WYW and GFY functional domains. Among them, the V (valine) of the TWVLIS domain in EuGID1 was replaced by I (isoleucine) (Fig. 3), which showed that these domains play a crucial role in the process of GA and DELLA binding. Through comparison, it was found that EuGID1 protein sequence was similar to *Jatropha curcas*, *Manihot esculenta*, *Camellia sinensis*, *Hevea brasiliensis*, *Populus trichocarpa*, *Capsicum annuum*, and *Durian*. The similarity of was 82.32%~85.17%. Among them, it had the highest homology with *Manihot esculenta*, up to 85.17%. It had the lowest homology with *Camellia sinensis*, only 82.32%.

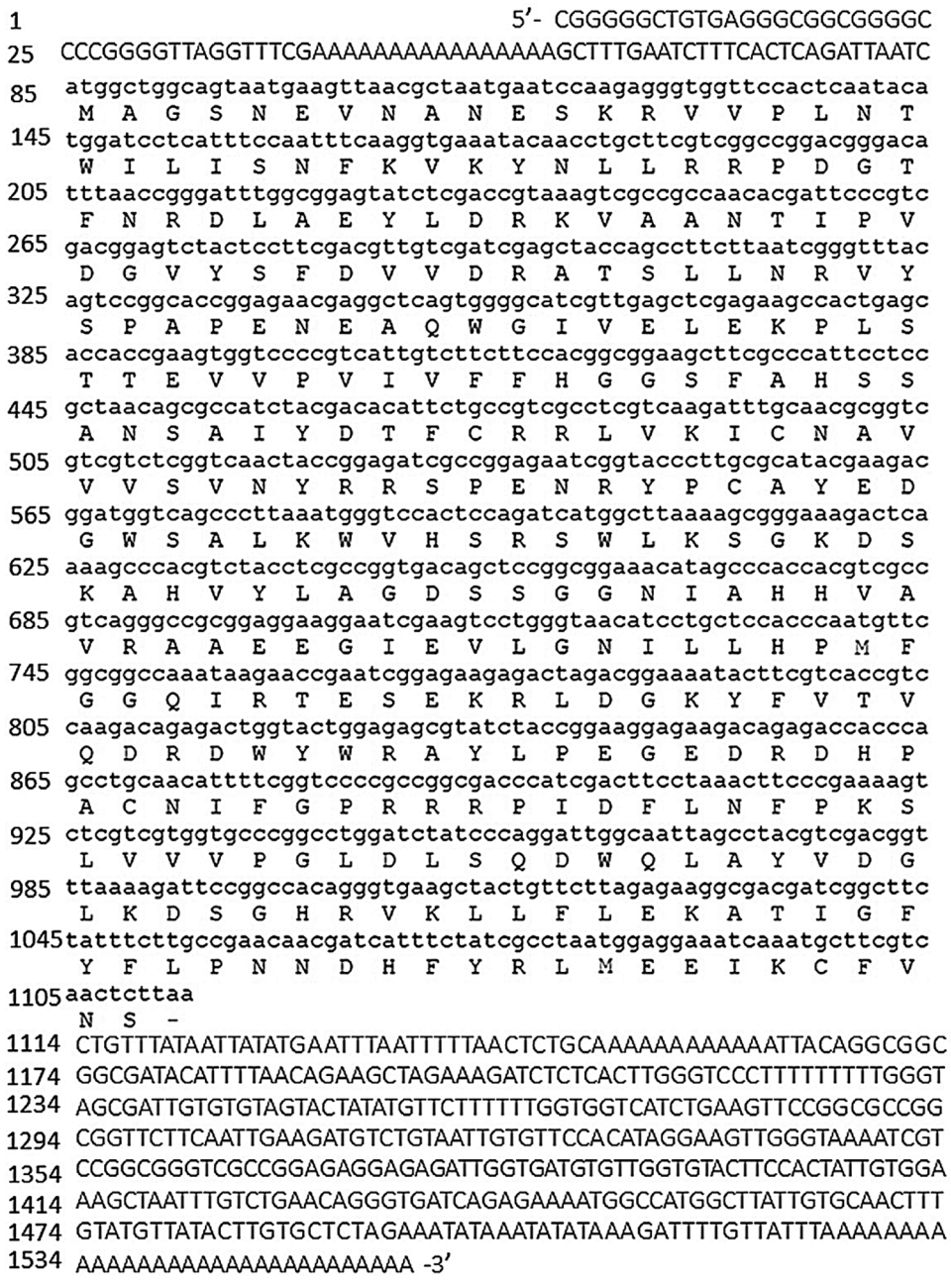

Figure 2: Full-length of *EuGlD1* gene

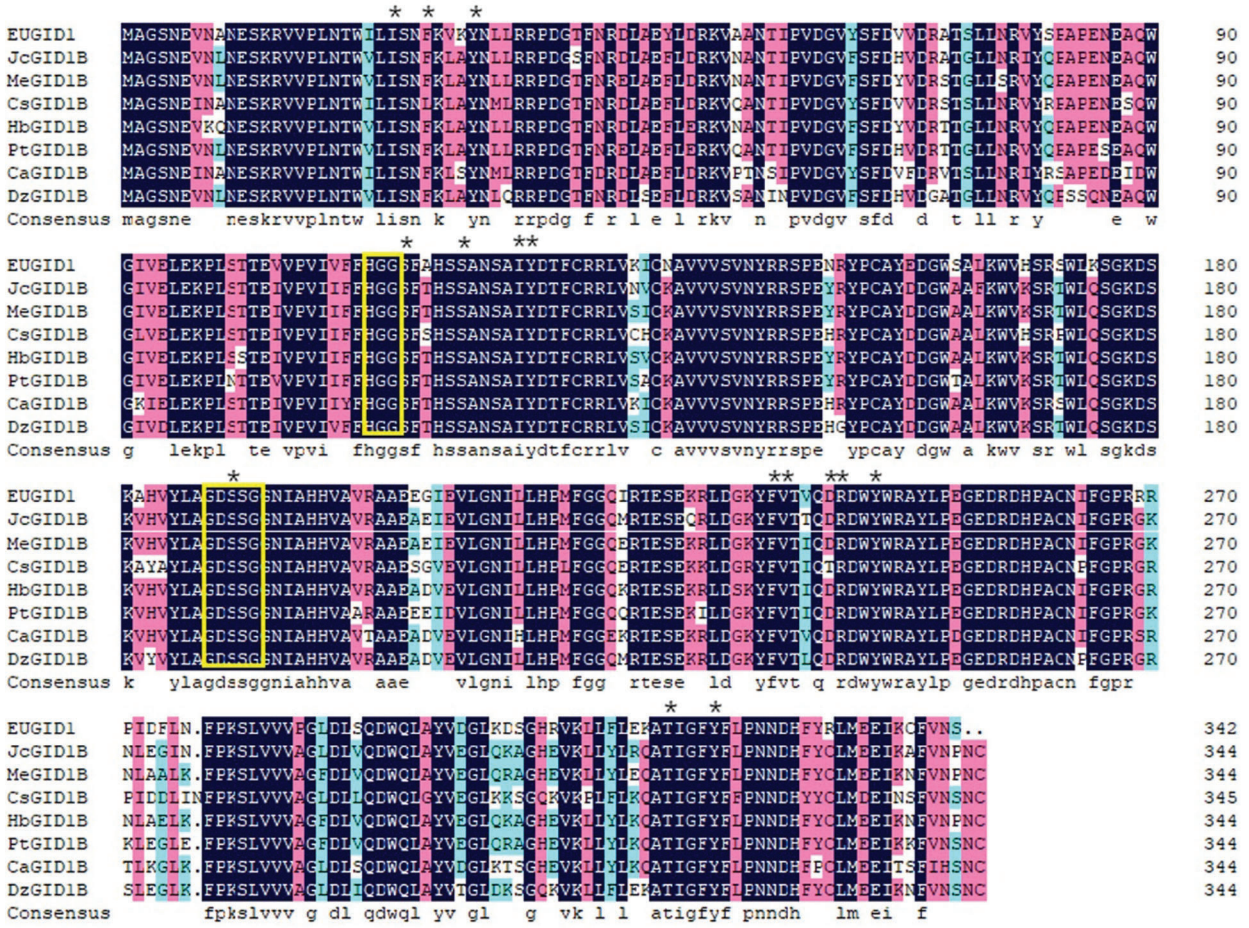

Figure 3: Amino acid sequence comparison between EuGID1 and other species

Jc: *Jatropha curcas*; Me: *Manihot esculenta*; Cs: *Camellia sinensis*; Hb: *Hevea brasiliensis*; Pt: *Populus trichocarpa*; Ca: *Capsicum annuum*; Dz: *Durian*. *Represented the key site where GID1 bind to active GA. The sequence in the yellow box was the conserved sequence of the hormone-sensitive lipase family (HSL).

3.4 Phylogenetic Analysis of EuGID1

A comparison of EuGID1 with the GID1 protein sequences of 28 representative plant species from NCBI (Fig. 4), which revealed that 29 species of plants were divided into 3 clusters: EuGID1 and *Camellia sinensis*, *Nyssa sinensis*, *Panax ginseng*, *Coffea arabica*, *Ipomoea triloba*, *Ipomoea nil*, *Capsicum chinense*, *Petunia x hybrida*, *Nicotiana attenuata*, *Solanum lycopersicum*, *Solanum tuberosum* were clustered into one branch; *Jatropha curcas*, *Ricinus communis*, *Manihot esculenta*, *Hevea brasiliensis*, *Populus euphratica*, *Populus tomentosa*, *Populus alba*, *Castanea mollissima*, *Hibiscus syriacus*, *Durio zibethinus*, *Gossypium hirsutum*, *Gossypium arboreum*, *Gossypium barbadense* and *Gossypium mustelinum* were the second cluster. *Momordica charantia*, *Cucumis sativus*, *Cucumis melo* were the third cluster.

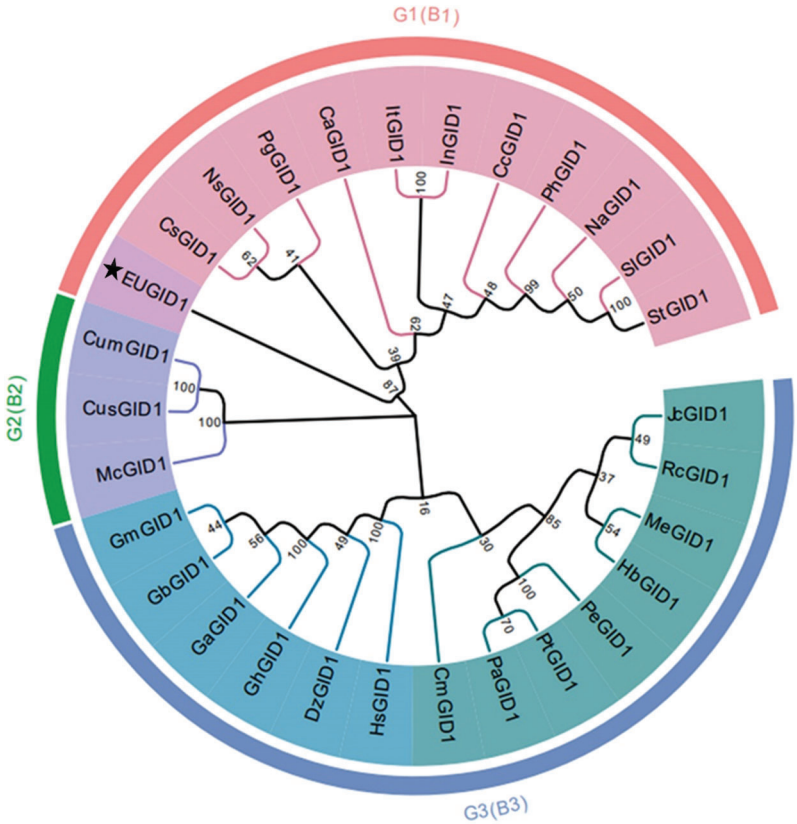

Figure 4: Phylogenetic tree of EuGID1

The following species were arranged into a phylogenetic tree using the neighbor connection method: *Camellia sinensis* KAF5930343.1 (CsGID1), *Nyssa sinensis* KAA8550923.1 (NsGID1), *Panax ginseng* QEX51270.1 (PgGID1), *Coffea arabica* XP_027097574.1 (CaGID1), *Ipomoea triloba* XP_031100613.1 (ItGID1), *Ipomoea nil* XP_019197492.1 (InGID1), *Capsicum chinense* PHU22188.1 (CcGID1), *Petunia x hybrida* AGN72649.1 (PhGID1), *Nicotiana attenuate* XP_019232495.1 (NaGID1), *Solanum lycopersicum* NP_001352577.1 (SlGID1), *Solanum tuberosum* XP_006362976.1 (StGID1), *Jatropha curcas* XP_012092333.1 (JcGID1), *Ricinus communis* XP_002524767.1 (RcGID1), *Manihot esculenta* XP_021613000.1 (MeGID1), *Hevea brasiliensis* XP_021681392.1 (HbGID1), *Populus euphratica* XP_011009471.1 (PeGID1), *Populus topulus alba* ANB66267.1 (PtGID1), *Populus alba* XP_034908255.1 (PaGID1), *Castanea mollissima* KAF3975015.1 (CmGID1), *Hibiscus syriacus* KAE8692101.1 (HsGID1), *Durio zibethinus* XP_022771057.1 (DzGID1), *Gossypium hirsutum* ABQ96123.1 (GhGID1), *Gossypium arboretum* XP_017627860.1 (GaGID1), *Gossypium barbadense* KAB2056790.1 (GbGID1), *Gossypium mustelinum* TYJ09289.1 (GmGID1), *Momordica charantia* XP_022150283.1 (McGID1), *Cucumis sativus* NP_001277149.1 (CusGID1), *Cucumis melo* XP_008451414.1 (CumGID1), ★ represented EuGID1 protein.

3.5 Overexpression Vector Construction and Subcellular Localization of EuGID1 Protein

An overexpression vector of 35S::EuGID1::EGFP was constructed (Fig. 5A). PCR verification and sequencing showed that *EuGID1* had been inserted into the vector (Fig. 5B). The laser confocal microscope was used to determine the accurate localization of EuGID1 protein subcellular, EuGID1 and 35S-sEGFP enhanced green fluorescent protein were fused, and the transient expression of the epidermal cells of leaves was observed under a laser confocal microscope (Fig. 6). With 35S-sGFP empty as a control, a 35S::EuGID1::EGFP overexpression vector was constructed. The experimental results showed that the fluorescence distribution was determined on the nucleus and cell membrane.

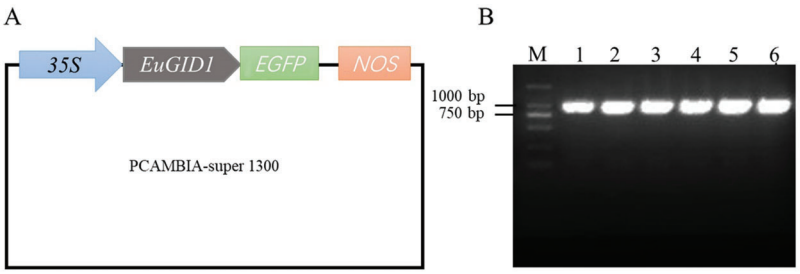

Figure 5: Construction of 35S::EuGID1::EGFP overexpression vector and PCR detection of agrobacterium liquid. (A) 35S::EuGID1::EGFP overexpression vector structure. In the PCAMBIA-super1300 vector, 35S was the strong promoter in the vector, EGFP was the green fluorescent protein signal, and NOS was the terminator. (B) Lanes 1–6 were PCR products of single colonies with 35S::EuGID1::EGFP, M was for DNA marker

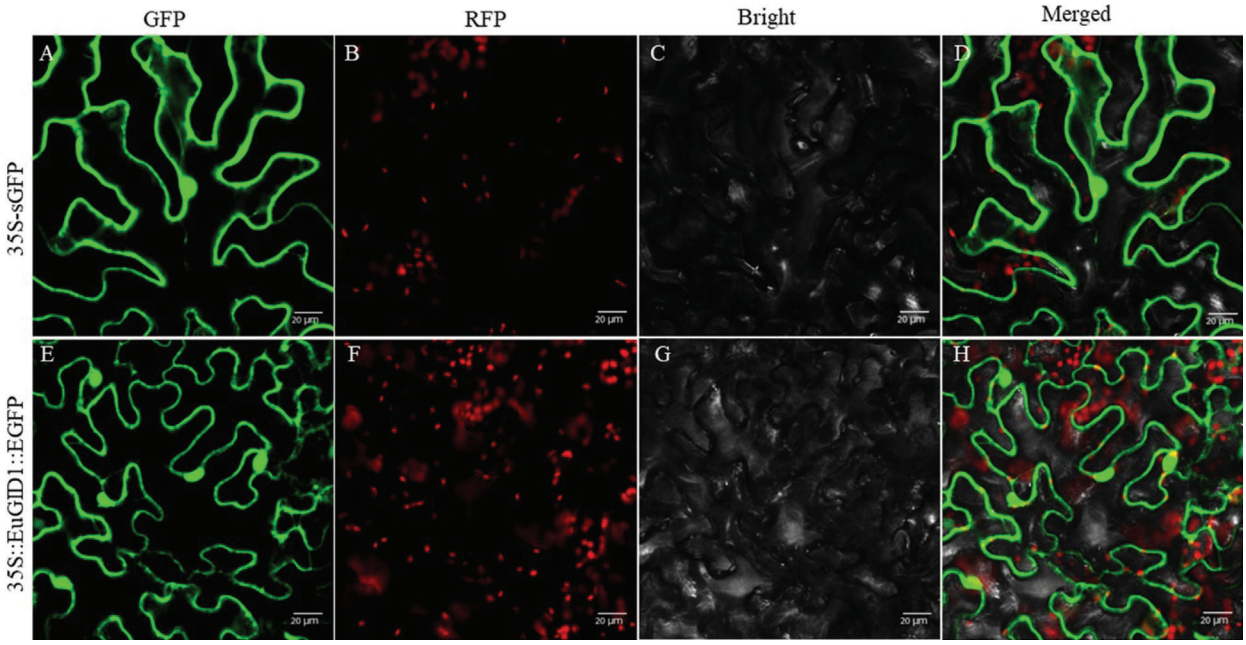

Figure 6: Analysis of subcellular localization of EuGID1 protein in *Nicotiana benthamiana* leaves. (A, E) GFP field; (B, F) RFP field; (C, G) Bright field; (D, H) Merged pictures. 35S-sGFP indicated the empty vector, and 35S::EuGID1::EGFP indicated the fusion protein with EuGID1. Bar: 20 μm

3.6 Relative Expression of GA Receptor Gene EuGID1

The expression of *EuGID1* gene was detected in four organs, that implied this gene has a role in these organs. Real-time fluorescent quantitative PCR was used to analyze the expression pattern of this gene in the roots, stems, leaves and fruits of *E. ulmoides* (Fig. 7), The research found: with higher expression in roots and fruits, followed by stems and leaves, that the expression level in the root was 2.17 times the expression level in the stem, and the expression level in the root was 2.46 times the expression level in the leaf. It indicated that *EuGID1* gene had the highest expression in roots in four organs, which might be an important effect gene in root development.

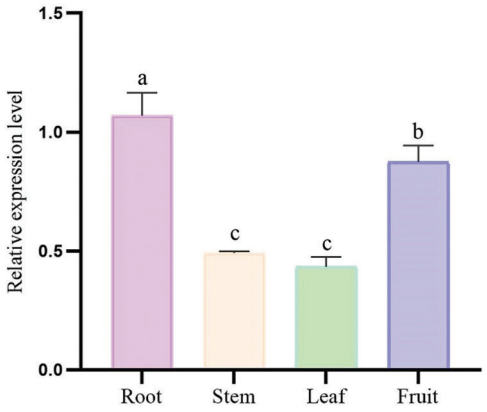

Figure 7: The relative expression of *EuGID1* in the organs of *E. ulmoides*. Data are mean SDs ($n = 3$). Different letters indicate the difference significance at the 0.05 level, respectively.

3.7 Genetic Transformation and Phenotype Analysis of EuGID1

EuGID1 gene might have similar functions to *AtGID1* in stem elongation and fruit development (Fig. 8B). Under the same growth conditions, the plant height and the number of siliques in the same period were measured (Fig. 8A). It was found that the average plant height of positive regenerated plants was 24.57 cm, and the average plant height of the wild-type plants in the same period was 19.37 cm (Fig. 8C), thus, the average plant height of the positive regenerated plants was 5.20 cm higher than the average plant height of the wild type. All transgenic types showed more significant elongation than the wild type, and positive regenerated plants had an average number of siliques of 171.80, while the average number of siliques of the wild type during the same period was 85.17 (Fig. 8C). The average number of siliques of the positive regenerated plants was about twice that of the wild type. All transgenic plants showed earlier silique maturation. There was no significant difference in the length of the siliques, the number of seeds in each silique and the size of the seeds. In *Arabidopsis*, heterologous transformation increased the number of gibberellin receptors and triggered the overall growth and development.

4 Discussion

Gibberellin receptors were widely in plants, which was considered a key factor in the gibberellin signal transduction pathway. Since the gibberellin receptor gene was first positionally cloned in rice mutants [21], it was found that the *GID1* gene played a significant role in regulating plant height, flower development and fruit length [34,35]. This study used *E. ulmoides* transcriptome and genome database as a reference to screen out homologous gene sequences [36], and RACE technology was used to amplify, sequence, and splice to obtain the full-length cDNA sequence of *EuGID1*. The full length of the amino acid sequence and the physical and chemical properties were similar to those of other species *GID1* [37,38].

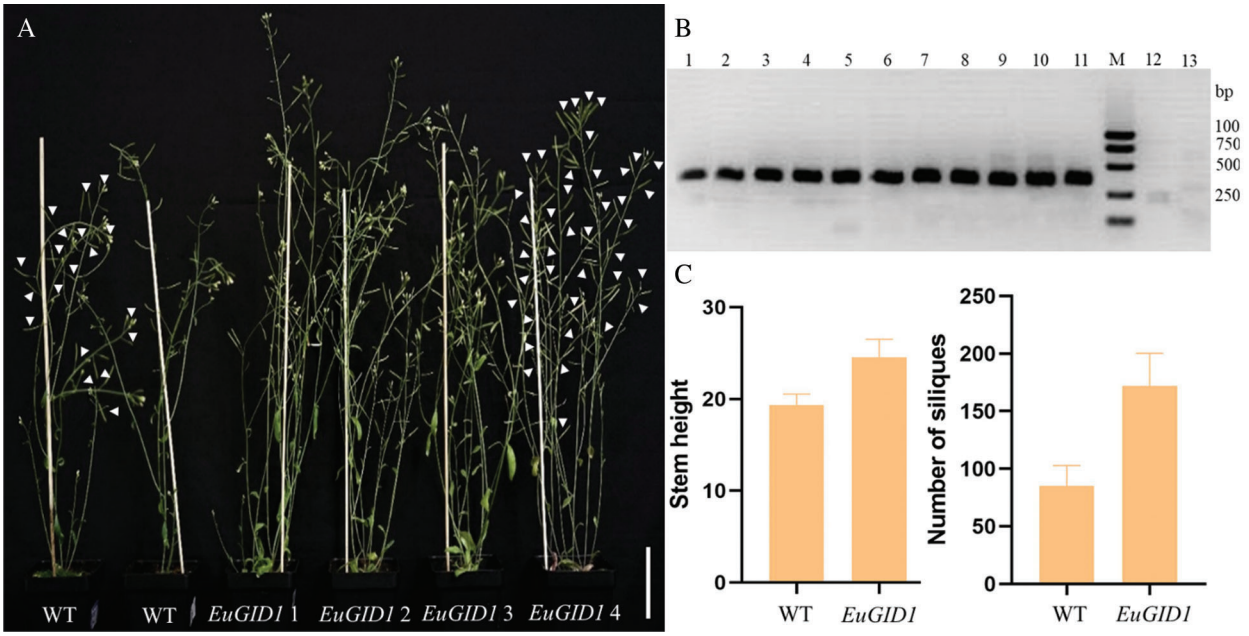

Figure 8: Phenotypic overexpression of *EuGID1* gene in wild-type *Arabidopsis*. (A) The gross morphology of the wild-type plants and transgenic plants at the life stage; arrows indicate the siliques; Bar = 5 cm. (B) Detection map of the target gene intermediate fragment of *Arabidopsis* positive plants. Lanes 1–10 were transformed plants with *EuGID1* gene, 11 was Plasmid control, M was DNA marker, 12–13 were wild type plants. (C) The comparison diagram of the stem height and the number of siliques between wild-type plants and positive plants

The *EuGID1* gene might be the receptor gene of active GA in *E. ulmoides*, it was involved in the recognition and transduction of GA signals. *EuGID1* protein belonged to the α/β hydrolase superfamily. The amino acid sequence of *EuGID1* and other species were compared, the protein sequence encoded by this gene contained the conserved sequence of the hormone-sensitive lipase family (HSL), included the conserved HSL motifs HGG and GXSXG [39]. Among the three important amino acids in the HSL family that could form a catalytic triad (Ser, Asp and His), Asp and Ser were conserved in *EuGID1* sequence, His was replaced by Ile, which might be the reason why they lost catalytic ability [14], which was consistent with the amino acid sequence of *GID1* in poplar and *Arabidopsis* [22,40]. Compared the amino acid sequence, it was found that the homology was greater than 82%, indicated that the gene had a high degree of conservation in evolution. The construction of amino acid phylogenetic tree analysis showed that it was divided into 3 clusters.

GA synthesis was carried out in different area of the cell. In the relevant research on the *GID1* gene report, the subcellular localization analysis found that the *GID1* protein of rice, grape, and newhall navel orange were all expressed in the nucleus [21,41,42]. The localization results of this study indicated that *EuGID1* protein was localized in the nucleus and cell membrane, result was for further study. Gibberellin was a hormone involved in multiple biological processes [43]. The results showed that *EuGID1* was expressed in all organs of *E. ulmoides*, which was similar to the *GID1* gene in *Arabidopsis* and grapes [35,41], this was consistent with GA being active during plant development [44]. Studies had found that the highest expression level in the roots, the maximum expression level of the *MsGID1b* gene in alfalfa and the *PttGID1.4* gene in hybrid poplars also appeared in the roots [40,45]. The expression level in the roots and fruits of *E. ulmoides* was significantly higher than that in the stems and leaves, which was consistent with the conclusion that *Arabidopsis AtGID1B* was a minor effect gene in stem elongation, but

AtGID1B was a major effect gene in root development [46]. Therefore, the expression of this gene was more advantageous in tissues with slow differentiation rate [47].

Gibberellin enhanced plant growth characteristics by stimulating the interaction between *GID1* and DELLA, which triggered the degradation of DELLA and activated the overall GA response. Nakajima found three *AtGID1* genes in *Arabidopsis* through homologous gene analysis [22]. Griffiths Jayne reported that the mutations of three *AtGID1* genes in *Arabidopsis* resulted in significant dwarfing characteristics, including height reduction, slow growth and low seed setting rate [35]. When genes from other species were overexpressed in *Arabidopsis*, the phenotype of *GID1* gene loss-of-function mutants might be appeared normal type or even larger type [46]. In this study, the average plant height of *Arabidopsis* positive regenerated plants was 5.20 cm higher than that of the wild type. The average siliques of the positive regenerated plants were about twice the average number of wild-type siliques, and all transgenic plants showed earlier silique maturation, which indicated that *EuGID1* was similar to *AtGID1* in function. This result was consistent with that El-Sharkawy transformed the *PslGID1* gene from the plum tree into the *gid1-ac* double mutant of *Arabidopsis*, the resulting transgenic plants were taller than the control plants and the leaves of the transgenic plants were longer than the control group. This conclusion was also basically consistent with Li Jun's conclusion: the *GoGID* gene from Galega orientalis Lam was transformed into the *Arabidopsis gid1ac* mutant, which showed increased plant height and increased germination rate [23,48]. Therefore, the overexpression of the *EuGID1* gene had a significant effect on the growth and development of the gibberellin. It was reasonable to speculate that overexpression of the *EuGID1* gibberellin receptor gene could speed up plant growth, increase plant height, and increase biomass.

5 Conclusion

In this work, a gibberellin receptor gene was obtained, *EuGID1* had the homology sequence with the hormone-sensitive lipase family, it was located in the nucleus and cell membrane. *EuGID1* had expression in four organs. Overexpression of *EuGID1* in transgenic *Arabidopsis* plants promoted plant elongation and increased siliques yield. It provided excellent candidate genes for molecular breeding of *E. ulmoides*. In the future, we will continue to determine the relative expression level of transgenic *Arabidopsis* and the morphological phenotype of transgenic *Arabidopsis* after GA treatment.

Author Contributions: Yan Li designed the research and conducted the experiments. Yulu Chen collected and analyzed the data. All authors approved the final version of manuscript.

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