



Article

Agave schidigera Transcriptome Reveals Stress-Responsive Phenylalanine ammonia-lyase Genes in *Agave*

Xuxia Wang ¹, Xiaoli Hu ^{2,3}, Chen Lin ⁴, Qingqing Liu ⁴, Yubo Li ^{2,5}, Dengxiang Du ⁶, Dietram Mkapu ^{2,3,7}, Weiyi Zhang ⁵, Xing Huang ^{2,8,9,*} and Kexian Yi ^{2,8,9,10}

¹ Urban Construction College, Wuchang Shouyi University, Wuhan 430064, China

² National Key Laboratory for Tropical Crop Breeding, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China; yikexian@126.com (K.Y.)

³ College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

⁴ Shanghai Key Laboratory of Plant Functional Genomics and Resources, Shanghai Chenshan Botanical Garden, Shanghai 201602, China

⁵ School of Tropical Agricultural and Forestry, Hainan University, Danzhou 571737, China

⁶ School of Life Science and Technology, Wuhan Polytechnic University, Wuhan 430023, China

⁷ Mlingano Centre, Tanzania Agricultural Research Institute (TARI), Tanga P.O. Box 5088, Tanzania

⁸ Key Laboratory of Integrated Pest Management on Tropical Crops, Ministry of Agriculture and Rural Affairs, Haikou 571101, China

⁹ Hainan Key Laboratory for Monitoring and Control of Tropical Agricultural Pests, Haikou 571101, China

¹⁰ Sanya Research Institute, Chinese Academy of Tropical Agricultural Sciences, Sanya 572025, China

* Correspondence: huangxing@catas.cn

Abstract: *Agave* is a significant fiber crop in tropical regions, known for its high fiber strength. Lignin is closely associated with fiber strength, and phenylalanine ammonia-lyase (PAL) serves as the initial enzyme in biosynthesis of lignin. Hence, it is of considerable significance to study the genes of *PAL* family to analyze the characteristics and mechanism of sisal fiber development. In this research, we conducted a transcriptomic analysis of *Agave schidigera*, a widely recognized ornamental plant in agave. Approximately 29.85 million clean reads were acquired through Illumina sequencing. In total, 116,602 transcripts including 72,160 unigenes were assembled, and 22.06–63.56% of those unigenes were annotated in public databases. Two, six, six and six *PAL* genes were successfully identified and cloned from *A. schidigera*, *A. deserti*, *A. tequilana* and *A. H11648*, respectively. After phylogenetic analysis, these genes were clustered into two branches. Genes *AhPLA2a* and *AhPLA2c* exhibited higher expression levels compared to other genes but had different expression patterns. Moreover, *AhPLA2a* and *AhPLA2c* were expressed at high levels under full-nutrient, nitrogen-free and phosphorus-free stresses. Most *PAL* genes were induced by *Phytophthora nicotianae* Breda, especially *AhPAL1a*, *AhPAL1b*, *AhPAL2b* and *AhPAL2c*. This research is the first work to present a de novo transcriptome dataset for *A. schidigera*, enriching its bioinformation of transcripts. The cloned *PAL* genes and the expression analyses will form the basis of future research on lignin biosynthesis, the relationship between lignin and fiber strength, and stress resistance in *Agave* species.

Keywords: transcriptome; *Agave schidigera*; *PAL* genes; phylogeny; stress; expression pattern



Citation: Wang, X.; Hu, X.; Lin, C.;

Liu, Q.; Li, Y.; Du, D.; Mkapu, D.;

Zhang, W.; Huang, X.; Yi, K.

Agave schidigera Transcriptome

Reveals Stress-Responsive

Phenylalanine ammonia-lyase Genes in

Agave. *Agronomy* **2024**, *14*, 2520.

[https://doi.org/10.3390/](https://doi.org/10.3390/agronomy14112520)

[agronomy14112520](https://doi.org/10.3390/agronomy14112520)

Academic Editor: Yingpeng Han

Received: 22 August 2024

Revised: 11 October 2024

Accepted: 24 October 2024

Published: 26 October 2024



Copyright: © 2024 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article

distributed under the terms and

conditions of the Creative Commons

Attribution (CC BY) license ([https://](https://creativecommons.org/licenses/by/4.0/)

[creativecommons.org/licenses/by/](https://creativecommons.org/licenses/by/4.0/)

4.0/).

1. Introduction

Agave (Agavaceae) plants are extensively cultivated in tropical regions around the world for their applications in food, beverage, fiber, and pharmaceutical uses [1,2]. *Agave* is a relatively young genus, estimated to be between 7.8 and 10.1 million years old. It encompasses approximately 210 species, making it the most diverse species in the family Agavaceae [3,4]. They are quintessential CAM (Crassulacean Acid Metabolism) plants, boasting huge biological yield and exhibiting high tolerance to abiotic stresses like drought, cold temperature and elevated heat [5]. Hybrid cultivar *A. H11648* is renowned for its robust natural fibers and high tensile strength, and is extensively cultivated in Brazil,

Tanzania and Kenya [6,7]. It is also known as “East No. 1” in China, and has been used in production for more than 60 years.

Alternatively, *A. schidigera*, which is native to Mexico, is not merely an important fiber crop but also possesses ornamental value, making it suitable for gardens or as a potted plant due to its unique morphological traits, with green leaves having curly white hairs on the edges. But the fiber characteristics of *A. schidigera* are not as favorable as those of *A. H11648*, hence the former serving as a negative control in the fiber characteristics studies. Sisal fibers are the fibers from the *Agave* plants. Due to different sources, ages, and measurement methods, there are large variations in the chemical compositions of sisal fibers [8]. Relevant studies indicate that the sisal fiber comprises approximately 43% to 56% cellulose, 7% to 9% lignin, 21% to 24% pentosan and 0.6% to 1.1% ash [9]. Furthermore, studies have shown that the cellulose and lignin contents in sisal can vary between 49.62% and 60.95%, and from 3.75% to 4.40% [8], contingent upon the plant’s developmental stage. Nevertheless, in *Agave*, the molecular mechanisms governing fiber biosynthesis are still unclear. Up to now, a number of research results concerning fiber characteristics have been reported in *Agave* plants. In particular, 6 valuable genes associated with fiber traits [10], 9 *expansin A* genes [11], 24 *SAUR* family genes [12], and 5 *CesA* (*cellulose synthase A*) genes [1] have been revealed and characterized. These studies provide an important basis for the analysis of the *Agave* fiber synthesis mechanism. However, the large genomes and very long life cycle of these plants have posed significant limitations on genetic research, presenting a big challenge in studying their molecular mechanism of fiber traits. Moreover, studies on the lignin anabolism of *Agave* have not been reported.

The plant cell wall mainly constitutes cellulose, hemicellulose, pectin, and lignin, along with a minor quantity of proteins and minerals. In a study involving the utilization of corn stalk wastes as reinforcement material for polypropylene (PP), it was demonstrated that the lignin content assumed a crucial role in the intrinsic modulus of the reinforced fiber and directly influenced the ultimate stiffness of the composite [13]. As a potential source of carbon fiber, lignin has attracted the attention of researchers in the field of composite materials, and is also considered a potential biological resource in the fields of concrete additives, thermoplastic materials, and fuel production [14]. Phenylalanine Ammonia-Lyase (PAL, EC 4.3.1.24) is prevalently found in various plants, a few microorganisms and filamentous fungus [15]. It is the first enzyme in the phenylpropanoid pathway that is responsible for regulating the transfer of primary metabolites into the secondary metabolic pathway, including the synthesis of lignin, flavonoids, and various other aromatic metabolites [16,17]. Secondary metabolites can improve plant tolerance to various stresses. Some research results have also confirmed that *PAL* genes significantly contribute to plant growth, development and tolerance to both biotic and abiotic stresses in the environment [18–20].

Consequently, further study of *PAL* gene family is of considerable significance for analyzing the characteristics and mechanism of sisal fiber strength as well as fiber stiffness and its function in plant growth and stress resistance. It was initially extracted from *Hordeum vulgare* in 1961. Since then, *PAL* has been successfully isolated and characterized from various plants, such as *Oryza sativa* [21], *Arabidopsis thaliana* [22], bean [23], *Nicotiana tabacum* [24] and *Malus pumila* [25]. In recent years, the decipherment of an increasing number of plant genomes and transcriptomes has established the scientific basis for the exploration of the *PAL* gene family. By employing a bioinformatics-based strategy, a comprehensive analysis has resulted in the identification of 11 GbPAL members of the *PAL* gene family in Ginkgo [26]. In total, 9 *PAL* genes from orchids [27] (*Apostasia shenzhenica*, *Dendrobium catenatum* and *Phalaenopsis equestris*), 12 *PAL* genes in *Juglans regia* L., 14 *StPAL* genes in *Solanum tuberosum* [28] and 8 *PAL* genes (*HbPAL1*–*HbPAL8*) in *Hevea brasiliensis* [29] were identified using genome-wide bioinformatics analysis. *PAL* is encoded by a multi-gene family, exhibiting variations observed among different plants. The gene copy number is greater in potatoes, while in species like *A. thaliana*, *O. sativa*, *Vitis vinifera* and *N. tabacum*, it is comparatively lower [26]. Additionally, we successfully sequenced and obtained full-length transcriptomes of five tissues of *Agave* leaves by PacBio sequencing [30]. It is

the inaugural full-length transcriptome of *Agave* that constitutes a valuable resource for the identification and characterization of genes in *Agave* plants.

In this work, we employed Illumina technology to sequence and assemble the leaf transcriptome of *A. schidigera*, thereby facilitating a comprehensive understanding of gene expression patterns correlated with fiber characteristics. We further conducted a comparative analysis of *PAL* genes in four *Agave* species, namely *A. H11648*, *A. schidigera*, *A. deserti* and *A. tequilana*. The results will offer novel insights into the molecular underpinnings of the underlying lignin traits in *Agave*. The cloned *PAL* genes and the expression analyses will provide a basis for future research on lignin biosynthesis, and the relationship between lignin and fiber strength, which is of paramount significance for breeding new *Agave* varieties with high fiber yield and various resistances against stresses.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

A. schidigera and sisal main cultivar *A. H11648* were cultivated and managed normally in the experimental field of Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences (Haikou, Hainan Province, China). Different developmental stages of shoots, unexpanded leaves and expanded leaves (with an angle greater than 45° to the central axis) were separately collected from two-year-old potted *A. schidigera* plants for transcriptome analysis. Abiotic and biotic stress treatments were carried out on one-year-old *A. H11648* plants. In order to study the effect of fertilization on the expression pattern of *Agave PAL* genes, the following treatments were designed for *A. H11648*: full-nutrition (F), nitrogen-free (N-), phosphorus-free (P-), and potassium-free (K-) Hoagland nutrient solutions, and sterile water (W). On a different note, zebra disease caused by *P. nicotianae* Breda is a serious disease that occurs frequently in *A. H11648* and has a great impact on its yield and fiber quality [31]. In this study, the young parts of the *A. H11648* leaves were inoculated by *P. nicotianae* Breda through the puncture inoculation approach as the biotic stress [32]. The strain of *P. nicotianae* Breda utilized for inoculation was previously isolated and preserved in our laboratory. Prior to inoculation, it was imperative to disinfect the inoculated leaves with alcohol, followed by washing them with sterilized water. After the water was completely dried, the leaves were inoculated. The control group merely underwent pricking without inoculation, and sampling was carried out at five days post-inoculation.

Every three *A. H11648* individual plants were chosen for each treatment and the negative control, and each *A. H11648* strain was repeated for three times. The leaf tissue was collected and promptly frozen in liquid nitrogen for subsequent RNA extraction. The total RNA of collected experimental samples was extracted following the protocol of the Tiangen Biomart RNA extraction kit (Beijing, China). Commercialized RNase-free DNase I (Takara Biomedical Technology, Beijing, China) was used to remove residual DNA from RNA. The RNA was purified and kept in an ultra-low-temperature refrigerator.

2.2. Transcriptome Sequencing, De Novo Assembly and Annotation

Illumina sequencing with the purified RNA samples of *A. schidigera* was conducted in Genoseq Technology Co., Ltd., located in Wuhan, Hubei province, China. The quality of the extracted RNA was assessed using a NanoDrop 2000 spectrophotometer (Termo Fisher Scientific, Waltham, MA, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). Library construction was initiated only when the detection results met the requirements. Once the sample quality and concentration were qualified, the library could be constructed. Firstly, mRNA was enriched with Oligo (dT) magnetic beads.

Then, the mRNA was randomly disrupted through a fragmentation buffer. The mRNA was employed as a template to synthesize the first cDNA strand with six-base random hexamers, followed by the addition of buffer solution dNTPs. The synthesis of the second cDNA strand was carried out using RNase H and DNA polymerase I, followed by purification using AMPure XP beads. Subsequently, the purified double-stranded cDNA

underwent end-repair, with the addition of an A-tail and the connection of sequencing joints, with AMPure XP beads being utilized for selecting fragment sizes. The *A. schidigera* cDNA library was obtained through general PCR technique and agarose gel purification. Qubit 2.0 (Invitrogen, Waltham, MA, USA) was employed for the preliminary quantitative detection of the library, and the Agilent Bioanalyzer 2100 system was utilized to determine the insert size of the library. The library's effective concentration was precisely measured by qPCR, ensuring it exceeded 2 nM. After the quality assessment, paired-end 150 bp (PE150) sequencing was conducted with the platform of Illumina HiSeq 4000.

The FASTQ-formatted raw data from Illumina sequencing were uploaded into the public Sequence Read Archive (SRA) database [30]. The clean reads were derived through removing the adaptors and low-quality sequences with the help of the Cutadapt and Trimmomatic Version 0.39 software, respectively. The de novo transcriptome of *A. schidigera* was assembled using Trinity software v2.8.5 (with the parameters `--min_kmer_cov2 --min_glue 5`), and it was annotated on the basis of some public databases. A total of five databases were selected for functional annotation, namely NCBI non-redundant protein database (NR) [33], Gene Ontology (GO) [34], Kyoto Encyclopedia of Genes and Genomes (KEGG) [35,36], Eukaryotic Ortholog Groups (KOG) [37], and the Swiss-Prot database [38].

2.3. Characterization and Phylogeny of PAL Genes

We employed the *PAL* sequences *Arabidopsis* (4) and *Oryza sativa* (9) to conduct a BLAST [39] search in *A. H11648* transcriptome for *PAL* orthologs in *Agave* species. ORF-FINDER was used to analyze the coding sequence of the searching genes, and only *PAL* genes with complete coding sequences were retained for subsequent analysis. Using the complete coding sequences of *PAL* genes from the *A. H11648* transcriptome as the search sequences, homologous sequences of *PAL* genes were obtained and identified from previously published transcriptomes of *A. deserti*, *A. tequilana* [5] and *A. schidigera* in this work. The full-length *PAL* gene sequences of *Arabidopsis* (4), *Oryza sativa* (9), *A. H11648* (6), *A. schidigera* (2), *A. deserti* (6) and *A. tequilana* (6) were aligned for phylogenetic analysis utilizing the ClustalX 2.0 method. A neighbor-joining evolutionary tree was constructed with a bootstrap consensus value derived from 1000 replicates [40].

2.4. Expression Patterns of *Agave PAL* Genes

Quantitative real-time PCR (qRT-PCR) was employed to test the expression of the *Agave PAL* genes during different developmental phases, including shoot development, and non-expanding and expanding leaves, and in response to the abiotic and biotic stresses. Using the cDNA obtained by reverse transcription as the template, QuantStudio 6 PCR System (Thermo Fisher Scientific, Waltham, MA, USA) was employed for real-time quantitative PCR analysis. The qRT-PCR reaction mixture totaling 20 μ L, comprised 0.5 μ L of the forward gene primer (10 μ M), 0.5 μ L of the reverse gene primer (10 μ M), 1 μ L of the cDNA template, 10 μ L of TransStart Tip Green qPCR Supermix, 0.4 μ L of Passive Reference Dye (50 $\times$) (Transgen Biotech, Beijing, China) and 7.6 μ L of ddH₂O. The qRT-PCR procedure consisted of an initial stage of 94 $^{\circ}$ C for 30 s, 40 cycles of 94 $^{\circ}$ C 5 s, 58 $^{\circ}$ C 30 s and a final melt curve analysis. Each sample was subjected to three experimental replications. Six pairs of qRT-PCR primers for *Agave PAL* genes and two pairs of primers for the *phosphatase 2A (PP2A)* gene and *TUB* gene as endogenous genes were designed (Table 1) by Primer v3.0 software [41]. The qRT-PCR results were analyzed through the $2^{-\Delta\Delta C_t}$ method [42].

Table 1. Primers utilized for qRT-PCR analysis.

Genes	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product Size (bp)
<i>AhPAL1a</i>	TCGCTCTGCCTCGCCAAGGA	GACAGCTCCACCGTCACCTC	194
<i>AhPAL1b</i>	CTCCTCTCTTCTCCGTTGTGCT	CCACCGGCTTCCTGAACTCCC	189
<i>AhPAL1c</i>	CCTTTCCGTTCCACCTACACA	ATCTAGTCTTACTGGTCGCTGT	226
<i>AhPAL2a</i>	CATCAAACACCTCTCGCTCA	GCTGCCTGAAATCCCTCACC	198
<i>AhPAL2b</i>	TCATCCAACGCCTCTCGCTCA	GCTGCCTGAAGTCCCTCACC	196
<i>AhPAL2c</i>	AAAGGCAACTGCGCTGCTAA	CCAGTCGCTGCTCGCCTTG	237
<i>PP2A</i>	CCTCCTCCTCTTCGGTTTG	GCCATGAATGTCACCGCAGA	235
<i>TUB</i>	TCCCATCACCAAAGGTCTC	CGCTCATTGTGGCAGAGATA	196

Notes: Primers for *Agave PAL* genes and endogenous genes were designed by Primer 3 software.

3. Results

3.1. Transcriptome Analysis of *A. schidigera*

In the transcriptome sequencing and analysis of *A. schidigera*, consequently, a total of 29,852,218 clean reads were generated, with a combined length of 8,955,665,400 bp. The GC content of the sequences was 46.77%. Rates of base quality above 20 and 30 were recorded at 98.57% and 96.2%. Exactly 72,160 unigenes were assembled, with a minimum length of 201 bp, an average length of 656.02 bp, a median length of 363 bp and an N50 length of 1082 bp. More than 50% of the unigenes ranged from 201 bp to 400 bp (Figure 1). Approximately 51.52% and 11.34% of the transcript's sequences were of lengths ≥ 601 bp and ≥ 2000 bp (additional file1: Table S1), with the retention of the largest sequence being 8949 bp, suggesting a greater likelihood of discovering a homolog with putative functions.

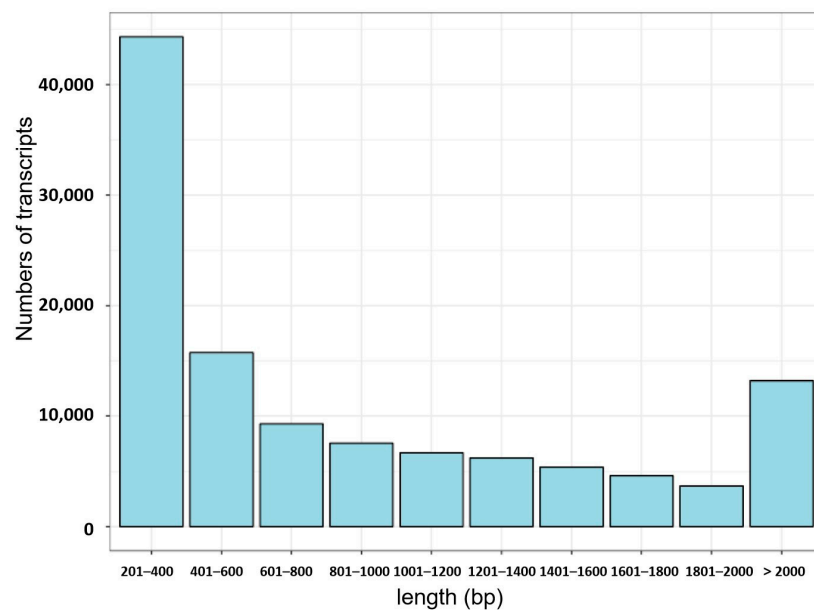


Figure 1. The length distribution of all transcripts. The chart presents data categorized by DNA sequence lengths of all transcripts (bp). The sequences are grouped into the following length ranges: 201–400, 401–600, 801–1000, 1001–1200, 1201–1400, 1401–1600, 1601–1800, 1801–2000, and greater than 2000. The y-axis, labeled as “count”, indicates the number of sequences within each length category.

All transcripts were also mapped to the NR, GO, KEGG, KOG and Swiss-Prot databases, and the annotations are shown in Table 2 below and the additional file1 in Table S1. The GO annotation categorizes the function of genes into three main categories: biological processes, cellular components and molecular functions (Figure 2). There are a large number of genes that have been classified into different biological functional processes, including ‘anatomical structure development’, ‘cellular processes’ and ‘metabolic processes’. In particular,

the ‘metabolic process’ has the highest number of genes of 3565. The cellular component domain shows that 11,738 genes are involved in the ‘cytoplasm’ and 18,883 are involved in ‘intracellular’ functions. In the molecular function category, a significant number of genes are associated with ‘catalytic activity’ and ‘binding’. The top eight KEGG pathways were found to be ‘Transcription’, ‘Folding, sorting and degradation’, ‘Carbohydrate metabolism’, ‘Transport and catabolism’, ‘Energy metabolism’, ‘Amino acid metabolism’, ‘Translation’ and ‘Lipid metabolism’ (Figure 3). The KOG function classification of transcripts shows that the top eight pathways were ‘Post-translational modification, protein turnover, chaperones’, ‘General function prediction only’, ‘Translation, ribosomal structure and biogenesis’, ‘Signal transduction mechanisms’, ‘RNA processing and modification’, ‘Intracellular trafficking, secretion, and vesicular transport’, ‘Transcription’, and ‘Energy production and conversion’ (Figure 4).

Table 2. Annotation rates of all transcripts against public databases.

Database	Number of Transcripts
NR	74,117 (63.56%)
GO	50,228 (43.08%)
KEGG	39,144 (33.57%)
KOG	25,721 (22.06%)
Swiss-Prot	54,024 (46.33%)

Note: The full names of the public databases were shown in Section 2.2.

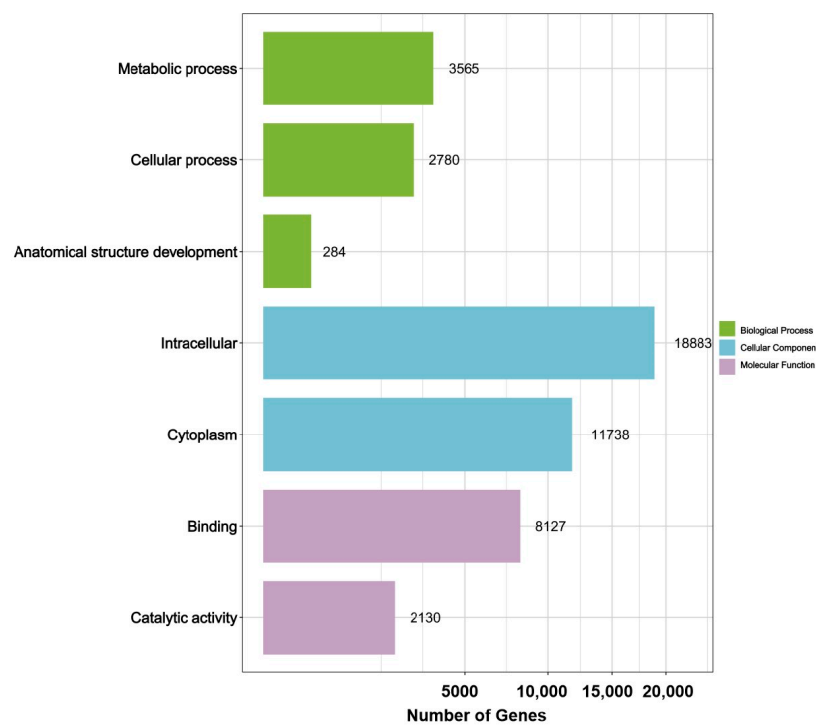


Figure 2. GO annotation of all transcripts. The GO annotation categorizes the function of genes into three main categories, denoted by green (biological processes), blue (cellular components), and purple (molecular functions).

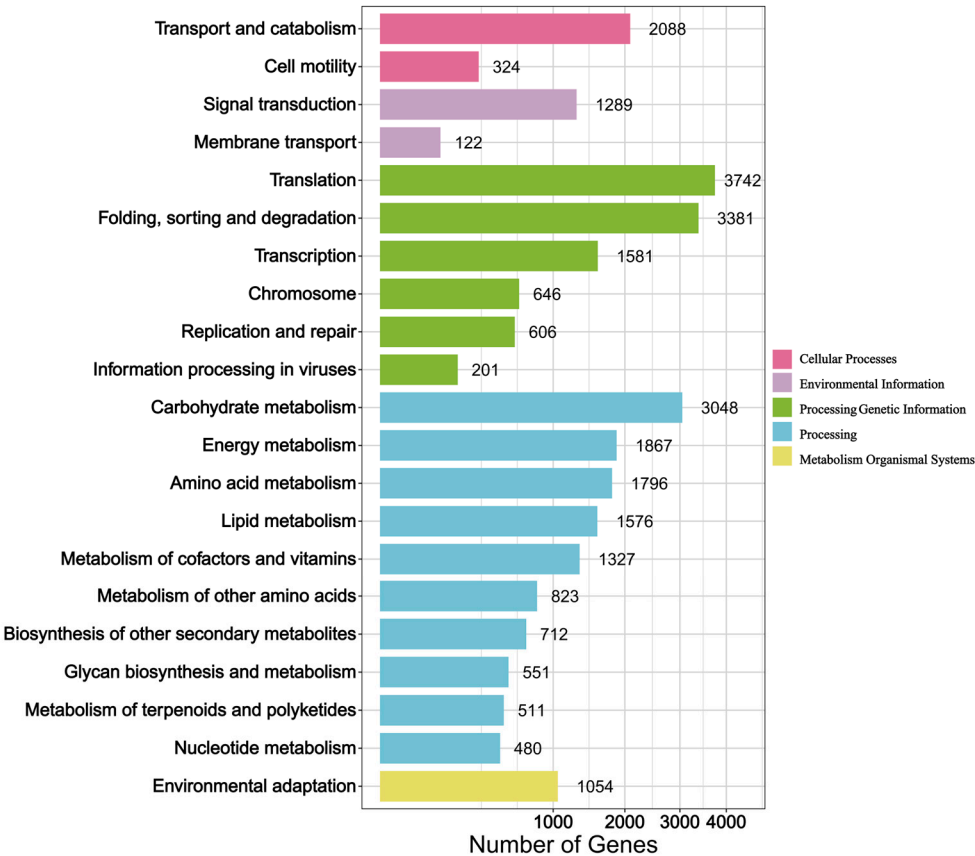


Figure 3. KEGG annotation of all transcripts.

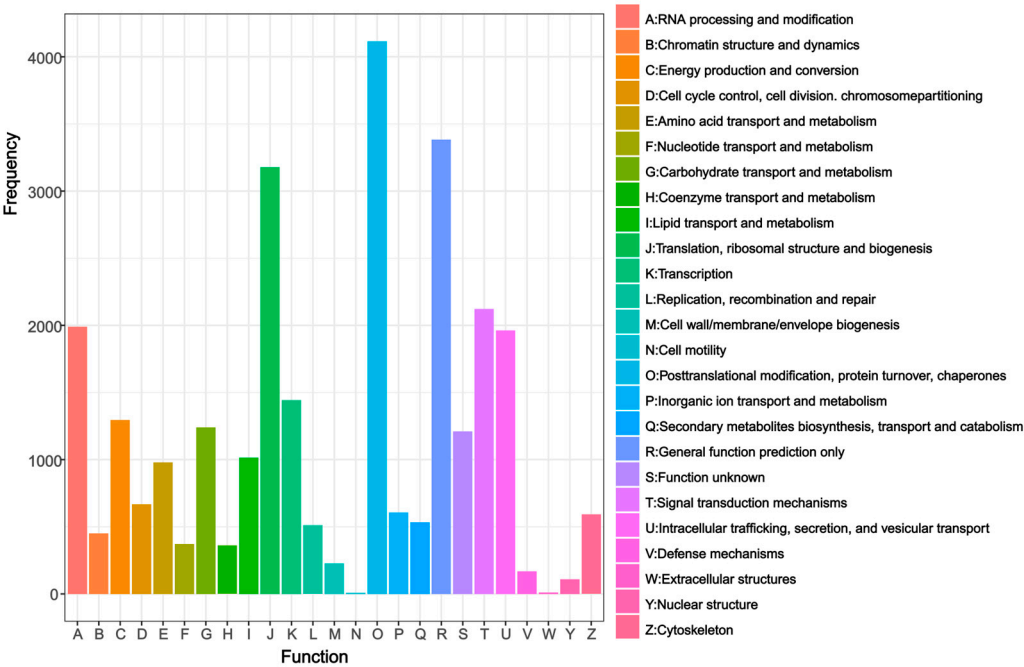


Figure 4. KOG functional classification of the transcripts.

3.2. Identification and Cloning of PAL Genes in Agave Species

The PAL genes of *Arabidopsis* (4) and *Oryza sativa* (9) [43] were selected for the identification of orthologs within the *A. H11648* transcriptome [1] using the TBlastx method [44]. Six genes (*AhPAL1a*, *AhPAL1b*, *AhPAL1c*, *AhPAL2a*, *AhPAL2b*, and *AhPAL2c*) were observed.

In addition, the identified genes were utilized to locate orthologs within the transcriptomic datasets of *Agave* species, encompassing *A. schidigera*, *A. deserti* and *A. tequilana*. Ultimately, two, six and six orthologs with complete coding regions were identified in *A. schidigera*, *A. deserti* and *A. tequilana*, respectively. These whole genes were subsequently amplified in the three *Agave* species mentioned above, and were named *AscPAL1*, *AscPAL2*, *AdPAL1a*, *AdPAL1b*, *AdPAL1c*, *AdPAL2a*, *AdPAL2b*, *AdPAL2c*, *AtqPAL1a*, *AtqPAL1b*, *AtqPAL1c*, *AtqPAL2a*, *AtqPAL2b* and *AtqPAL2c* with the sequence lengths varying from 1818 to 2136 bp (additional file1: Table S2).

3.3. Phylogenetic Analysis of PAL Genes in *Agave* Species

The 33 PAL genes (referred to in Section 2.3) from *Arabidopsis*, *Oryza sativa* and four *Agave* species were chosen for phylogenetic analysis. Following the analysis, these 33 PAL genes were clustered into two branches (Figure 5). The first branch contain PAL genes from *Arabidopsis* and rice, while *Agave* species have PAL sequences clustered in the second branch. The same class of PAL genes from different sisal species were also clustered together.

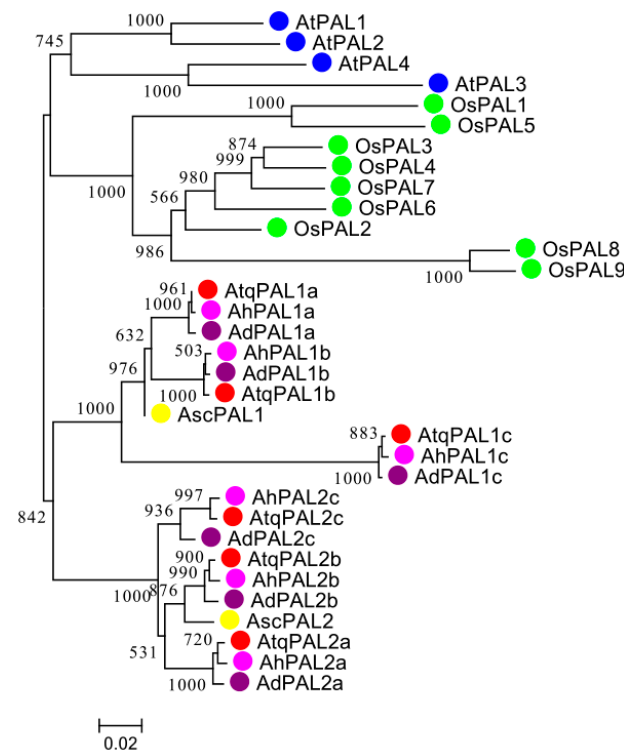


Figure 5. Phylogenetic tree of the PAL gene family. The PAL genes of *Arabidopsis thaliana* (At), *Oryza sativa* (Os), *Agave tequilana* (Atq); *Agave* H11648 (Ah), *Agave deserti* (Ad) and *Agave schidigera* (Asc) are marked in blue, green, red, pink, purple and yellow, respectively.

3.4. Expression Patterns of PAL Genes in *A. H11648*

To detect expression patterns of PAL genes, six genes (*AhPAL1a*, *AhPAL1b*, *AhPAL1c*, *AhPAL2a*, *AhPAL2b*, *AhPAL2c*) from *A. H11648* were selected for further expression analysis. Expression patterns were estimated at different leaf developmental stages (Figure 6). Gene *AhPAL2a* was highly expressed in the shoot as well as in both unexpanded and expanded leaves. Similarly, the gene *AhPAL2c* exhibited elevated expression levels in the shoot and unexpanded leaves. The expression levels of gene *AhPAL1a*, *AhPAL1b* and *AhPAL2a* initially increased before experiencing a decrease, while the expression levels of the *AhPAL2c* gene exhibited a consistent downward trend. Additionally, genes *AhPAL1c* and *AhPAL2b* demonstrated low expression levels at all three leaf stages.

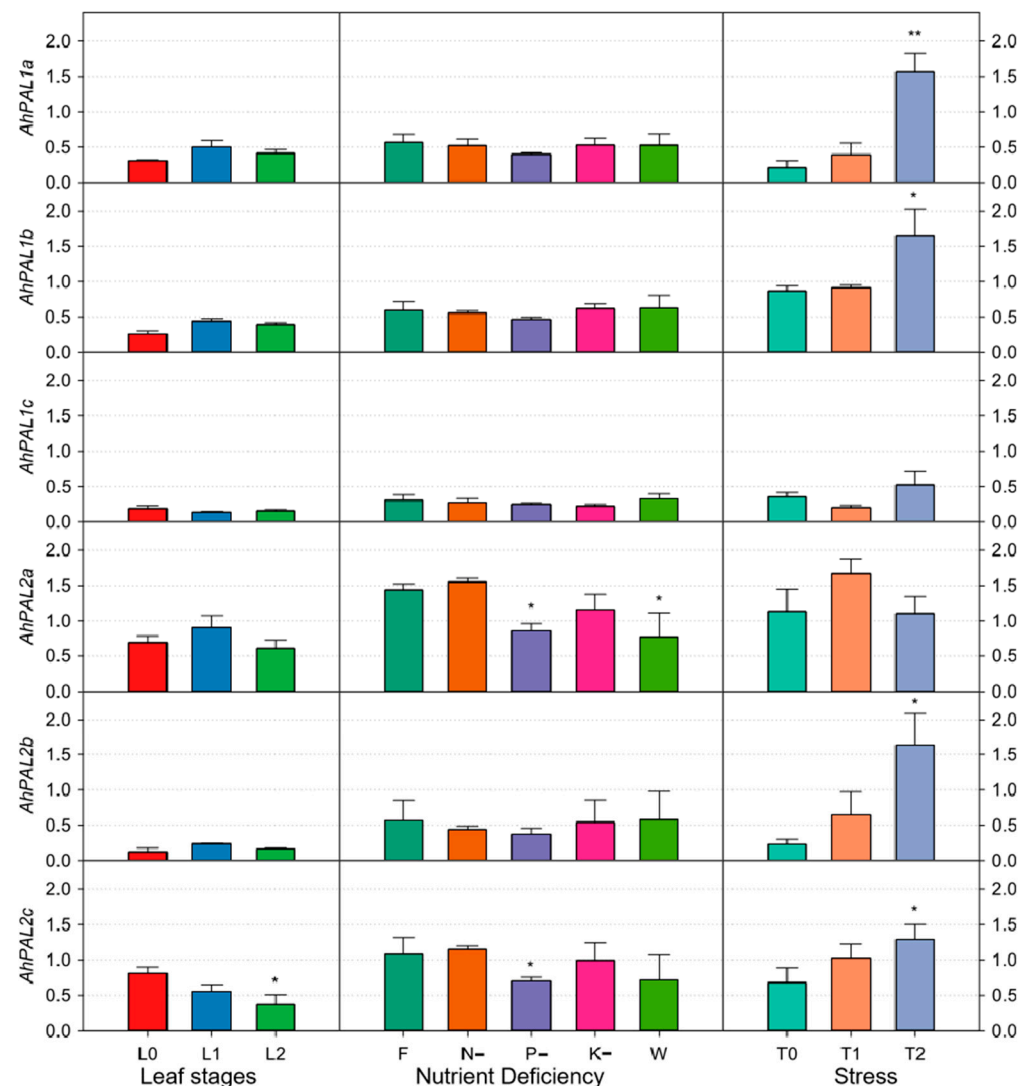


Figure 6. The relative expression of *PAL* genes in *A. H11648* at different leaf developmental stages and under different stresses. Y-axis: expression level. X-axis: L0 (shoot), L1 (unexpanded leaf), L2 (expanded leaf). F (full nutrient), N- (nitrogen free), P- (phosphorus free), K- (potassium free) and W (Sterile water); T0 (Negative control), T1 (*P. nicotianae* Breda inoculation stress 24 h) and T2 (*P. nicotianae* Breda inoculation stress 48 h). The relative expression quantities were obtained with *PP2A* as an endogenous reference gene under the qRT-PCR technique. All data were generated from three biological replicates. The error bar represents the standard error. In each column of the bar charts, L0, F and T0 of the X-axis indicate the control. The expression values of L0, F and T0 were normalized as 1. “*” and “**” demonstrate differences at the 0.05 and 0.01 probability levels, respectively.

In the nutrient deficiency experiment, the full Hoagland nutrient solution was selected as a control (F), while Hoagland nutrient solutions without nitrogen (N-), phosphorus (P-), potassium (K-) and water (W) were used as other treatments. The experimental results indicated that *AhPLA2a* and *AhPLA2c* were sensitive to nutrient phosphorus, while other genes were less sensitive under nutrient deficiency treatments (Figure 5). It is worth noting that *AhPLA2a* and *AhPLA2c* were expressed at high levels under the full-nutrient, nitrogen-free (N-) and potassium-free (K-) treatments, whereas *AhPLA1a*, *AhPLA1b*, *AhPLA1c* and *AhPLA2b* were expressed at low levels in each nutrient deficiency treatment.

Guided by the knowledge that *A. H11648* is often subjected to pathogenic microorganisms during its growth and development, with *P. nicotianae* Breda being the most prevalent infectious agent. Our aim was to evaluate the expression of the *PAL* genes in *A. H11648* by

inoculating *P. nicotianae* Breda as one form of biotic stress. The results demonstrated that the expression levels of *AhPAL1a*, *AhPAL1b*, *AhPAL2b* and *AhPAL2c* increased progressively with the duration of the infection, displaying a significant increase after 48 h. The expression level of *AhPAL2a* increased first and then decreased, but maintained a high level the whole time. There was no significant expression change in gene *AhPAL1c* before or after *P. nicotiana* Breda induction (Figure 6).

4. Discussion

4.1. Characterization of *A. schidigera* Transcriptome

PAL is the first enzyme of the phenylpropanoid metabolic pathway that exerts a vital role in plant growth, development and environmental adaptability. However, lignin is produced from several metabolites of the phenylpropanoid pathway. Previous studies have affirmed that PAL is directly associated with lignin content [45]. Thus, cloning the *PAL* gene and analyzing its function in the development of *Agave* fiber are of great significance for improving the related traits of sisal fiber. Though some chloroplast genomes of *Agave* were submitted to the GenBank database [6], their large genome and high heterozygosity make them challenging to assemble. The EST sequences of *Agave* species available in the NCBI database have been limited and scarce, which has impeded the research on the *Agave* genome and transcriptome, and even the study of the agave fiber development mechanism. Next-generation sequencing (NGS) is an efficient method for acquiring gene sequences in plant species that lack complete genome data. In previous transcriptomic studies of *A. H11648* [1], *A. deserti* [10], *A. amanuensis* [11], *A. angustifolia* [46], *A. tequilana*, and *A. sisalana*, a collection of potential genes associated with fructans, fiber traits and stress response, were mined and analyzed using Illumina sequencing technology. However, as of now, no genetic sequence or transcriptome information for *A. schidigera* has been submitted to any public database, which represents a gap that needs to be filled on a molecular basis.

Accordingly, the transcriptome was assembled with 72,160 unigenes, which will serve as the molecular basis for the study of *A. schidigera*. According to our assembly, the unigene number was higher than that in *A. angustifolia* (66,314) [46] and *A. amaniensis* (66,746) [11] but lower than that in *A. H11648* (148,046) [1]. Here, we successfully obtained 20 *PAL* genes from four *Agave* species with complete coding sequence according to the published transcriptomes of *Agave* leaves, indicating that the main expression sites of these genes were the leaf. Based on the whole-genome-wide analysis of the *Juglans regia* L. *PAL* genes, it is hypothesized that sisal may contain multiple *PAL* genes [45]. The results of phylogenetic analysis showed that *PALs* of *Agave*, *Arabidopsis* and rice are clustered in different branches or sub-branches, indicating that *PAL* genes had species-specific evolution. Previous reports have also confirmed the existence of species-specific *PAL* gene family expansion in *Juglans regia* and *Gramineae* [47].

4.2. Candidate *PAL* Genes in Development of *Agave*

The genes *AhPLA2a* and *AhPLA2c* exhibited high expression levels in the shoot, unexpanded leaf and expanded leaf, though they had different expression patterns (Figure 6). The expression levels of gene *AhPLA1a*, *AhPLA1b*, *AhPLA2a* and *AhPLA2b* initially increased and subsequently decreased. In ramie, the lignin content of ramie fiber increased gradually from development to maturity, while PAL activity increased first and then decreased. The level of stem lignin development in ramie showed a positive relationship with the expression of genes such as *PAL*, *4CL1* and *C4H* within the phenylpropanoid pathway [48]. Sisal leaves were mainly harvested with fully expanded leaves (with an angle greater than 45° to the central axis), and the fibers tended to mature when the leaves were fully unfolded. Therefore, three leaf development stages were selected in this study, corresponding to the first, middle and last three stages of sisal fiber development. During this process, the expression patterns of *AhPLA1a*, *AhPLA1b*, *AhPLA2a* and *AhPLA2b* in sisal were consistent with those in ramie, indicating that the *PAL* gene of sisal is closely related to lignin synthesis in sisal.

It was discovered that diverse nitrogen application could enhance the activity of superoxide dismutase (SOD), PAL and polyphenol oxidase (PPO), as well as the expression of defense genes in faba bean [49]. In contrast, in our study, six *PAL* genes remained insignificantly altered under the nitrogen-free and potassium-free conditions, suggesting post-transcriptional regulation in sisal. While *AhPLA2a* and *AhPLA2c* were sensitive to phosphorus nutrient, four other genes (*AhPAL1a*, *AhPAL1b*, *AhPAL1c*, *AhPAL2b*) were less sensitive under phosphorus deficiency treatments, suggesting that sisal exhibits distinct expression patterns under phosphorus deficiency (Figure 6).

4.3. *PAL* Genes in Biotic/Abiotic Resistance

Meanwhile, some studies have shown that the rice genome encompasses nine *PAL* genes, among which *OsPAL4* is implicated in mediating broad-spectrum resistance to various fungal diseases [50]. In our research, most of the *PAL* genes of sisal were significantly up-regulated subsequent to infection by *P. nicotianae* Breda (Figure 6). Sisal *PAL*s may play a role in the biosynthetic processes of secondary metabolites associated with disease resistance in the phenylpropanoid pathway, as well as in immunity mediated by plant cell walls. Nevertheless, how the *PAL* genes participate in the disease resistance mechanism by regulating the phenylpropanoid metabolic pathway remains elusive and needs to be further investigated.

Phenylalanine ammonia-lyase (*PAL*) serves as a crucial enzyme for regulating the transfer of primary metabolites into the secondary metabolic pathway that plays a significant role in the defense mechanisms of plants against pathogens and other forms of insect resistance [51]. On the invasion of pathogens and insects, the configuration of the plant cell wall is compromised easily. In such a situation, the plant triggers an innate defense mechanism and restores its impaired structure caused by its undermined integrity [52]. Additionally, the *PAL* genes govern lignin biosynthesis for cell wall repair and participate in the synthesis of numerous secondary metabolites related to defense-related phytohormone salicylic acid (SA) and flavonoid-type phytoalexins sakuranetin and naringenin [53].

It is well known that soil pollution is a global problem; however, most solutions are too expensive for large-scale applications or may lead to secondary pollution [54]. In contrast to conventional techniques like chemical solidification, stabilization, or soil washing, phytoremediation is characterized by its low invasiveness and cost-effectiveness. In a study regarding the potential of soil remediation by different plants in mine, *A. americana* demonstrated a strong enrichment of metals such as Cd, Cr, Pb and Cu aboveground, underground, and in the rhizosphere of soils. Moreover, its biomass and phenotype were not significantly influenced, suggesting that this *Agave* plant has high tolerance to heavy metals [55]. The *Agave* plant has very strong tolerance to heavy metals stresses, which is closely related to the secondary metabolites in it, and *PAL* is the initial enzyme of secondary metabolites. Thus, the study of this *PAL* gene family is crucial for the future analysis of the mechanism of heavy metal resistance.

5. Conclusions

This research presents a study on the *A. schidigera*, a prominent fiber crop in tropical regions, and provides the first comprehensive transcriptome data for *A. schidigera*, revealing 72,160 unigenes and acting as a rich resource for understanding lignin traits in *Agave* species. The identification and expression pattern analysis of *PAL* genes may not only deepen the research on the development mechanism of sisal fiber but also enhance the understanding of the disease resistance mechanism in sisal. The up-regulation of *PAL* genes in response to biotic and abiotic stresses indicates their role in the plant's defense mechanisms, potentially through the regulation of secondary metabolite biosynthesis by *PAL* genes. The distinct expression patterns of *PAL* genes in different developmental stages and under nutrient deficiencies indicate the complexity of their regulation and their significance in the adaptation of *Agave* species to their environment. The research results will lay a molecular basis for future research on lignin biosynthesis, the relationship

between lignin and fiber strength, and stress resistance in *Agave* species. The results will also provide a basis for breeding sisal varieties with high fiber quality and strong stress resistance, and even support potential applications in heavy metal adsorption and soil remediation work.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy14112520/s1>, Table S1: Annotations of all transcripts; Table S2: Identification and cloning of agave *PAL* genes.

Author Contributions: Conceptualization, X.W., X.H. (Xiaoli Hu) and X.H. (Xing Huang); methodology, X.W., X.H. (Xiaoli Hu), C.L., Q.L., Y.L. and X.H. (Xing Huang); software, X.W., Y.L., D.D. and X.H. (Xing Huang); formal analysis, X.W., X.H. (Xiaoli Hu), D.D., W.Z. and X.H. (Xing Huang); investigation, X.W., X.H. (Xiaoli Hu), Y.L. and W.Z.; resources, C.L. and Q.L.; writing—original draft preparation, X.W.; writing—review and editing, D.M., X.H. (Xing Huang) and K.Y.; supervision, K.Y.; project administration, X.H. (Xing Huang) and K.Y.; funding acquisition, X.H. (Xing Huang) and K.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Hainan Provincial Natural Science Foundation of China (323RC544), National Natural Science Foundation of China (32001598), China Agriculture Research System of MOF, MARA (CARS-16) and Central Public-interest Scientific Institution Basal Research Fund (1630042022005), Scientific research plan of Education Department of Hubei Province of China (B2023360).

Data Availability Statement: All data are contained within the article or Supplementary Materials.

Acknowledgments: We would like to thank Xiaohan Yang from Oak Ridge National Laboratory (Oak Ridge, TN 37831, USA) for his thorough suggestions about the experiment's design.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Huang, X.; Xiao, M.; Xi, J.; He, C.; Zheng, J.; Chen, H.; Yi, K. De novo transcriptome assembly of *Agave* H11648 by Illumina sequencing and identification of cellulose synthase genes in *Agave* species. *Genes* **2019**, *10*, 103. [CrossRef] [PubMed]
- Bermudez-Bazan, M.; Castillo-Herrera, G.A.; Urias-Silvas, J.E.; Escobedo-Reyes, A.; Estarron-Espinosa, M. Hunting bioactive molecules from the *Agave* genus: An update on extraction and biological potential. *Molecules* **2021**, *26*, 6789. [CrossRef] [PubMed]
- Good-Avila, S.V.; Souza, V.; Gaut, B.S.; Eguiarte, L.E. Timing and rate of speciation in *Agave* (*Agavaceae*). *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 9124–9129. [CrossRef] [PubMed]
- Lopez-Romero, J.C.; Ayala-Zavala, J.F.; Gonzalez-Aguilar, G.A.; Pena-Ramos, E.A.; Gonzalez-Rios, H. Biological activities of *Agave* by-products and their possible applications in food and pharmaceuticals. *J. Sci. Food Agric.* **2018**, *98*, 2461–2474. [CrossRef]
- Gross, S.M.; Martin, J.A.; Simpson, J.; Abraham-Juarez, M.J.; Wang, Z.; Visel, A. De novo transcriptome assembly of drought tolerant CAM plants, *Agave deserti* and *Agave tequilana*. *BMC Genom.* **2013**, *14*, 563. [CrossRef]
- Xu, B.; Tan, S.; Qin, X.; Huang, X.; Xi, J.; Chen, H.; Yi, K. The complete chloroplast genome of *Agave amaniensis* (*Asparagales: Asparagaceae: Agavoideae*). *Mitochondrial DNA B Resour.* **2022**, *7*, 1519–1521. [CrossRef]
- Lu, Z.; Hou, X.; Ke, Z.; Zhang, Y.; Yang, Z.; Zhou, W. A newly identified glycosyltransferase AsRCOM provides resistance to purple curl leaf disease in agave. *BMC Genom.* **2023**, *24*, 669. [CrossRef]
- Li, Y.; Mai, Y.; Ye, L. Sisal fibre and its composites: A review of recent developments. *Compos. Sci. Technol.* **2000**, *60*, 2037–2055. [CrossRef]
- Schultz, T.P.; Narayan, R.; Rowell, R.M. *Emerging Technologies for Materials and Chemicals from Biomass*; American Chemical Society: Washington, DC, USA, 1992.
- Huang, X.; Wang, B.; Xi, J.; Zhang, Y.; He, C.; Zheng, J.; Yi, K. Transcriptome comparison reveals distinct selection patterns in domesticated and wild *Agave* species, the important CAM plants. *Int. J. Genom.* **2018**, *2018*, 5716518. [CrossRef]
- Wang, X.; Huang, X.; Chen, L.; Xie, Z.; Tan, S.; Qin, X.; Yi, K. Transcriptome sequencing of *Agave amaniensis* reveals shoot-related expression patterns of *Expansin A* genes in *Agave*. *Plants* **2023**, *12*, 2020. [CrossRef]
- Deng, G.; Huang, X.; Xie, L.; Tan, S.; Gbokie, T.J.; Bao, Y.; Yi, K. Identification and expression of *SAUR* genes in the CAM Plant *Agave*. *Genes* **2019**, *10*, 555. [CrossRef] [PubMed]
- Serra-Parareda, F.; Tarres, Q.; Espinach, F.X.; Vilaseca, F.; Mutje, P.; Delgado-Aguilar, M. Influence of lignin content on the intrinsic modulus of natural fibers and on the stiffness of composite materials. *Int. J. Biol. Macromol.* **2020**, *155*, 81–90. [CrossRef] [PubMed]
- Chatterjee, S.; Saito, T. Lignin-derived advanced carbon materials. *ChemSusChem* **2015**, *8*, 3941–3958. [CrossRef]
- Lin, W.; Liu, A.; Weng, C.; Li, H.; Sun, S.; Song, A.; Zhu, H. Cloning and characterization of a novel *phenylalanine ammonia-lyase* gene from *Inonotus baumii*. *Enzym. Microb. Technol.* **2018**, *112*, 52–58. [CrossRef] [PubMed]

16. Huang, X.; Bai, X.; Xie, Z.; Fahad, S.; Gbokie, T., Jr.; Lu, Y.; Guo, T.; Li, J.; Zhang, Z.; Wu, W.; et al. De novo transcriptome assembly of *Coffea liberica* reveals phylogeny and expression atlas of phenylalanine ammonia-lyase genes in *Coffea* species. *Ind. Crops Prod.* **2023**, *192*, 116029. [\[CrossRef\]](#)
17. Rohde, A.; Morreel, K.; Ralph, J.; Goeminne, G.; Hostyn, V.; De Rycke, R.; Boerjan, W. Molecular phenotyping of the *pal1* and *pal2* mutants of *Arabidopsis thaliana* reveals far-reaching consequences on phenylpropanoid, amino acid, and carbohydrate metabolism. *Plant Cell.* **2004**, *16*, 2749–2771. [\[CrossRef\]](#)
18. Wu, X.; Cui, Z.; Li, X.; Yu, Z.; Lin, P.; Xue, L.; Yu, F. Identification and characterization of PAL genes involved in the regulation of stem development in *Saccharum spontaneum* L. *BMC Genom. Data* **2024**, *25*, 38. [\[CrossRef\]](#)
19. Zhang, H.; Zhang, X.; Zhao, H.; Hu, J.; Wang, Z.; Yang, G.; Wan, H. Genome-wide identification and expression analysis of phenylalanine ammonia-lyase (PAL) family in rapeseed (*Brassica napus* L.). *BMC Plant Biol.* **2023**, *23*, 481. [\[CrossRef\]](#)
20. Huang, J.; Gu, M.; Lai, Z.; Fan, B.; Shi, K.; Zhou, Y.H.; Chen, Z. Functional analysis of the *Arabidopsis* PAL gene family in plant growth, development, and response to environmental stress. *Plant Physiol.* **2010**, *153*, 1526–1538. [\[CrossRef\]](#)
21. Minami, E.; Ozeki, Y.; Matsuoka, M.; Koizuka, N.; Tanaka, Y. Structure and some characterization of the gene for phenylalanine ammonia-lyase from rice plants. *Eur. J. Biochem.* **1989**, *185*, 19–25. [\[CrossRef\]](#)
22. Wanner, L.A.; Li, G.; Ware, D.; Somssich, I.E.; Davis, K.R. The phenylalanine ammonia-lyase gene family in *Arabidopsis thaliana*. *Plant Mol. Biol.* **1995**, *27*, 327–338. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Elkind, Y.; Edwards, R.; Mavandad, M.; Hedrick, S.A.; Ribak, O.; Dixon, R.A.; Lamb, C.J. Abnormal plant development and down-regulation of phenylpropanoid biosynthesis in transgenic tobacco containing a heterologous phenylalanine ammonia-lyase gene. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9057–9061. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Reichert, A.I.; He, X.Z.; Dixon, R.A. Phenylalanine ammonia-lyase (PAL) from tobacco (*Nicotiana tabacum*): Characterization of the four tobacco PAL genes and active heterotetrameric enzymes. *Biochem. J.* **2009**, *424*, 233–242. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Lister, C.E.; Lancaster, J.E.; Walker, J.R.L. Phenylalanine ammonia-lyase (PAL) activity and its relationship to anthocyanin and flavonoid levels in New Zealand-grown apple cultivars. *J. Am. Soc. Hortic. Sci.* **1996**, *2*, 281–285. [\[CrossRef\]](#)
26. Gao, X.; Hu, Y.; Xu, Z.; Peng, D.; Guo, Q. Expression profiling of the phenylalanine ammonia-lyase (PAL) gene family in *ginkgo biloba* L. *Plant Signal. Behav.* **2023**, *18*, 2271807. [\[CrossRef\]](#)
27. Vishwakarma, S.K.; Singh, N.; Kumaria, S. Genome-wide identification and analysis of the PAL genes from the orchids *Apostasia shenzhenica*, *Dendrobium catenatum* and *Phalaenopsis equestris*. *J. Biomol. Struct. Dyn.* **2023**, *41*, 1295–1308. [\[CrossRef\]](#)
28. Mo, F.; Li, L.; Zhang, C.; Yang, C.; Chen, G.; Niu, Y.; Chen, Y. Genome-wide analysis and expression profiling of the phenylalanine ammonia-lyase gene family in *Solanum tuberosum*. *Int. J. Mol. Sci.* **2022**, *23*, 6833. [\[CrossRef\]](#)
29. Liu, H.; He, Q.; Hu, Y.; Lu, R.; Wu, S.; Feng, C.; Wang, Z. Genome-wide identification and expression profile analysis of the phenylalanine ammonia-lyase gene family in *Hevea brasiliensis*. *Int. J. Mol. Sci.* **2024**, *25*, 5052. [\[CrossRef\]](#)
30. Huang, X.; Hu, X.; Liu, Q.; Xie, Z.; Tan, S.; Qin, X.; Yi, K. Full-length agave transcriptome reveals candidate glycosyltransferase genes involved in hemicellulose biosynthesis. *Int. J. Biol. Macromol.* **2024**, *274*, 133508. [\[CrossRef\]](#)
31. Gao, J.; Luoping; Guo, C.; Li, J.; Liu, Q.; Chen, H.; Yi, K. AFLP analysis and zebra disease resistance identification of 40 sisal genotypes in China. *Mol. Biol. Rep.* **2012**, *39*, 6379–6385. [\[CrossRef\]](#)
32. Wang, P.; Gao, J.; Yang, F.; Zheng, J.; Liu, Q.; Chen, H.; Yi, K. Transcriptome of sisal leaf pretreated with *Phytophthora nicotianae* Breda. *Chin. J. Trop. Crops* **2014**, *35*, 576–582.
33. Pruitt, K.D.; Tatusova, T.; Maglott, D.R. NCBI reference sequence (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res.* **2005**, *33*, D501–D504. [\[CrossRef\]](#)
34. Ashburner, M.; Ball, C.A.; Blake, J.A.; Botstein, D.; Butler, H.; Cherry, J.M.; Sherlock, G. Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* **2000**, *25*, 25–29. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Ogata, H.; Goto, S.; Sato, K.; Fujibuchi, W.; Bono, H.; Kanehisa, M. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **1999**, *27*, 29–34. [\[CrossRef\]](#)
36. Kanehisa, M.; Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **2000**, *28*, 27–30. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Tatusov, R.L.; Fedorova, N.D.; Jackson, J.D.; Jacobs, A.R.; Kiryutin, B.; Koonin, E.V.; Natale, D.A. The COG database: An updated version includes eukaryotes. *BMC Bioinformatics* **2003**, *4*, 41. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Bairoch, A.; Apweiler, R. The SWISS-PROT protein sequence database: Its relevance to human molecular medical research. *J. Mol. Med.* **1997**, *75*, 312–316. [\[PubMed\]](#)
39. Mount, D.W. Using the basic local alignment search tool (BLAST). *CSH Protoc.* **2007**, *2007*, pdb.top17. [\[CrossRef\]](#)
40. Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; McGettigan, P.A.; McWilliam, H.; Higgins, D.G. Clustal W and Clustal X version 2.0. *Bioinformatics* **2007**, *23*, 2947–2948. [\[CrossRef\]](#)
41. Untergasser, A.; Cutcutache, I.; Koressaar, T.; Ye, J.; Faircloth, B.C.; Remm, M.; Rozen, S.G. Primer3—New capabilities and interface. *Nucleic Acids Res.* **2012**, *40*, e115. [\[CrossRef\]](#)
42. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔC_T} Method. *Methods* **2001**, *25*, 402–408. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Yu, X.Z.; Fan, W.J.; Lin, Y.J.; Zhang, F.F.; Gupta, D.K. Differential expression of the PAL gene family in rice seedlings exposed to chromium by microarray analysis. *Ecotoxicology* **2018**, *27*, 325–335. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [\[CrossRef\]](#) [\[PubMed\]](#)

45. Lu, J.; Shi, Y.; Li, W.; Chen, S.; Wang, Y.; He, X.; Yin, X. *RcPAL*, a key gene in lignin biosynthesis in *Ricinus communis* L. *BMC Plant Biol.* **2019**, *19*, 181. [\[CrossRef\]](#)
46. Huang, X.; Xu, B.; Tan, S.; Huang, Y.; Xi, J.; Qin, X.; Yi, K. Transcriptome sequencing of *Agave angustifolia* reveals conservation and diversification in the expression of *cinnamyl alcohol dehydrogenase* genes in *Agave* Species. *Agriculture* **2022**, *12*, 1003. [\[CrossRef\]](#)
47. Yan, F.; Li, H.; Zhao, P. Genome-wide identification and transcriptional expression of the *PAL* gene family in common walnut (*Juglans Regia*, L.). *Genes* **2019**, *10*, 46. [\[CrossRef\]](#)
48. Tang, Y.; Liu, F.; Xing, H.; Mao, K.; Chen, G.; Guo, Q.; Chen, J. Correlation analysis of lignin accumulation and expression of key genes involved in lignin biosynthesis of ramie (*Boehmeria nivea*). *Genes* **2019**, *10*, 389. [\[CrossRef\]](#)
49. Hu, B.; Zheng, Y.; Wang, D.; Guo, Y.; Dong, Y. Managing faba bean wilt disease through intercropping with wheat and reasonable nitrogen application: Enhancing nutrient absorption and biochemical resistance in faba beans. *Physiol. Mol. Biol. Plants* **2014**, *30*, 1029–1046. [\[CrossRef\]](#)
50. Wang, R.; Wang, G.L.; Ning, Y. PALs: Emerging key players in broad-spectrum disease resistance. *Trends Plant Sci.* **2019**, *24*, 785–787. [\[CrossRef\]](#)
51. He, J.; Liu, Y.; Yuan, D.; Duan, M.; Liu, Y.; Shen, Z.; Wan, J. An R2R3 MYB transcription factor confers brown planthopper resistance by regulating the phenylalanine ammonia-lyase pathway in rice. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 271–277. [\[CrossRef\]](#)
52. Bacete, L.; Melida, H.; Miedes, E.; Molina, A. Plant cell wall-mediated immunity: Cell wall changes trigger disease resistance responses. *Plant J.* **2018**, *93*, 614–636. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Duan, L.; Liu, H.; Li, X.; Xiao, J.; Wang, S. Multiple phytohormones and phytoalexins are involved in disease resistance to *Magnaporthe oryzae* invaded from roots in rice. *Physiol. Plant.* **2014**, *152*, 486–500. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Jiang, M.; Wang, K.; Wang, Y.; Zhao, Q.; Wang, W. Technologies for the cobalt-contaminated soil remediation: A review. *Sci. Total Environ.* **2022**, *813*, 151908. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Niu, X.; Jia, Y.; Wu, X.; Wang, S.; Hou, J.; Zhang, W. Phytoremediation potential of indigenous plants growing in soils affected by mine activities in Gejiu City, Yunnan Province. *Int. J. Phytoremediation* **2023**, *25*, 880–888. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.