forests

MDPI

Review

Dragon's Blood from *Dracaena cambodiana* in China: Applied History and Induction Techniques toward Formation Mechanism

Xupo Ding [1,2], Jiahong Zhu [2], Hao Wang [1], Huiqin Chen [1] and Wenli Mei [1,2,*]

[1] Hainan Key Laboratory for Research and Development of Natural Products from *Li folk* Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, No. 4 Xueyuan Road, Haikou 571101, Hainan, China; dingxupo@itbb.org.cn (X.D.); wanghao@itbb.org.cn (H.W.); chenhuiqin@itbb.org.cn (H.C.)

[2] Key Laboratory of Biology and Genetic Resources of Tropical Crops of Ministry of Agriculture and Rural Affairs, Institute of Tropical Bioscience and Biotechnology; Chinese Academy of Tropical Agriculture Sciences, No. 4 Xueyuan Road, Haikou 571101, Hainan, China; zhujiahong@itbb.org.cn

* Correspondence: meiwenli@itbb.org.cn; Tel.: +86-898-6698-7529

Received: 19 January 2020; Accepted: 25 March 2020; Published: 26 March 2020

Abstract: Dragon's blood that is extracted from *Dracaena* plants has been widely used as traditional medicine in various ancient cultures. The application of dragon's blood has a cherished history in China, even though the original plants were not discovered for some period. *Dracaena cochinchinensis* and *Dracaena cambodiana* were successively discovered in southern China during the 1970s–1980s. In the last half of the century, Chinese scientists have extensively investigated the production of dragon's blood from these two *Dracaena* species, whereas these results have not been previously systematically summarized, as in the present paper. Herein, we present the applied history in ancient China and artificially induced technologies for dragon's blood development based on these two *Dracaena* species, in particular, using tissue cultures seedlings and tender plants of *D. cambodiana*. Big data research, including transcriptomic and genomic studies, has suggested that dragon's blood might be a defense substance that is secreted by *Dracaena* plants in response to (a)biotic stimuli. This review represents an effort to highlight the progress and achievements from applied history as well as induction techniques that are used for the formation of dragon's blood that have taken place in China. Such knowledge might aid in the global conservation of wild *Dracaena* species and contribute to understanding dragon blood formation mechanisms, eventually assisting in the efficient utilization of limited *Dracaena* plant resources for the sustainable production of dragon's blood.

Keywords: dragon's blood; *Dracaena cambodiana*; big data; genetic background; formation mechanism; organelle genome; survey sequencing

1. Introduction

Dragon's blood, a crimson resin that is exuded from the injured branch or trunk of *Dracaena* plants from Asparagaceae family [1,2], has been broadly utilized in the histories of many cultures as a traditional medicine for curing fractures, wounds, diarrhea, piles, and stomach and intestinal ulcers [3,4]. Modern pharmacological research has demonstrated that dragon's blood also has anti-inflammatory, antimicrobial, antioxidant, antitumor, and cytotoxic activities [4]. Further studies have indicated that flavonoids, saponins, terpenes, and steroids that are biosynthesized in plant secondary metabolism pathways are some of the main pharmacodynamic compounds found in dragon's blood [5–10].

The sources and identification of dragon's blood have always generated great confusion. Being used as a traditional name for many different resins, dragon's blood has been described in the

medical literature as being obtained from the stems of *Dracaena draco* (L.) L, *Dracaena cinnabari* Balf.f, *Pterocarpus draco* L., *Croton lechleri* Müll.Arg., *Croton gossypifolium* Vahl, and the fruit of *Daemonorops draco* (Willd.) Blume, in these cases being respectively referred to as Canary dragon's blood, Socotran dragon's blood, West Indian dragon's blood, Mexican dragon's blood, Venezuelan dragon's blood, and East Indian dragon's blood [11,12], but dragon's blood was originally produced from *D.cinnabari*, later from *Dracaena draco* and more recently from *Daemonorops draco* [4,13]. In China, dragon's blood had always been considered to be exotic before the source species were discovered in Yunnan Province of China. Even now, a large proportion of dragon's blood used in China is dependent on import from the countries of Southeast Asia.

Resource shortages and medical requirements have resulted in an abundance of research in the last half-century regarding the production of dragon's blood based on limited *Dracaena* plants. Here, we elaborate on the history regarding the discovery and exploitation of dragon's blood in China, and the two species from which it was first obtained. Subsequently, we outline the artificial inducing techniques for dragon's blood formation, based on *D. cochinchinensis* (Lour.) S.C. Chen and *D. cambodiana* Pierre ex Gagnep and developed by Chinese scientists, in particular, for those technologies that are involved in the plant tissue cultures seedling of *D. cambodiana* for producing dragon's blood. Most importantly, we examine the current transcriptomic and survey genomic approaches that are used to study the mechanisms of dragon's blood formation in *D. cambodiana* plants in China.

2. The History of Dragon's Blood as a Traditional Chinese Medicine in China

De Materia Medica, edited by Pedanius Dioscorides (approximately 40–90 AD), who was a Greek physician, pharmacologist, and botanist, lists the earliest record of dragon's blood and *Dracaena* plants [14]. He first encountered *Dracaena* plants and the production of dragon's blood in the Middle East and North African regions while he was employed as a physician in the Roman army. Therefore, the history of dragon's blood use might pre-date the first century in these local ancient cultures. Dragon's blood use as a traditional medicine in China can be dated from the fifth century AD, which is later than its use by Greeks and Egyptians, on account of the lack of source plants. Dragon's blood was first documented in "Lei Gongs Treatise on Preparation and Boiling of Materia Medica (Lei Gong Pao Zhi Lun)" by Xiao Lei in the Liu-Song Period (approximately 420–479 AD) of the Northern and Southern Dynasties of China; the pharmacologists of the time considered dragon's blood to have the functions of promoting blood circulation and removing stasis in blood, healing bleeding and sores, and to function as an anti-inflammatory and analgesic. However, the source plant was not described until approximately the 1060s AD during the Northern Song Dynasty, in which "Illustrated Classics of Materia Medica (Ben Cao Tu Jing)" of Song Su appears to have been published. Furthermore, dragon's blood was also listed in the New Revised Materia Medica (Xin Xiu Ben Cao), Chu-Fan Chih (Zhu Fan Zhi), and Materia Medica of South Yunnan (Dian Nan Ben Cao), all of which are classical traditional Chinese medicine (TCM) records. The most famous of these is the Compendium of Materia Medica, edited by Shizhen Li, who was the most distinguished pharmacist in ancient China and East Asia, confirming that dragon's blood was an effective medicine for stimulating blood circulation [15]. Modern natural production chemistry and pharmacological researches has indicated that the monomeric compounds extracted from dragon's blood also have anti-inflammatory, antimicrobial, antioxidant, antitumor, and cytotoxic activities, such as Loureisin A, Loureisin B, 7,4'- dihydroxyflavone, and 5,7,4'-trihydroxyflavone [6–9]. The first source species of dragon's blood were *Dracaena* plants in ancient China, followed by *Daemonorops draco* from Southeast Asia [4,13]. Even now, the fruits of exotic *Daemonorops draco* represent the main source of dragon's blood in China [16]. Excitement arose when two source species for dragon's blood, *D.cochinchinensis* and *D.cambodiana*, were found in China, and scientific research that was based on these two species began in the 1970s. Wild *Dracaena* plants have been excessively exploited due to their medicinal and economic importance, and many of them have been considered to be endangered [17], including *D.cochinchinenesis* and *D. cambodiana*.

3. The *Dracaena* Source Species and Their Conservation in China

3.1. Dracaena cochinchinensis

In 1972, the team of Prof. Xitao Cai from Kunming Institute of Botany, Chinese Academy of Sciences, found *D. cochinchinensis* in Yunnan Province of China and determined its dragon's blood to have equal medicinal efficacy to that sourced from other *Dracaena* species that were in use. The sapling of *D. cochinchinensis* can increase by around 10–20 cm in nature, or approximately 23–29 cm under cultivation conditions, every year [18]. The ensuing investigation indicated that *D. cochinchinensis* was distributed in the subtropical and tropical regions between approximately 21.5°–23.6° N, such as in southern China, Vietnam, Cambodia, and Laos [19]. A large number of *D. cochinchinensis* plants were found in southern Yunnan and Guangxi Province of China in the 1980s, being distributed at altitudes that ranged from approximately 400 to 1700 m. Subsequently, the dragon's blood from *D. cochinchinensis* and its associated medicines were also developed and named as dragon's blood of Guangxi Xue Jie or Long Xue Jie [20].

3.2. Dracaena cambodiana

Dracaena cambodiana was discovered in China during the investigation of sustainable traditional medicine resources in the 1980s, and this *Dracaena* species was distributed in jungle, stone cracks, cliffs, or desert islands in Hainan Province and southeast Asia, for instance, in Vietnam and Cambodia [21]. As a cork tree, the *D. cambodiana* plant could not be used as firewood or furniture, and a significant level of moisture was stored in its stem and branch, thus showing excellent drought tolerance. These immense adaptations indicated that the population of *D. cambodiana* predominantly formed in or around tropical forests and the individuals of *D.cambodiana* tree are often several hundred years old [18].

3.3. Conservation of D. cochinchinensis and D. cambodiana in China

Dracaena plants have been exhausted for their economic and medicinal importance in the period of 1980s ~ 2000s, and many of them were considered to be endangered. In 1998, many of the *Dracaena* species were list in the Red List of IUCN (The International Union for Conservation of Nature) [22–24]. Soon afterwards, wild *D.cochinchinensis* and *D.cambodiana* species in China had been list as endangered species in 2001 and were then prohibited to felling. The stem of these two *Dracaena* species thicken slowly and only the 30~50 years old trees can produce a small amount of dragon's blood [25]. There are the two main causes of raw material of dragon's blood in China relying on import commerce. *D.cambodiana* is also a horticultural plant species and its tender plant is widely cultured in chamber as bonsai or planted in courtyard as an ornamental tree [26]. The scientists have been focusing on producing dragon's blood while using these tender of *D.cambodiana* since *Dracaena* plants discovered in China and many inducing experiments have been performed. These artificial inducing technologies might contribute to reduce the demand for wild harvested dragon's blood.

4. The Technology for Artificial Inducing Dragon's Blood Formation

4.1. Wounds and Dragon's Blood Formation

The initial research of Prof. Cai revealed that artificial wounds could promote red resin accumulation in the stem of *D. cochinchinensis*; hence, the wound became a controlled experiment for further studies on dragon's blood formation in *Dracaena* species that is still used now. This dragon's blood has both similar chemical constituents and clinical curative effect as dragon's blood from Africa, but its constituents are different to dragon's blood from Southeast Asia, although it has same clinical efficacy as Indonesian dragon's blood that is extracted from the fruit of *Daemonorops* species [27]. Further textual criticism demonstrated that the dragon's blood that was used throughout the ancient history of Traditional Chinese Medicine was extracted from the *Dracaena* species planted in the regions

of Africa or West Asia, and that dragon's blood from *Daemonorops* species was the succedaneum of African dragon's blood [28,29].

4.2. Microorganisms and Dragon's Blood Formation

Previous studies in model plants have indicated that defense components, such as benzoxazine, camalexins, capsidiol, momilactone, piceids, sakuranetin, and resveratrol, can be induced by microorganisms, and that many of them are flavonoids [30–34]. Modern natural product chemistry has demonstrated that dragon's blood is composed of flavonoids, sterol, lignin, stilbene, and saponin, and that flavonoids are the major constituents [35–37]. Microorganisms may also be involved in the formation of dragon's blood. The earliest studies on the relationship between microorganisms and dragon's blood focused on *D. cochinchinensis*. 303 fungi strains, divided into 23 genera, were isolated from xylem containing dragon's blood from *D. cochinchinensis* plants, and 52% of strains were *Fusarium* species. Of these *Fusarium* strains, 38% were identified as *F. graminearum* Schw. The other *Fusarium* strains were *F.culmorum* (W.G.M.) Sacc. (20%), *F. tricinctum* (Cd.) Snyd. et Hans (18%), *F. solani* f. sp. pisi (13%), *F. sporotrichioides* Sherb. (10%), and with an isolation frequency below 5% were *Fusarium* strains *F. oxysporum* Schl., *F. moniliforme* Sheld., and *F. lateritium* (Nees) emend. Snyder and Hansen. Although *Aureobasidium* and *Cladosporium* strains were also isolated, the *Fusarium* genus was considered as the predominant strain on account of its high isolation frequency, and subsequent studies also revealed that the *Fusarium* species could induce dragon's blood formation in *D. cochinchinensis* stems [38]. Furthermore, 172 fungi strains were isolated from the leaf, stem, or roots of *D. cambodiana*, with the stem showing the highest abundance of *Fusarium* species [39].

After being infected by *F. graminum* at 0, 5, 10, and 15 days, 7,4′-dihydroxyflavanone, 7-hydroxy-4′-methoxyflavane, and loureirin A-the main active components of dragon's blood from *D. cochinchinensis*—continuously accumulated in the stems of *D. cochinchinensis*-7,4′-dihydroxyflavanone synthesis occurred later than that of 7-hydroxy-4′-methoxyflavane and loureirin A, but its content was the highest. Six months later, the yield of dragon's blood increased to 67%–120% [38]. *F. proliferatum* and *F. oxysporum* can also induce dragon's blood formation in the stems of *D. cochinchinensis* and *D. cambodiana*. When compared to the wounded only control, the application of two strains of *F. proliferatum* increased the yield of dragon's blood in the stem of *D. cochinchinensis* plants by 2.7 and 3.3 times [40], and it effectively elicited dragon's blood formation from the surrounding leaves and in the inoculation spots in *D. cambodiana* [41]. Their chemical fingerprint showed that the components of artificial dragon's blood from *Fusarium* species induction were similar to natural dragon's blood [40,41]. Since then, in about 2007, research on dragon's blood in China gradually shifted its focus to *D. cambodiana* plant, as it is easier to cultivate than *D. cochinchinensis* plant.

4.3. Plant Hormones and Dragon's Blood Formation

Microorganism infection can activate plant hormone synthesis and increase endogenous hormone content, and exogenous hormones might also induce dragon's blood formation in *D. cambodiana*. The production of dragon's blood increased by 2.57, 1.6, 2.64, and 4.57 times in response to treatment with gibberellin (GA), indole-3-acetic acid (IAA), brassinolide (BR), and kinetin (KT), respectively. Furthermore, the combination of any two of these three plant hormones can also lead to synergistically increased dragon's blood formation. The application of two of the most important hormones in plants, jasmonic acid (JA) and salicylic acid (SA), did not result in any increases in the yield of dragon's blood at any of the tested concentrations. In addition, 2,4-dichlorophenoxyacetic acid (2,4-D) was demonstrated to have a lethal effect on *D. cambodiana* plants in experiments to induce dragon's blood formation [42]. The application of the common cytokinin 6-benzylaminopurine (6-BA) could also significantly induce dragon's blood production in the branch or stem of three-year old *D. cambodiana* plants. In experiments examining the interaction of plant growth regulators (PGRs) with tissue-cultured seedlings of *D. cambodiana*, KT, GA, IAA, 2,4-D, and 1-naphthylacetic acid (NAA) were respectively mixed in Murashige–Skoog (MS) medium. In tissue cultured seedlings, only 6-BA could promote the

secretion of loureirin A and loureirin B into MS medium in culture bottles [43], and this effect did not appear to be dose-dependent, but rather illumination intensity-dependent. In the case of tissue-cultured seedlings of *D. cambodiana*, none of the PGR treatments resulted in the secretion of dragon's blood into medium under dark conditions, including 6-BA, which was able to induce tissue-cultured seedlings of *D. cambodiana* in order to secrete dragon's blood into medium under illumination intensities of 1000–3000 Lx, and the inducing effect was positively related to illumination intensity [43].

4.4. Small Molecules and Dragon's Blood Formation

Being inspired by the induction of exogenous plant hormones, chemical molecules might induce dragon's blood formation more immediately according to their small MW (molecular·weight). Seventeen small molecules (analytical reagent), including oxalic acid ($H_2C_2O_4$), sodium oxalate ($Na_2C_2O_4$), zinc sulfate ($ZnSO_4$), magnesium nitrate ($Mg(NO_3)_2{\cdot}6H_2O$), hydrochloric acid (HCl), leucine (Leu), sodium thiosulfate ($Na_2S_2O_3{\cdot}5H_2O$), sodium 2-nitrophenoxide, sodium 4-nitrophenoxide, sodium 2-methoxy-5-nitrophenol, compound sodium nitrophenolate, hydrogen peroxide (H_2O_2), sodium bromide ($Na_2Br{\cdot}2H_2O$), sodium molybdate (Na_2MoO_4), copper sulfate ($CuSO_4{\cdot}5H_2O$), barium chloride ($BaCl_2$), and sodium pyrophosphate ($Na_4P_2O_7$), were used as chemical inducers on *D. cambodiana* plants, and three concentration (10%, 1%, and 0.1%) were tested, followed by the harvesting of dragon's blood for counting three months after treatment. The statistics show that > 100% induction was observed for $H_2C_2O_4$ (1%), $ZnSO_4$, $Mg(NO_3)_2$ (0.1%), sodium 4-nitrophenoxide (0.1%), Leu (10%), and $H_2C_2O_4$ (10%), while $ZnSO_4$ (10% and 0.1%), $Mg(NO_3)_2$ (10% and 1%), and sodium 2-nitrophenoxide (0.1%) could induce a > 50% and < 100% increase in the yield of dragon's blood. By contrast, Na_2Br, Na_2MoO_4, $CuSO_4$, and $BaCl_2$ at 10% concentration decreased the dragon's blood formation [42]. These results suggest that acid and sodium salt can significantly increase the accumulation of dragon's blood. Subsequent research also found that compounds that are mixed with sodium chloride (NaCl) and acetic acid (HOAc) can induce dragon's blood formation in the stem of *D. cambodiana* rapidly, and even flavanones-the special constituents of dragon's blood-could be detected using high-performance liquid chromatography (HPLC) after treatment for nine days [44], in particular, (2S)7,4′-dihydroxyflavanone, 4,4′-dihydroxy-2-methoxydihydrochalcone, (2S)-7,3′-dihydroxy-4′-methoxyflavane, (2R)-7,4′-dihydroxy-8-methylflavane, (2S)-3′,7-dihydroxy-4′-methoxy-8-methylflavane, or (3R)-3,5,7-trihydroxy-4′-methoxy-6-methoxydihydro-homoisoflavone [45]. Based on this phenomenon, the mechanism of dragon's blood formation was preliminarily explored while using transcriptome analysis.

5. The Mechanism of Dragon's Blood Formation

5.1. Suppression Subtractive Hybridization

Before the large-scale application of transcriptomics, suppression subtractive hybridization (SSH) was the popular technology in molecular biology for priming genes with different expression levels [46]. In 2005, 431 recombinants were obtained through SSH by comparing the samples with and without the production of dragon's blood in *D. cambodiana* tissue culture plantlets. Subsequently, 51 fragments showing differential expression were indicated via sequencing and reverse Northern blot. Of these 51 fragments, 12 cDNA fragments were related to metabolism and energy transfer, five cDNA fragments were involved in signaling transduction, five cDNA fragment were related to transcription and translation, one cDNA fragment was related to photosynthesis, and the other 23 cDNAs were not homologous to genes in the Nr database [47]. In this SSH library, the complete open reading frame encoding calcium-dependent protein kinase 2 (*CDPK2*) was cloned with RACE, and its expression profile was found to be consistent with dragon's blood accumulation in the stem of *D. cambodiana* via semi-quantitative RT-PCR. Now we know that the CDPKs play vital roles in plant hormone signaling, growth and development, plant (a)biotic stress responses [48], and they even contribute to plant secondary metabolism by activating calcium binding to their calmodulin regulatory domains [49,50].

Frustratingly, the genes that are directly related to flavonoid or terpene synthesis in plants were not detected by SSH [47].

5.2. Transcriptome

In 2014, the transcriptome for the accumulation of dragon's blood was generated while using the stems of three-year-old *D. cambodiana* plants [44]. Three cDNA libraries were constructed, with the stems being injected by the inducer on the *D. cambodiana* plant at 0, 3, and 6 days, and 266.57 million raw data from Illumina HiSeq 2000 were assembled into 198,204 unigenes while using Trinity, of which 34,873 unigenes were annotated in the public database. Totals of 2724, 1698, and 2155 unigenes were respectively expressed in the treatments for 0, 3, and 6 days, and the DESeq determined 6986, 7106, and 6085 differentially expressed genes (DEGs) corresponding to the respective pairs of 0 and 3 days, 0 and 6 days, and 3 and 6 days. The KEGG (Kyoto Encyclopedia of Genes and Genomes) classification found 76 DEGs to be involved in flavonoid biosynthesis from the phenylpropanoid pathway, including phenylalanine ammonia-lyase (PAL, six DEGs), cinnamate-4-hydroxylase (C4H, one DEG), 4-coumarate CoA ligase (4CL, 18 DEGs), chalcone synthase (CHS, 10 DEGs), chalcone isomerase (CHI, six DEGs), flavanone 3-hydroxylase (F3H, seven DEGs), flavonol synthase (FLS, 10 DEGs), dihydroflavonol 4-reductase (DFR, 16 DEGs), and leucoanthocyanidin reductase (LAR, one DEG), and of these, 34 DEGs are involved in the catalysis of flavanones into flavonols [44]. As another important chemical component, the total concentration of steroidal saponin, was significantly decreased during dragon's blood formation, and we also detected 122 unigenes that were involved in steroidal saponin biosynthesis, 29 of which encoded 24 kinds of enzymes that had complete open reading frames (ORF) and differential expression [51]. The plant chemical diversity of flavonoids and saponin is dependent on their modification through methylation, glycosylation, or hydroxylation. A total of 27 unigenes encoding O-methyltransferase (OMT, 2 DEGs), UDP-glycosyltransferase (UGT, 14 DEGs), or cytochrome P450 (CYP450, 11 DEGs) were significantly upregulated upon treatment with inducer [44].

Flavonoids are synthesized in the plant cytosol [52], whereas the precursors of steroidal saponin can be synthesized in cytosol or plastid [53]. After synthesis, they are all transported into vacuoles for storage, or to other destinations [54]. The analysis of transcript expression demonstrated that 13 DEGs encoding multidrug resistance-associated proteins (ABC, MRP-type) were significantly induced, while only three DEGs encode ATP-binding cassette transporters (ABC, G-type)—the results might suggest that MRP-type ABC transporters play key roles in secondary metabolism transport during the accumulation of dragon's blood. Furthermore, two DEGs encoding H^+PPase, two DEGs encoding vacuolar sorting receptor (VSR), four DEGs encoding soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE), 18 DEGs encoding H^+ATPase, and 18 DEGs encoding multidrug and toxic compound extrusion protein transporters (MATE) were also found to be significantly upregulated in dragon's blood formation [44]. Previous studies have indicated that glutathione S-transferase (GST) genes contribute to the transportation of flavonoids from the site of cytosolic synthesis to vacuolar accumulation in plant cells. A total of 20 GST genes were identified based on the transcriptome database, and their transcript profiles were strongly correlated with those of genes that were involved in dragon's blood formation and flavonoid synthesis [55].

As essential transcription factors (TFs), *MYB*, *bHLH*, and *WD40* play essential roles in flavonoid synthesis and transport [56]. Our transcriptomic analysis also detected 86 TFs with differential expression profiles in the process of dragon's blood accumulation, including 41 DEGs of *MYB*, 33 DEGs of *bHLH*, and 12 DEGs of *WD40*, whereas not all of the TFs selected were induced [41]. The ternary complex of MYB–bHLH–WD40 generally regulating the expression of numerous structural genes might cause these asymmetrical expression profiles [57]. Furthermore, a putative *bHLH* transcription factor was identified, named as *DcHLH1*. *DcHLH1* could activate the transcription of *DcF3′H* via binding and regulate the promoter activities of *DcF3′H*, as shown by yeast one-hybrid screening [58]. F3′H (flavonoid 3′-hydroxylase) can catalyze B-ring hydroxylation of flavonoid derivatives at the 3′-position, forming 3′-hydroxylated flavonoids [59,60].

A model for dragon's blood accumulation in *D. cambodiana* was proposed based on our transcriptome analysis and previous studies in the model plants [61,62]. It assumes that different environmental factors can induce transient fluctuations in cytosolic Ca^{2+} levels in the cell of *D. cambodiana*, interact with Ca^{2+} sensors and resulting in calcium signaling, which always involves transcription factors or upstream kinases, such as, for instance, calcineurin B-like proteins (CBLs), Ca^{2+}-dependent protein kinases (CDPKs), and calmodulin-like proteins (CMLs). These sensors subsequently activate or restrain transcriptional activators, such as promoters, enhancers, or suppressors, resulting in the activation of the phenylpropane pathway and suppression of the terpene biosynthetic route. Subsequently, the main chemical constituents of dragon's blood are transferred out of intracellular space to respond to various stimuli through three potential transport mechanisms, including vesicle trafficking-mediated transport, GST transport, or membrane transport. Our present and future studies will focus on verifying this hypothesis via multiple approaches involving omics and genome editing techniques.

6. The Genome Structure Characterization of *Dracaena cambodiana*

6.1. Organelle Genome Sequencing of Dracaena cambodiana

Plants generally harbor two independent organellar genomes (chloroplast and mitochondria) that provide invaluable resources for a range of functional, evolutionary, and comparative genomic studies [63]. The *D. cambodiana* chloroplast (cp) genome can be found in GenBank, with accession number MH293451 [64]. The *D. cambodiana* plant harbors a circular cp genome 156,697 bp in length. It exhibits the typical plant cp genome structure, including two inverted repeat regions (IRs, 26,526 bp) that are separated by a large single-copy region (LSC, 84,988 bp) and a small single-copy region (SSC, 18,657 bp). The base composition of the *D. cambodiana* cp genome was uneven (30.81% A, 18.46% G, 19.14% C, 31.59% T), with an overall GC content of 37.6%, while those of the IRs, LSC, and SSC regions were 43%, 35.7%, and 31.1%, respectively. The phylogenetic analyses of entire cp genomes indicated that *D. cambodiana* might be classified into the Asparagaceae family, and this result was supported by the taxonomy systems of National Center for Biotechnology Information (NCBI) and Angiosperm Phylogeny Group (APG) IV [65]. The cp genome sequence of *D. cambodiana* encodes a total of 113 cp genes, 76 of which are protein-coding genes, while four are rRNA genes, 30 are tRNA genes, and three (*infA*, *matK*, and *ndhf*) are pseudogenes. Of these genes, *atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rps16*, *rpoC1*, *trnA*, *trnG*, *trnL*, *trnK*, and *trnV* contained a single intron while *clpP*, *rps12*, and *ycf3* have two introns. These intron sequences might contribute to special DNA barcoding development and species identification with an internal transcribed spacer from rDNA, especially ITS2 (Internal Transcribed Spacer 2) [66]. Moreover, the cp genome of *D. cochinchinensis* was also released [67]. However, until now, none of the mitochondrial genomes of the *Dracaena* species have been characterized.

6.2. Genome SurveySsequencing of Dracaena cambodiana

The genome of *D. cambodiana* was surveyed with next-generation sequencing on an Illumina HiSeq 2500 platform, and then the data were assembled and roughly annotated, for obtaining a comprehensive understanding of the genetic characterization of *Dracaena* species and the formation of dragon's blood. The genome size of *D. cambodiana* was estimated as 1.12 Gb by k-mer analysis, and its assembly was considered to be straightforward with the PacBio platform, due to the 53.45% of repetitive sequences and 0.38% heterozygosis from k-mer distribution. After assembly with SOAPdevnovo [68], the N50s of the contigs and scaffolds were found to be 1.87 and 3.19 kb. The longest scaffold was 348.12 kb. Although this is neither the draft nor final genome of *D. cambodiana*, it is the first report of the genome-wide characterization of a *Dracaena* species [69]. The LTR_FINFER [70] indicated that 30.15% (80,584) scaffolds contained a total of 26,246 simple sequence repeats (SSRs) and the mono-, di-, and tri- nucleotides comprised nearly 98% of the SSRs. The SciRoKo software [71] identified 208 types motif and the most abundant motif was hexanucleotide repeats (106 types). The comprehensive

analysis of TRE and RepeatMasker [72] indicated that there were 37.11% retroelements and 2.85% DNA transposons in this *D. cambodiana* genome, with a total of 39.96% TEs (transposable elements).

The annotation with GeneID [73] predicted 53,700 protein-encoding genes in the survey genome of *D. cambodiana* and 38,162 (71.07%) genes could be mapped in the public database, such as Swiss-Prot, Pfam, GO, KEGG, KOG, COG, TrEMBL, Nt, or Nr [74–78]. *Elaeis guineensis*, *Phoenix dactylifera*, and *Musa acuminate* were the top three hits for species distribution homologues with *D. cambodiana* when surveying genome annotation in the Nr database, for which the percentages were 27.80% (6,202), 24.76% (5,524), and 11.45% (2,555), respectively. A total of 38,162 predicated genes were clustered for the gene family analysis with *Dendrobium officinale*, *Asparagus officinalis*, *Populus euphratica,* and *Arabidopsis thaliana* as the model plant. The results showed that 5375 gene families were mutual among five plant species and 1139 gene families were unique to *D. cambodiana*.

Interestingly, of the 12,510 genes that matched within Gene Ontology [75], 2,620 genes (20%) were classified into response to stimulus, after cellular process, and metabolic process in the biological process. This revelation suggested that the plant defense system might be involved in dragon's blood formation and the hypothesis was then supported by KEGG mapping [74] and the transcriptome [44]. Of these 38,162 annotated genes, 9258 could be mapped to 125 KEGG pathways and 355 (3.83%) genes corresponded to environment information processing, which was much more than the 267 (2.89%) genes that were mapped to the secondary metabolite biosynthesis and the 184 (1.99%) genes that were involved in terpenoid and polyketide metabolism. Furthermore, this hypothesis guided us to reanalyze the transcriptome of dragon's blood formation by screening the expression profiles of genes that were related to plant defense.

Plant cell walls, antioxidants, and immune systems all contribute to plant defense [79–82]. For the plant's physical defense, cellulose, chitin, pectin, and polysaccharides are the main structural components of plant cell walls [83]. We detected 41 DEGs that were involved in component synthesis of plant cell walls, including 25 DEGs encoding pectin esterase, 11 DEGs encoding chitinase, four DEGs encoding pectate lyase, one DEG encoding cellulase, and four DEGs encoding galactosidase. The physiological and biochemical barriers of plant defense include antioxidants and immune systems [81,82]. As an antioxidant, ROS is not only a defense substance, but also an important signaling factor in plants, and it might be linked to hormone signaling, programmed cell death, and systemic acquired resistance [82]. However, ROS can also injure normal plant cells, so the plant antioxidant system will be primed during a ROS burst to protect healthy cells from redundant ROS [84]. A total of 79 DEGs that were related to the plant antioxidant system were screened in the RNA-seq data, comprising eight DEGs encoding ascorbate peroxidase, 10 DEGs encoding glutathione s-transferase, two DEGs encoding superoxide dismutase, 22 DEGs that were involved in peroxidase synthase, 12 DEGs encoding permease, 21 DEGs related to plant P450 system, and one DEG for synthesis of each antioxidant, such as glutathione, phytoene, or naringenin. As important defense components, JA, SA, proline, and trehalose regulate plant defense via signaling transduction or relieving osmotic stress [85]. We detected seven DEGs encoding lipoxygenase (LOX), two DEGs encoding allene oxide synthase (AOS), one gene encoding allene oxide cyclase (AOC), and six DEGs encoding 12-Oxo-phytodienoic acid-10,11-reductase (OPAR) in the JA pathway, which are all encoding key enzymes in JA biosynthesis [86]. The SA-related DEGs included one *PAL* (phenylalanine ammonia lyase), three *PBS3* (acyl-adenylate/thioester-forming enzyme), one *ICS* (isochorismate synthase), and six *EPS1* (enhanced pseudomonas susceptibility 1) [87]. In addition, three *PDG* (proline dehydrogenase) and seven *TPS* (trehalose phosphate synthase) genes might participate in the formation of dragon's blood via osmotic stress.

These initial results that are based on a genomic survey of *D. cambodiana* are expected to contribute to further progress in the form of genome sequencing and genetic studies of *D. cambodiana* and may improve our understanding of the mechanisms of dragon's blood formation. However, most importantly, these results demonstrate effective strategies that involve sequencing platforms and assembly software selection for the construction of the final chromosome-scale genome of *D. cambodiana*.

7. Conclusions

This paper presents a summary review of the applied history and current progress in understanding the mechanisms of dragon's blood formation based on *Dracaena cambodiana* in China. Hence, when considering the vulnerable and severely endangered status of the *Dracaena* tree population that results from the heavy exploitation of its stem for the production of dragon's blood, the research into artificial induction technologies and dragon's blood formation mechanisms based on *D. cambodiana* in China, as presented here, will provide valuable information to aid in the global conservation of the wild *Dracaena* species and contribute to understanding the mechanisms of dragon's blood formation, eventually helping us to understand the evolution of flavonoid genes and *Dracaena* plants.

Author Contributions: Conceptualization, X.D. and W.M.; Writing—Original Draft Preparation & Editing, X.D.; Writing—Review & Editing, W.M., J.Z., H.W. and H.C.; Funding acquisition, X.D. and W.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Key Research and Development Project of Hainan Province (ZDYF2018226 for X.D.), the National Natural Science Foundation of China (81803663 for X.D.) and the Central Public-interest Scientific Institution Basal Research Fund for Chinese Academy of Tropical Agricultural Sciences (1630052020003 for X.D. and 17CXTD-15 for W.M.).

Acknowledgments: We would like to acknowledge the invitation from 1st World Conference Focused on Dragon Trees and Petr Maděra who suggested our topic in the content of special issue "Dragon Trees - Tertiary Relicts in Current Reality" in *Forests*.

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

References

1. Adolt, R.; Pavlis, J. Age structure and growth of *Dracaena cinnabari* populations on Socotra. *Trees-Struct. Funct.* **2004**, *18*, 43–53. [CrossRef]
2. Aslam, J.; Mujib, A.; Sharma, M.P. In vitro micropropagation of *Dracaena sanderiana* Sander ex Mast: An important indoor ornamental plant. *Saudi J. Biol. Sci.* **2013**, *20*, 63–68. [CrossRef] [PubMed]
3. Fan, J.Y.; Yi, T.; Sze-To, C.M.; Zhu, L.; Peng, W.L.; Zhang, Y.Z.; Zhao, Z.Z.; Chen, H.B. A systematic review of the botanical, phytochemical and pharmacological profile of *Dracaena cochinchinensis*, a plant source of the ethnomedicine "Dragon's Blood". *Molecules* **2014**, *19*, 10650–10669. [CrossRef] [PubMed]
4. Gupta, D.; Bleakley, B.; Gupta, R.K. Dragon's blood: Botany, chemistry and therapeutic uses. *J. Ethnopharmacol.* **2008**, *115*, 361–380. [CrossRef]
5. Chen, H.Q.; Zuo, W.J.; Wang, H.; Shen, H.Y.; Luo, Y.; Dai, H.F.; Mei, W.L. Two new antimicrobial flavanes from dragon's blood of *Dracaena cambodiana*. *J. Asian Nat. Prod. Res.* **2012**, *14*, 436–440. [CrossRef] [PubMed]
6. Mei, W.L.; Luo, Y.; Wang, H.; Shen, H.Y.; Zeng, Y.B.; Dai, H.F. Two new flavonoids from dragon's blood of *Dracaena cambodiana*. *Bull. Korean Chem. Soc.* **2013**, *34*, 1791–1794. [CrossRef]
7. Tapondjou, L.A.; Ponou, K.B.; Teponno, R.B.; Mbiantcha, M.; Djoukeng, J.D.; Nguelefack, T.B.; Watcho, P.; Cadenas, A.G.; Park, H.J. In vivo anti-inflammatory effect of a new steroidal saponin, mannioside A, and its derivatives isolated from *Dracaena mannii*. *Arch. Pharm. Res.* **2008**, *31*, 653. [CrossRef]
8. Kougan, G.B.; Miyamoto, T.; Tanaka, C.; Paululat, T.; Mirjolet, J.F.; Duchamp, O.; Sondengam, B.L.; Lacaille-Dubois, M.A. Steroidal saponins from two species of *Dracaena*. *J. Nat. Prod.* **2010**, *73*, 1266–1270. [CrossRef]
9. Zheng, D.J.; Xie, L.S.; Wang, Y.; Zhang, Z.L.; Zhang, W. Research advances in dragon's blood plants in China. *Chin. Wild Plant Res.* **2009**, *28*, 15–20.
10. Luo, Y.; Wang, H.; Zhao, Y.X.; Zeng, Y.B.; Shen, H.Y.; Dai, H.F.; Mei, W.L. Cytotoxic and antibacterial flavonoids from dragon's blood of *Dracaena cambodiana*. *Planta Med.* **2011**, *77*, 2053–2056. [CrossRef]
11. Sollmann, T. A sketch of the medical history of Dragon's blood. *J. Am. Pharm. Assoc.* **1920**, *9*, 141–144. [CrossRef]
12. Zheng, Q.A.; Yang, C.R. Dracaenoside A and B, new C-22 steroidal lactone glycosides from the stem of *Dracaena cochinchinensis*. *Chin. Chem. Lett.* **2003**, *14*, 1261–1264.
13. Mabberley, D.J. *Mabberley's Plant-Book: A Portable Dictionary of Plants, Their Classification and Uses*; Cambridge University Press: Cambridge, UK, 2008; pp. 287–288.

14. Dioscorides, P. *De Materia Medica (Translated by Beck L.Y)*; Olms Press: Hildesheim, Germany, 2005; p. 374.
15. Krishnaraj, P.; Chang, Y.; Ho, T.J.; Lu, N.C.; Lin, M.D.; Chen, H.P. In vivo pro-angiogenic effects of dracorhodin perchlorate in zebrafish embryos: A novel bioactivity evaluation platform for commercial dragon blood samples. *J. Food Drug Anal.* **2019**, *27*, 259–265. [CrossRef]
16. Rustiami, H.; Setyowati, F.M.; Kartawinata, K. Taxonomy and uses of *Daemonorops draco* (Willd.) Blume. *J. Trop. Ethnobiol.* **2004**, *1*, 65–75.
17. Nadezhdina, N.; Plichta, R.; Nadezhdin, V.; Gebauer, R.; Jupa, R.; Habrova, H.; Madera, P. A comparative structural and functional study of leaf traits and sap flow in *Dracaena cinnabari* and *Dracaena draco* seedlings. *Funct. Plant Biol.* **2015**, *42*, 1092–1105. [CrossRef]
18. Chen, X.Q.; Xu, J.M.; Liang, S.Y.; Ji, Z.H.; Lang, K.Y. Liliaceae. *Flora China* **1978**, *14*, 276–277.
19. Hu, Y.; Tu, P.; Li, R.; Wan, Z.; Wang, D. Studies on stilbene derivatives from *Dracaena cochinchinensis* and their antifungal activities. *Chin. Tradit. Herb. Drugs* **2001**, *32*, 104–106.
20. Yi, T.; Chen, H.B.; Zhao, Z.Z.; Yu, Z.L.; Jiang, Z.H. Comparison of the chemical profiles and anti-platelet aggregation effects of two "dragon's blood" drugs used in traditional Chinese medicine. *J. Ethnopharmacol.* **2011**, *133*, 796–802. [CrossRef]
21. Van Minh, C.; Dat, N.T.; Dang, N.H.; Nam, N.H.; Ban, N.K.; Van Tuyen, N.; Huong, L.M.; Huong, T.T.; Van Kiem, P. Unusual 22S-spirostane steroids from *Dracaena cambodiana*. *Nat. Prod. Commun.* **2009**, *4*, 1934578X0900400908. [CrossRef]
22. Mwachala, G.; Cheek, M.; Fischer, E.; Muasya, A.M. A new species of Dracaena L. (*Dracaenaceae-Ruscaceae*) from Mt Kupe and the Bakossi Mts, Cameroon. *Kew Bull.* **2007**, *62*, 613–616.
23. Kamel, M.; Ghazaly, U.M.; Callmander, M.W. Conservation status of the endangered Nubian dragon tree *Dracaena ombet* in Gebel Elba national park, Egypt. *Oryx* **2018**, *49*, 704–709. [CrossRef]
24. Sousa, M.M.; Melo, M.J.; Parola, A.J.; de Melo, J.S.S.; Catarino, F.; Pina, F.; Cook, F.E.M.; Simmonds, M.S.J.; Lopes, J.A. Flavylium chromophores as species markers for dragon's blood resins from *Dracaena* and *Daemonorops* trees. *J. Chromatogr. A* **2008**, *1209*, 153–161. [CrossRef] [PubMed]
25. Edwards, H.G.M.; Farwell, D.W.; Quye, A. 'Dragon's blood'I-characterization of an ancient resin using Fourier transform Raman spectroscopy. *J. Raman Spectrosc.* **1997**, *28*, 243–249. [CrossRef]
26. Xue, Y.; Huang, B.L.; Lü, C.Q.; Zhao, Y.P.; Zhou, C.M.; Li, Y. Rapid propagation of *Dracaena cambodiana* in vitro. *Guihaia* **2007**, *27*, 937–940.
27. Cai, X.T.; Xu, Z.F. A study on the resource of Chinese Dragon's Blood. *Acta Bot. Yunnanica* **1979**, *1*, 1–10.
28. Zheng, Q.A.; Zhang, Y.J.; Li, H.Z.; Yang, C.R. Flavonoids from Dragon's Blood of *Dracaena cochinchinensis*. *Helv. Chim. Acta* **2004**, *87*, 1167–1171. [CrossRef]
29. Zheng, Q.A.; Zhang, Y.J.; Li, H.Z.; Yang, C.R. Steroidal saponins from fresh stems of *Dracaena cochinchinensis*. *Steroids* **2004**, *69*, 111–119. [CrossRef]
30. Ma, C.J. Cellulase elicitor induced accumulation of capsidiol in *Capsicum annumm* L. suspension cultures. *Biotechnol. Lett.* **2008**, *30*, 961–965. [CrossRef]
31. Jasiński, M.; Kachlicki, P.; Rodziewicz, P.; Figlerowicz, M.; Stobiecki, M. Changes in the profile of flavonoid accumulation in *Medicago truncatula* leaves during infection with fungal pathogen *Phoma medicaginis*. *Plant Physiol. Biochem.* **2009**, *47*, 847–853. [CrossRef]
32. Mialoundama, A.S.; Heintz, D.; Debayle, D.; Rahier, A.; Camara, B.; Bouvier, F. Abscisic acid negatively regulates elicitor-induced synthesis of capsidiol in wild tobacco. *Plant Physiol.* **2009**, *150*, 1556–1566. [CrossRef]
33. Yang, M.H.; Lin, Y.J.; Kuo, C.H.; Ku, K.L. Medicinal mushroom *Ganoderma lucidum* as a potent elicitor in production of t-resveratrol and t-piceatannol in peanut calluses. *J. Agric. Food Chem.* **2010**, *58*, 9518–9522. [CrossRef] [PubMed]
34. Ding, X.; Yang, M.; Huang, H.; Chuan, Y.; He, X.; Li, C.; Zhu, Y.; Zhu, S. Priming maize resistance by its neighbors: Activating 1, 4-benzoxazine-3-ones synthesis and defense gene expression to alleviate leaf disease. *Front. Plant Sci.* **2015**, *6*, 830. [CrossRef] [PubMed]
35. Li, F.X.; Wang, H.; Gai, C.J.; Chen, H.Q.; Li, W.; Mei, W.L.; Dai, H.F. Three new flavanoids from artificially induced dragon's blood of *Dracaena cambodiana*. *J. Asian Nat. Prod. Res.* **2018**, *20*, 55–61. [CrossRef] [PubMed]
36. Shen, H.Y.; Wang, H.; Zuo, W.J.; Zhao, Y.X.; Luo, Y.; Mei, W.L.; Dai, H.F. A new phenylpropanoid glycoside from dragon's blood of *Dracaena cambodiana*. *Chem. Res. Chin. Univ.* **2013**, *29*, 483–486. [CrossRef]

37. Shen, H.Y.; Zuo, W.J.; Wang, H.; Zhao, Y.X.; Guo, Z.K.; Luo, Y.; Li, X.N.; Dai, H.F.; Mei, W.L. Steroidal saponins from dragon's blood of *Dracaena cambodiana*. *Fitoterapia* **2014**, *94*, 94–101. [CrossRef] [PubMed]
38. Wang, J.L.; Cheng, Z.Y. Chinese dragon' blood and *Fusarium graminearum*. *Plant Mag.* **1997**, *1*, 15–16.
39. Gong, L.; Guo, S. Endophytic fungi from *Dracaena cambodiana* and *Aquilaria sinensis* and their antimicrobial activity. *Afr. J. Biotechnol.* **2009**, *8*, 731–736.
40. Wang, X.H.; Zhang, C.; Yang, L.L.; Gomes-Laranjo, J. Production of dragon's blood in *Dracaena cochinchinensis* plants by inoculation of *Fusarium proliferatum*. *Plant Sci.* **2011**, *180*, 292–299. [CrossRef]
41. Ou, L.; Wang, X.; Zhang, C.H. Production and characterization of dragon's blood from leaf blades of *Dracaena cambodiana* elicited by *Fusarium proliferatum*. *Ind. Crop. Prod.* **2013**, *45*, 230–235. [CrossRef]
42. Yang, X.H.; Zheng, S.; Wang, X.H. Preliminary study on chemical inducers for dragon's blood formed by *Dracaena cambodiana*. *Amino Acid Biot. Resour.* **2014**, *36*, 49–54.
43. Yang, B.P.; Zhang, S.Z.; Cai, W.W.; Song, Q.S.; Wang, X.H.; Wang, D.; Yang, X.; Gu, L.H.; Feng, C.L.; Wang, J.G.; et al. Induced formation of dragon's blood in the process of tissue culture of cambodia dragonblood (*Dracaena cambodiana* Pierre ex Gagnep). *Chin. J. Trop. Crop.* **2009**, *30*, 181–185.
44. Zhu, J.H.; Cao, T.J.; Dai, H.F.; Li, H.L.; Guo, D.; Mei, W.L.; Peng, S.Q. *De novo* transcriptome characterization of *Dracaena cambodiana* and analysis of genes involved in flavonoid accumulation during formation of dragon's blood. *Sci. Rep.-U. K.* **2016**, *6*, 38315. [CrossRef] [PubMed]
45. Wang, H.; Liu, J.; Wu, J.; Mei, W.L.; Dai, H.F. Flavonoids from *Dracaena cambodiana*. *Chem. Nat. Compd.* **2011**, *47*, 624. [CrossRef]
46. Diatchenko, L.; Lau, Y.F.; Campbell, A.P.; Chenchik, A.; Moqadam, F.; Huang, B.; Lukyanov, S.; Lukyanov, K.; Gurskaya, N.; Sverdlov, E.D.; et al. Suppression subtractive hybridization: A method for generating differentially regulated or tissue-specific cDNA probes and libraries. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6025–6030. [CrossRef] [PubMed]
47. Cai, W.; Zhang, S.; Yang, B.; Feng, C.; Yang, X.; Gu, H. Isolation of Genes Related to Dragon Biosynthesis from *Dracaena cambodiana* Pierre ex Gagnep. *Mol. Plant Breed.* **2008**, *6*, 881–885.
48. Zhang, K.; Han, Y.T.; Zhao, F.L.; Hu, Y.; Gao, Y.R.; Ma, Y.F.; Zheng, Y.; Wang, Y.J.; Wen, Y.Q. Genome-wide identification and expression analysis of the CDPK gene family in grape, *Vitis* spp. *BMC Plant Biol.* **2015**, *15*, 164. [CrossRef]
49. Veremeichik, G.N.; Grigorchuk, V.P.; Shkryl, Y.N.; Bulgakov, D.V.; Silantieva, S.A.; Bulgakov, V.P. Induction of resveratrol biosynthesis in *Vitis amurensis* cells by heterologous expression of the Arabidopsis constitutively active, Ca^{2+}-independent form of the *AtCPK1* gene. *Process Biochem.* **2017**, *54*, 144–155. [CrossRef]
50. Gupta, R.; Min, C.W.; Kramer, K.; Agrawal, G.K.; Rakwal, R.; Park, K.H.; Wang, Y.M.; Finke Kim, S.T. A multi-omics analysis of glycine max leaves reveals alteration in flavonoid and isoflavonoid metabolism upon ethylene and abscisic acid treatment. *Proteomics* **2018**, *18*, 1700366. [CrossRef]
51. Zhu, J.H.; Li, H.L.; Guo, D.; Wang, Y.; Dai, H.F.; Mei, W.L.; Peng, S.Q. Identification, characterization and expression analysis of genes involved in steroidal saponin biosynthesis in *Dracaena cambodiana*. *J. Plant Res.* **2018**, *131*, 555–562. [CrossRef]
52. Petrussa, E.; Braidot, E.; Zancani, M.; Peresson, C.; Bertolini, A.; Patui, S.; Vianello, A. Plant flavonoids-biosynthesis, transport and involvement in stress responses. *Int. J. Mol. Sci.* **2013**, *14*, 14950–14973. [CrossRef]
53. Singh, P.; Singh, G.; Bhandawat, A.; Singh, G.; Parmar, R.; Seth, R.; Sharma, R.K. Spatial transcriptome analysis provides insights of key gene (s) involved in steroidal saponin biosynthesis in medicinally important herb *Trillium govanianum*. *Sci. Rep-U. K.* **2017**, *7*, 45295. [CrossRef] [PubMed]
54. Zhao, J. Flavonoid transport mechanisms: How to go, and with whom. *Trends Plant Sci.* **2015**, *20*, 576–585. [CrossRef] [PubMed]
55. Zhu, J.H.; Li, H.L.; Guo, D.; Wang, Y.; Dai, H.F.; Mei, W.L.; Peng, S.Q. Transcriptome-wide identification and expression analysis of glutathione *S*-transferase genes involved in flavonoids accumulation in *Dracaena cambodiana*. *Plant Physiol. Biochem.* **2016**, *104*, 304–311. [CrossRef] [PubMed]
56. Schaart, J.G.; Dubos, C.; Romero De La Fuente, I.; Van Houwelingen, A.M.; de Vos, R.C.; Jonker, H.H.; Xu, W.J.; Routaboul, J.M.; Lepiniec, L.; Bovy, A.G. Identification and characterization of MYB-b HLH-WD 40 regulatory complexes controlling proanthocyanidin biosynthesis in strawberry (*Fragaria* × *ananassa*) fruits. *New Phytol.* **2013**, *197*, 454–467. [CrossRef] [PubMed]

57. Hichri, I.; Barrieu, F.; Bogs, J.; Kappel, C.; Delrot, S.; Lauvergeat, V. Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. *J. Exp. Bot.* **2011**, *62*, 2465–2483. [CrossRef]
58. Zhu, J.; Chen, P.; Guo, D.; Li, H.; Wang, Y.; Dai, H.; Mei, W.L.; Peng, S. Identification and Functional Characterization of the *DcF3′H* Promoter from *Dracaena cambodiana*. *Trop. Plant Biol.* **2018**, *11*, 192–198. [CrossRef]
59. Jeong, S.T.; Goto-Yamamoto, N.; Hashizume, K.; Esaka, M.J.P.S. Expression of the flavonoid 3′-hydroxylase and flavonoid 3′, 5′-hydroxylase genes and flavonoid composition in grape (*Vitis vinifera*). *Plant Sci.* **2006**, *170*, 61–69. [CrossRef]
60. Sun, R.Z.; Pan, Q.H.; Duan, C.Q.; Wang, J. Light response and potential interacting proteins of a grape flavonoid 3′-hydroxylase gene promoter. *Plant Physiol. Biochem.* **2015**, *97*, 70–81. [CrossRef]
61. Nesi, N.; Jond, C.; Debeaujon, I.; Caboche, M.; Lepiniec, L. The Arabidopsis TT2 gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed. *Plant Cell* **2001**, *13*, 2099–2114. [CrossRef]
62. Yan, J.; Wang, B.; Zhong, Y.; Yao, L.; Cheng, L.; Wu, T. The soybean R2R3 MYB transcription factor GmMYB100 negatively regulates plant flavonoid biosynthesis. *Plant Mol. Biol.* **2015**, *89*, 35–48. [CrossRef]
63. Petit, R.J.; Vendramin, G.G. Evolutionary perspectives on the origins and conservation of European biodiversity. In *Phylogeography of Southern European Refugia*; Weiss, S., Ferrand, N., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp. 23–97.
64. Zhu, Z.X.; Mu, W.X.; Wang, J.H.; Zhang, J.R.; Zhao, K.K.; Ross Friedman, C.; Wang, H.F. Complete plastome sequence of *Dracaena cambodiana* (Asparagaceae): A species considered "Vulnerable" in Southeast Asia. *Mitochondrial DNA B* **2018**, *3*, 620–621. [CrossRef]
65. Chase, M.W.; Christenhusz, M.J.M.; Fay, M.F.; Byng, J.W.; Judd, W.S.; Soltis, D.E.; Mabberley, D.J.; Sennikov, A.N.; Soltis, P.S.; Stevens, P.F. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.* **2016**, *181*, 1–20.
66. Gong, L.; Qiu, X.H.; Huang, J.; Xu, W.; Bai, J.Q.; Zhang, J.; Su, H.; Xu, C.M.; Huang, Z.H. Constructing a DNA barcode reference library for southern herbs in China: A resource for authentication of southern Chinese medicine. *PLoS ONE* **2018**, *13*, e0201240. [CrossRef] [PubMed]
67. Wang, Y.; Sun, J.; Yuan, Q.; Guo, L. Complete chloroplast genome sequence of *Dracaena cochinchinensis*: Genome structure and genomic resources. *Mitochondrial DNA B* **2019**, *4*, 1750–1751. [CrossRef]
68. Bazinet, A.L.; Mitter, K.T.; Davis, D.R.; Van Nieukerken, E.J.; Cummings, M.P.; Mitter, C. Phylotranscriptomics resolves ancient divergences in the Lepidoptera. *Syst. Entomol.* **2017**, *42*, 305–316. [CrossRef]
69. Ding, X.; Mei, W.; Huang, S.; Wang, H.; Zhu, J.; Hu, W.; Ding, Z.; Tie, W.; Peng, S.; Dai, H. Genome survey sequencing for the characterization of genetic background of *Dracaena cambodiana* and its defense response during dragon's blood formation. *PLoS ONE* **2018**, *13*, e0209258. [CrossRef]
70. Xu, Z.; Wang, H. LTR_FINDER: An efficient tool for the prediction of full-length LTR retrotransposons. *Nucleic Acids Res.* **2007**, *35*, W265–W268. [CrossRef]
71. Kofler, R.; Schlötterer, C.; Lelley, T. SciRoKo: A new tool for whole genome microsatellite search and investigation. *Bioinformatics* **2007**, *23*, 1683–1685. [CrossRef]
72. Tarailo-Graovac, M.; Chen, N. Using RepeatMasker to identify repetitive elements in genomic sequences. *Curr. Protoc. Bioinform.* **2009**, *25*, 4–10. [CrossRef]
73. Blanco, E.; Parra, G.; Guigó, R. Using geneid to identify genes. *Curr. Protoc. Bioinform.* **2007**, *18*, 4.3.1–4.3.28.
74. Kanehisa, M.; Furumichi, M.; Tanabe, M.; Sato, Y.; Morishima, K. KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* **2016**, *45*, D353–D361. [CrossRef]
75. Gene Ontology Consortium. Gene ontology consortium: Going forward. *Nucleic Acids Res.* **2014**, *43*, D1049–D1056.
76. Boeckmann, B.; Bairoch, A.; Apweiler, R.; Blatter, M.C.; Estreicher, A.; Gasteiger, E.; Martin, M.J.; Michoud, K.; O'Donovan, C.; Phan, I.; et al. The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. *Nucleic Acids Res.* **2003**, *31*, 365–370. [CrossRef] [PubMed]
77. Finn, R.D.; Bateman, A.; Clements, J.; Coggill, P.; Eberhardt, R.Y.; Eddy, S.R.; Heger, A.; Hetherington, K.; Holm, L.; Mistry, J.; et al. Pfam: The protein families database. *Nucleic Acids Res.* **2013**, *42*, D222–D230. [CrossRef] [PubMed]
78. Yu, K.; Zhang, T. Construction of customized sub-databases from NCBI-nr database for rapid annotation of huge metagenomic datasets using a combined BLAST and MEGAN approach. *PLoS ONE* **2013**, *8*, e0059831. [CrossRef] [PubMed]

79. Hématy, K.; Cherk, C.; Somerville, S. Host-pathogen warfare at the plant cell wall. *Curr. Opin. Plant Biol.* **2009**, *12*, 406–413. [CrossRef]
80. Rejeb, K.B.; Abdelly, C.; Savouré, A. How reactive oxygen species and proline face stress together. *Plant Physiol. Biochem.* **2014**, *80*, 278–284. [CrossRef]
81. Jones, J.D.; Dangl, J.L. The plant immune system. *Nature* **2006**, *444*, 323–329. [CrossRef]
82. Qi, J.; Wang, J.; Gong, Z.; Zhou, J.M. Apoplastic ROS signaling in plant immunity. *Curr. Opin. Plant Biol.* **2017**, *38*, 92–100. [CrossRef]
83. Underwood, W. The plant cell wall: A dynamic barrier against pathogen invasion. *Front. Plant Sci.* **2012**, *3*, 85. [CrossRef]
84. Choudhury, F.K.; Rivero, R.M.; Blumwald, E.; Mittler, R. Reactive oxygen species, abiotic stress and stress combination. *Plant J.* **2017**, *90*, 856–867. [CrossRef] [PubMed]
85. Alazem, M.; Lin, N.S. Roles of plant hormones in the regulation of host-virus interactions. *Mol. Plant Pathol.* **2015**, *16*, 529–540. [CrossRef] [PubMed]
86. Browse, J. Jasmonate: An oxylipin signal with many roles in plants. *Vitam. Horm.* **2005**, *72*, 431–456. [PubMed]
87. Ahuja, I.; Kissen, R.; Bones, A.M. Phytoalexins in defense against pathogens. *Trends Plant Sci.* **2012**, *17*, 73–90. [CrossRef] [PubMed]