Tobacco rattle virus -induced gene silencing in Hevea brasiliensis

Hui-Liang Li
   Institute of Tropical Bioscience and Biotechnology

Dong Guo
   Institute of Tropical Bioscience and Biotechnology

Ying Wang
   Institute of Tropical Bioscience and Biotechnology

Jia-Hong Zhu
   Institute of Tropical Bioscience and Biotechnology

Long Qu
   Hainan University

Shi-Qing Peng (✉ shqpeng@163.com)
   Institute of Tropical Bioscience and Biotechnology   https://orcid.org/0000-0002-3984-1902

Methodology

Abstract

Background

Since it is very difficult to obtain gene knockouts in rubber tree (*Hevea Brasiliensis)* due to low genetic transformation efficiency. Virus-induced gene silencing (VIGS) is a powerful gene silencing tool that has been intensively applied in plant. Up to now, the application of VIGS in rubber tree has not yet been reported.

Results

Hevea brasiliensis phytoene desaturase (*HbPDS*) was identified in *H. brasiliensis* genome. The prediction of small interfering RNAs (siRNAs) from *HbPDS* and the silencing gene fragment (SGF) were predicted and a length of 409 bp SGF was chosen to be tested. We show that the tobacco rattle virus (TRV) -VIGS is able to induce effective *HbPDS* silencing in rubber tree. The TRV-VIGS system has the potential for functional gene studies in rubber tree.

Conclusions

This is the first time to report VIGS in rubber tree. The present TRV-VIGS method could be further applied to produce gene silenced rubber tree plants, to advance functional gene of rubber tree. The applied TRV-VIGS method will achieve deeper underground into the natural rubber biosynthesis and regulation in this important rubber-producing plant.

Background

Rubber tree (*Hevea brasiliensis* Muell. Arg.) is an important rubber-producing plant of *Euphorbiaceae* [1, 2]. The valuable of rubber tree as a sole commercial source rubber-producing plant led to enormous interest in understanding the natural rubber biosynthesis and regulation [3–5]. Natural rubber (NR) is regarded as a valuable industry raw material which is synthesized in latex of laticifers [6, 7]. The physiological functions of rubber molecule and latex remain intrigue [7, 8]. To date, rubber biosynthesis pathway has been well characterized [8], the whole-genome data and transcriptome database became available in rubber tree [9–11], but only a few NR biosynthesis-related genes functions are evaluated by transgenic rubber tree due to low genetic transformation efficiency [12–15]. The development of an efficient and reliable rubber tree transformation system is still challenging [15, 16].

Virus-induced gene silencing (VIGS) has been intensively utilized to investigate the gene function in plant [17]. Tobacco rattle virus (TRV)is a RNA virus that belongs to the bipartite viruses with the TRV1 and TRV2 genomes [18]. Compared with other applied plant viruses including tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), barley stripe mosaic virus (BSMV), potato virus X (PVX) in previous studies, TRV could energetically propagate throughout the whole-plant and reveal milder infection phenotypes[18, 19]. TRV-induced VIGS (TRV-VIGS) has been widely utilized for silencing genes in various

plants, including tobacco [19], poplar [20], wheat, maize [21], tomato [22], rose [23], cassava [24], cotton [25], and so on. However, it is no reported that VIGS was used in rubber tree.

Here, we utilized TRV to develop a VIGS system in rubber tree. We used *H. brasiliensis* phytoene desaturase (HbPDS) gene as a reporter gene resulting in the photo-bleaching phenotype by silencing *HbPDS* in rubber tree plants. This VIGS using TRV system suggested the potential for silencing gene of rubber tree, to advance functional gene of rubber tree.

Results

Identification and expression of HbPDS

The reference sequence from *NbPDS* was used to search for homologies in the local *H. brasiliensis* genome database, a homologous sequence was found. The predicted *HbPDS* was amplified by PCR and then was sequenced (GenBank accession: XP 021691516). The identified HbPDS sequence share over 82.51% homology compared to the sequence of NbPDS (Fig. 1). The phylogenetic analyses revealed that HbPDS was the closest to that from *Manihot esculenta* and *Jatropha curcas* which belong to *Euphorbiaceae* (Fig. 2).

The expression profiles of *HbPDS* was investigated in latex, bark, leaves, roots, flowers and seeds by qPCR. *HbPDS* showed the highest transcript level in the leaves, while *HbPDS* transcripts accumulated at relatively low levels in seeds and latex (Fig. 3). The expression level of *HbPDS* in the leaves was increased from juvenile leaves stage to mature leaves stage during leaf development (Fig. 4).

siRNAs prediction

To complete high-efficiency gene silencing vector, it is very important to choose the silencing gene fragment that can create efficient siRNAs. For this purpose, the *HbPDS* sequence was analyzed by using pssRNAit tool. Nineteen siRNAs and 312 off-targets for *HbPDS* were predicted (data not shown). All predicted siRNAs were then further scanned against the rubber tree database to filter out off-target sites. Finally, one fragment, containing a length of 409 bp and a 53% GC content, was designed as a silencing gene fragment to construct the VIGS vector.

Silencing of HbPDS in rubber tree

Ten rubber tree plants were infiltrated with Agrobacterium strain carrying pTRV2-HbPDS and pTRV1. Nine plants showed the desired photo-bleaching on the upper young leaves at 60 days post infiltration, while no control plants showed desired phenotype. Notably, in rubber tree plants infiltrated with TRV2-HbPDS, the partial photo-bleaching appeared in fully developed leaves, and then photo-bleaching were observed in the upper newly developing leaves (Fig. 5). These results suggested that *HbPDS* was exactly silenced in pTRV-HbPDS infected plants.

Detection of virus in infiltrated rubber tree plants

To investigate the presence of virus, the sequences of TRV1 and pTRV2 were amplified by PCR. PCR result indicated that the sequences of TRV could be confirmed in the upper newly developing leaves of the infitrated rubber tree plants, not in the WT rubber tree (Fig. 6A). Moreover, the expression of TRV1 and TRV2 was examined by RT-PCR. TRV1 and TRV2 were present in VIGS-HbPDS plants as well as in VIGS-GFP plants, not in WT rubber tree (Fig. 6B), indicating TRV1 and TRV2 in leaves of the VIGS-HbPDS plants.

Validation of the silencing of HbPDS

The transcript level of *HbPDS* was validated in leaves of the VIGS-HbPDS plants by qPCR. qPCR results demonstrated that the transcript level of *HbPDS* in the upper leaves of VIGS-HbPDS plants was 5.1% -15.3% - of that in the VIGS-GFP plants (Fig. 7), displaying significantly lower chlorophyll and carotenoids content than that in the VIGS-GFP plants (Fig. 8). These results indicates that the photo-bleaching is be closely associated with the down-regulated the expression of *HbPDS* by VIGS, not caused by infection-induced, suggesting that the established TRV-VIGS system can silence *HbPDS* in rubber tree.

Discussion

Although a large number of genomic data and transcriptome data of rubber tree can be obtained [9–11], but few functional genes have been proved in rubber tree[12–15]. Functional gene analysis is still a major challenge lacking efficient and reliable genetic transformation system[15, 16]. In this study, we examined Agrobacterium-mediated TRV-based VIGS in rubber tree. VIGS is an effective tool to study gene function in vivo and more than 40 viruses have been applied as VIGS vectors [17, 28–30]. TRV has been used in VIGS to silence genes in many plants [19–25]. Rubber tree is the most important rubber-producing crops, it is no reported that VIGS was applied in rubber tree. An efficient and reliable TRV-VIGS system for gene function analysis in rubber would be extremely valuable.

Phytoene desaturase (PDS) gene, encoding the firs enzyme catalyzing in the carotenoid biosynthetic pathway [31], has been applied as a reporter gene in VIGS systems. The silencing of *PDS* transcript will result in easily-detected photo-bleaching symptoms [19–25]. Thus, we used pTRV-HbPDS to silence *HbPDS* in rubber tree. Inoculation of *Agrobacterium* strain carrying pTRV was applied to silence *HbPDS*. Compare to some plants, it is difficult that Agrobacterium inoculated into leaves by a syringe. We employed rubbed leaves for agroinfiltration. Agroinfiltration of rubbed leaves resulted in higher VIGS efficiency and obvious albino symptoms in rubber tree. Agroinfiltration of rubbed leaves might increase the percentage of infected rubber tree cells, resulted higher silencing efficiencies.

In pTRV2-HbPDS infiltrated rubber tree plant developed leaves showed partial bleaching phenotypes at 60 days post infiltration. The all photo-bleaching could be observed on the upper newly developed at leaves at 80 days post infiltration. In contrast, pTRV2-MePDS-infiltrated cassava showed a photo-bleaching in

the distal leaves at 20 days post infiltration [24], suggesting that different plants have different sensitivity to TRV. The typical photo-bleaching was observed in the tobacco top young leaves [32]. But in rubber tree, the photo-bleaching appeared in fully expanded leaves, and then were observed in the upper newly leaves. This is consistent with in poplar plants infiltrated with TRV2-*PePDS* [20], suggesting that the virus transfers from the inoculation site to the upper newly developing leaves.

In Addition, the transcript level of *HbPDS* in the upper leaves of HbPDS-VIGS plants was 5.1% -15.3% of that in the pTRV-GFP plants (Fig. 7), and 1.5–16.5% of chlorophyll and carotenoids content in the upper plant leaves (Fig. 8). In TRV-VIGS system, the silencing of reporter gene was 30–70% in rose [23], 28–38% in tomato [22], 37.9–53.1% in cassava [24], and 55–64% of in poplar [20]. Although reporter genes could not silenced completely, the photo-bleaching was obvious. Consistently, the transcript level of *HbPDS* in HbPDS-VIGS rubber trees was 5.1% -15.3% of that in the control, with photo-bleaching phenotype and a 1.5–16.5% level of chlorophyll and carotenoids content.

Conclusions

An Agrobacterium-mediated TRV-VIGS system was the first established in rubber tree, as shown by the expression of TRV and *HbPDS*, as well as the photo-bleaching phenotype. The established TRV-VIGS system will help gene functions study to elucidate the natural rubber biosynthesis and regulation in rubber tree.

Methods

Identification of HbPDS and prediction of siRNAs

The sequence of *Nicotiana benthamiana* phytoene desaturase gene (*NbPDS*) was downloaded from NCBI (ABE99707). The local rubber tree genome database was used to search for homologous sequence of *NbPDS* with Blastn according to Li et al [31]. The pss-RNAit (https://plant grn.noble .org/pssRNAit/) was used to predict of siRNAs from the identified *HbPDS* according to Schachtsiek et al [32].

Plant cultivation

Rubber tree (cultivar Reyan 7-33-97) mature seeds were collected from the experimental plantation of Chinese Academy of Tropical Agricultural Sciences. The seeds were sowed into pots with soil mixed with nutrient matrix. The seedlings were grown in a greenhouse.

TRV-VIGS vectors construction and TRV inoculation into rubber tree plants

pTRV1 and pTRV2 VIGS vectors were established as described protocol previously[18]. For the construct of pTRV2-HbPDS, a 409-bp cDNA of *HbPDS* was amplified from rubber tree cDNA using PCR primers (Table 1). The PCR product was ligated into pTRV2 vector at the site of *Kpn*I-*Bam*HI, generating pTRV2-HbPDS. For the construct of pTRV2-HbGFP, the 450 bp *GFP* fragment (+ 101−+552-bp) was amplified

from pCAMIBA1302 carrying *GFP* gene. The *GFP* fragment was ligated into the TRV2 vector, generating TRV2-GFP. pTRV1, pTRV2-GFP and pTRV2-HbPDS were transformed into *Agrobacterium tumefaciens* GV3101. The *Agrobacterium* carrying pTRV2-HbPDS was mixed with the one carrying pTRV1, or the ones carrying pTRV2-GFP and pTRV1 at a 1:1 ratio (vol/vol). The 2-month-old rubber tree plants were used to inoculate *Agrobacterium* cultures by needleless syringe in accordance with a published protocol [18]. Firstly the low leaves were gently rubbed with arenaceous quartz, then 0.3 ml of *Agrobacterium* mix culture was slowly dripped into the injured area of each leaf, and two or three leaves per plant were inoculated. The inoculated plants were in the greenhouse.

Table 1
List of primers used in this research

Primer name	Primer sequence(5'→3')	Usage
HbPDSF	ATGACTTTGCACGGGAGTGTTTC	Cloning of *HbPDS*
HbPDSR	TCAACTAATAGTTGCCTCCACCA	
HbPDSQF	ATGACTTTGCACGGGAGTGTTTC	Expression analysis of *HbPDS* by qPCR
HbPDSQR	GCATGGCCCATCGATTCACTTAC	
HbACTF	CACCACCAGAGAGAAAGTACAG	For the internal control by qPCR
HbACTR	GATGGACCAGACTCATCGTATTC	
pTRV2HbPDSF	GAATTCGGCGCTTAACTTTATTAACCCT	Construction of VIGS-HbPDS vector
pTRV2HbPDSR	CTCGAGCTTTAGTTTCCTGTCGAACCATATG	
pTRV2-GFPF	GAATTCCAATTCTTGTTGAATTAGATGG	Construction of VIGS-GFP vector
pTRV2-GFPR	CTCGAGCTTTGATTCCATTCTTTTGTTTGTC	
DpTRV1F	GAAGCATATTAAGAGTTTTACAG	Detection of TRV1 using DNA by PCR
DpTRV1R	GATAACAACACAGACAAACCATCCAC	
DpTRV2F	GCACCTATGGTGTTGCCCTGGCTG	Detection of TRV2 using DNA by PCR
DpTRV2R	ACAAAAGACTTACCGATCAATCAAGA	
cDpTRV1F	GAAGCATATTAAGAGTTTTACAG	Detection of TRV1 using cDNA by RT-PCR
cDpTRV1R	GATAACAACACAGACAAACCATCCAC	
cDpTRV2F	TTGCCCTGGCTGGGGTATGTCA	Detection of TRV2 using cDNA by RT-PCR
cDpTRV2R	AATTCGGTAACCTTACTCACAGA	

Isolation of DNA and RNA

Isolation of DNA was performed using previously described CTAB method [33]. Total RNA was isolated in accordance with the described protocol [34]. The total RNA was digested by RNase-free DNase I. The concentration of RNA and DNA was measured using a spectrophotometer.

The expression analysis of HbPDS

Quantitative real-time PCR (qRT-PCR) was used to analysis the expression level of *HbPDS* in accordance with the described protocol [35]. *HbACT7* was used as the reference gene as describe previously [35]. The primers used for qPCR were listed in Table 1. qRT-PCR was conducted in accordance with the manufacturer's instructions of SYBR Premix Taq Kit (TaKaRa, Dalian, China). The $2^{-\Delta\Delta Ct}$ method was employed to analysis the expression level of *HbPDS*.

Detection of TRV1 and TRV2

Detection of TRV1 and TRV2 was performed by PCR using infiltrated rubber tree plant genomic DNA and cDNA with the TRV1 and TRV2 specific primers (Table 1), respectively. PCR program is following as: 94 °C for 5 min; followed by 32 cycles of 94 °C for 25 s, 57 °C for 25 s, and 72 °C for 50 s; and then 72 °C for 10 min and 4 °C for storage.

Quantification of chlorophyll and carotenoids

The quantification of chlorophyll and carotenoids was performed in accordance with the described protocol [36]. In brief, 0.5 g leaves was grounded with 80% acetone. After centrifugation, the optical density of supernatant solution were measured at 470, 665, 647 nm.

Declarations

Availability of data and materials

Not applicable

Ethics approval and consent to participate

Local, National and International guidelines were followed in this study with virus-induced gene silencing in plants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

Authors' contributions

HLL and SQP planned the experimental studies. DG carried out bioinformatics analysis. JHZ cloned the constructs. HLL, YW and LQ performed VIGS and validation of the knockdown. HLL and SQP wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Backhaus RA. Rubber formation in plants. Israel J Bot. 1985;34:283–93.

2. van Beilen JB, Poirier Y. Establishment of new crops for the production of natural rubber. Trends Biotechnol. 2007;25:522–9.

3. Deng X, Guo D, Yang S, Shi M, Chao J, Li H, et al. Jasmonate signalling in the regulation of rubber biosynthesis in laticifer cells of rubber tree, *Hevea brasiliensis*. J Exp Bot. 2018;69:3559–71.

4. Guo D, Li HL, Wang Y, Zhu JH, Peng SQ. A myelocytomatosis transcription factor from *Hevea brasiliensis* positively regulates the expression of the small rubber particle protein gene. Ind Crop Prod. 2019;133:90–7.

5. Li HL, Qu L, Guo D, Wang Y, Zhu JH, Peng SQ. Histone deacetylase interacts with a WRKY transcription factor to regulate the expression of the small rubber particle protein gene from *Hevea brasiliensis*. Ind Crop Prod. 2020;145:111989.

6. d'Auzac J, Jacob JL, Chrestin H. The composition of latex from *Hevea brasiliensis* as laticiferous cytoplasm. In: d'Auzac J, Jacob JL, editors. Physiology of the rubber tree latex. Boca Raton: CRC Press; 1989. pp., 35–42.

7. Ramos MV, Demarco D, da Costa Souza IC, de Freitas CDT. Laticifers, latex, and their role in plant defense. Trends Plant Sci. 2019;24:553–67.

8. Chow KS, Wan KL, Isa MN, Bahari A, Tan SH, Harikrishna K, et al. Insights into rubber biosynthesis from transcriptome analysis of *Hevea brasiliensis* latex. J Exp Bot. 2007;58:2429–40.

9. Tang C, Yang M, Fang Y, Luo Y, Gao S, Xiao X, et al. The rubber tree genome reveals new insights into rubber production and species adaptation. Nat Plants. 2016;2:16073.

10. Makita Y, Kawashima M, Lau NS, Othman AS, Matsui M. Construction of Pará rubber tree genome and multi-transcriptome database accelerates rubber researches. BMC Genom. 2018;19:922.

11. Liu J, Shi C, Shi CC, Li W, Zhang QJ, Zhang Y, et al. The chromosome-based rubber tree genome provides new insights into spurge genome evolution and rubber biosynthesis. Mol Plant. 2020;13:336–50.

12. Leclercq J, Martin F, Sanier C, Clement-Vidal A, Fabre D, Oliver G, Lardet L, et al. Over-expression of a cytosolic isoform of the HbCuZnSOD gene in *Hevea brasiliensis* changes its response to a water deficit. Plant Mol Biol. 2012;80:255–72.

13. Lestari R, Rio M, Martin F, Leclercq J, Woraathasin N, Roques S, et al. Overexpression of *Hevea brasiliensis* ethylene response factor HbERF-IXc5 enhances growth and tolerance to abiotic stress and affects laticifer differentiation. Plant Biotech J. 2018;16:322–36.

14. Jayashree R, Nazeem PA, Rekha K, Sreelatha S, Thulaseedharan A, Krishnakumar R, et al. Over-expression of 3-hydroxy-3- methylglutaryl-coenzyme A reductase 1 (hmgr1) gene under super-promoter for enhanced latex biosynthesis in rubber tree (*Hevea brasiliensis* Muell. Arg.). Plant Physiol Biochem. 2018;127:414–24.

15. Martina F, Abatia V, Burela A, Clément-Vidala A, Saniera C, Fabrea D, et al. Overexpression of EcGSH1 induces glutathione production and alters somatic embryogenesis and plant development in *Hevea brasiliensis*. Ind Crop Prod. 2018;112:803–14.

16. Fan Y, Xin S, Dai X, Yang X, Huang H, Hua Y. Efficient genome editing of rubber tree (*Hevea brasiliensis*) protoplasts using CRISPR/Cas9 ribonucleoproteins. Ind Crop Prod. 2020;146:112146.

17. Burch-Smith TM, Anderson J, Martin GB, Dinesh-Kumar SP. Applications and advantages of virus-induced gene silencing for gene function studies in plants. Plant J. 2004;39:734–46.

18. Senthil-Kumar M, Mysore KS. Tobacco rattle virus-based virus-induced gene silencing in *Nicotiana benthamiana*. Nat Protoc. 2014;9:1549–62.

19. Bachan S, Dinesh-Kumar SP. Tobacco rattle virus (TRV)-based virus-induced gene silencing. Methods Mol Biol. 2012;894:83–92.

20. Shen Z, Sun J, Yao J, Wang S, Ding M, Zhang H, et al. High rates of virus-induced gene silencing by tobacco rattle virus in Populus. Tree Physiol. 2015;35:1016–29.

21. Zhang J, Yu D, Zhang Y, Liu K, Xu K, Zhang F, et al. Vacuum and co-cultivation agroinfiltration of (germinated) seeds results in tobacco rattle virus (TRV) mediated whole-plant virus-induced gene silencing (VIGS) in wheat and maize. Front Plant Sci. 2017;8:393.

22. Zheng Y, Ding B, Fei Z, Wang Y. Comprehensive transcriptome analyses reveal tomato plant responses to tobacco rattle virus-based gene silencing vectors. Sci Rep. 2017;7:9771.

23. Cheng C, Gao J, Ma N. Investigation of petal senescence by TRV-mediated virus-induced gene silencing in Rose. Methods Mol Biol. 2018;1744:49–63.

24. Zeng H, Xie Y, Liu G, Wei Y, Hu W, Shi H. Agrobacterium-mediated gene transient overexpression and tobacco rattle virus (TRV)-based gene silencing in cassava. Int J Mol Sci. 2019;20:3976.

25. Cai C, Wang X, Zhang B, Guo W. Tobacco rattle virus-induced gene silencing in cotton. Methods Mol Biol. 2019; 1902: 105–119.

26. Unver T, Budak H. Virus-induced gene silencing, a post transcriptional gene silencing method. Int J Plant Genomics. 2009; 2009:198680.

27. Lange M, Yellina AL, Orashakova S, Becker A. Virus-induced gene silencing (VIGS) in plants: An overview of target species and the virus-derived vector systems. Methods Mol Biol. 2013;975:1–14.

28. Ramegowda V, Mysore KS, Senthil-Kumar M. Virus-induced gene silencing is a versatile tool for unraveling the functional relevance of multiple abiotic-stress-responsive genes in crop plants. Front Plant Sci. 2014;5:323.

29. Kumagai M, Donson J, Harvey D, Hainley K, Grill LK. Cytoplasmic inhibition of carotenoid biosynthesis with virus-derived RNA. Proc Natl Acad Sci. 1995;92:1679–83.

30. Jiang Y, Ye S, Wang L, Duan Y, Lu W, Liu H, et al. Heterologous gene silencing induced by tobacco rattle virus (TRV) is efficient for pursuing functional genomics studies in woody plants. Plant Cell Tissue Organ Cult. 2014;116:163–74.

31. Li HL, Guo D, Zhu JH, Wang Y, Peng SQ. Identification and expression analysis of genes involved in histone acetylation in *Hevea brasiliensis*. Tree Genet Genomes. 2017;13:98.

32. Schachtsiek J, Hussain T, Azzouhri K, Kayser O, Stehle F. Virus–induced gene silencing (VIGS) in *Cannabis sativa* L. Plant Methods. 2019;15:157.

33. Allen GC, Flores-Vergara MA, Krasynanski S, Kumar S, Thompson WF. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nat Protoc. 2006;1:2320–5.

34. Tang C, Qi J, Li H, Zhang C, Wang Y. 2007. A convenient andefficient protocol for isolating high-quality RNA from latex of *Hevea brasiliensis* (para rubber tree). J Biochem Bioph Meth.2007; 70:749–754.

35. Li HL, Guo D, Zhu JH, Wang Y, Chen XT, Peng SQ. Comparative transcriptome analysis of latex reveals molecular mechanisms underlying increased rubber yield in *Hevea brasiliensis* self-rooting juvenile clones. Front Plant Sci. 2016;7:1204.

36. Wellburn AR. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol. 1994;144:307–13.
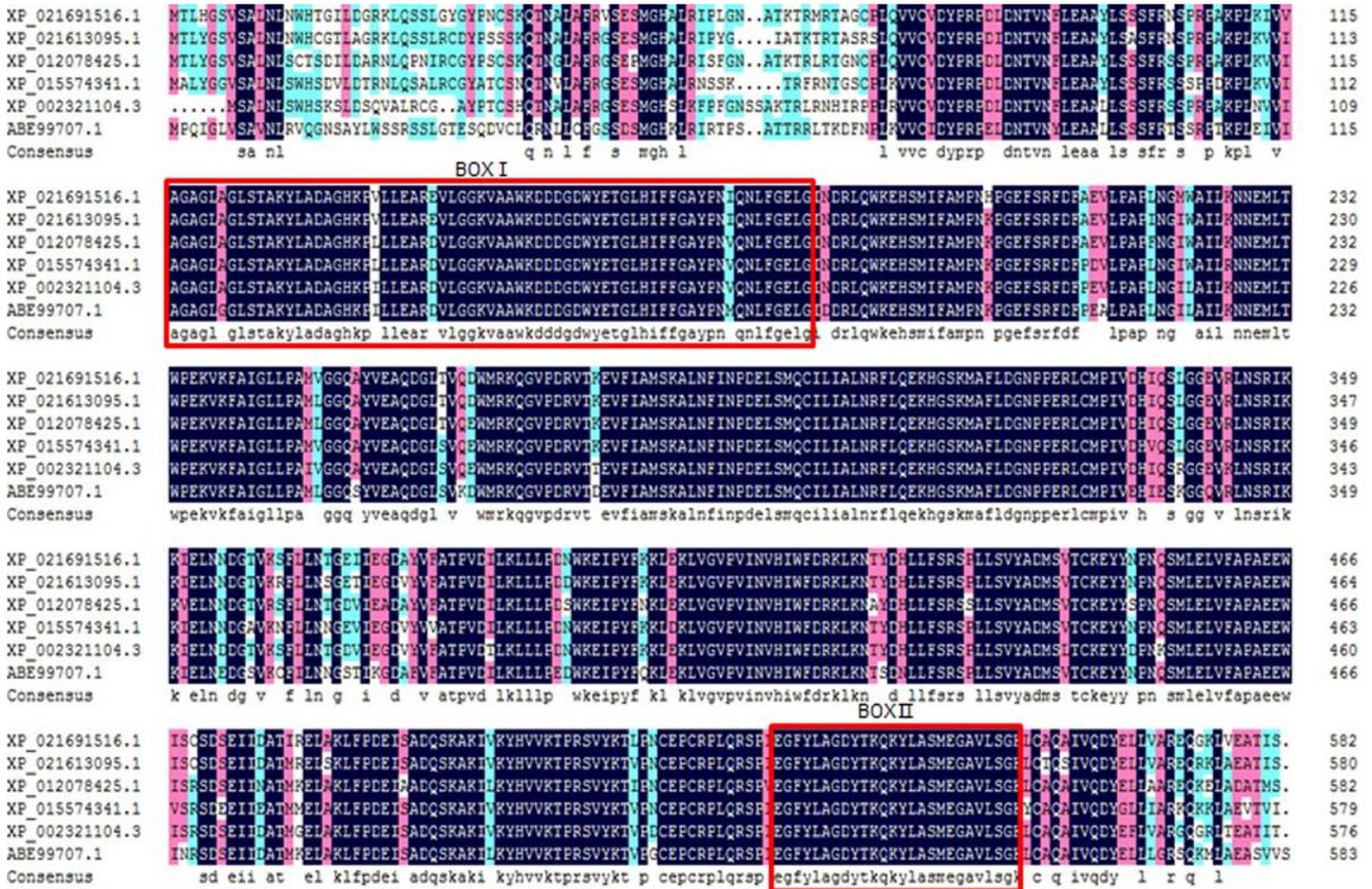
Figures

Figure 1

Alignment of HbPDS with other PDSs from other plant species. The PDS used in the alignment analysis are retrieved from Genbank including Hevea rasiliensis (XP_021691516.1), Manihot sculenta (XP_021613095.1), Jatropha curcas (XP_012078425.1), Ricinus communis (XP_015574341.1), Populus trichocarpa (XP_002321104.3), Nicotiana benthamiana (ABE99707.1). BOXⅠⅠNAD(P)-binding rossmann-like domain; BOXⅡⅡThe carotenoid-binding domain.
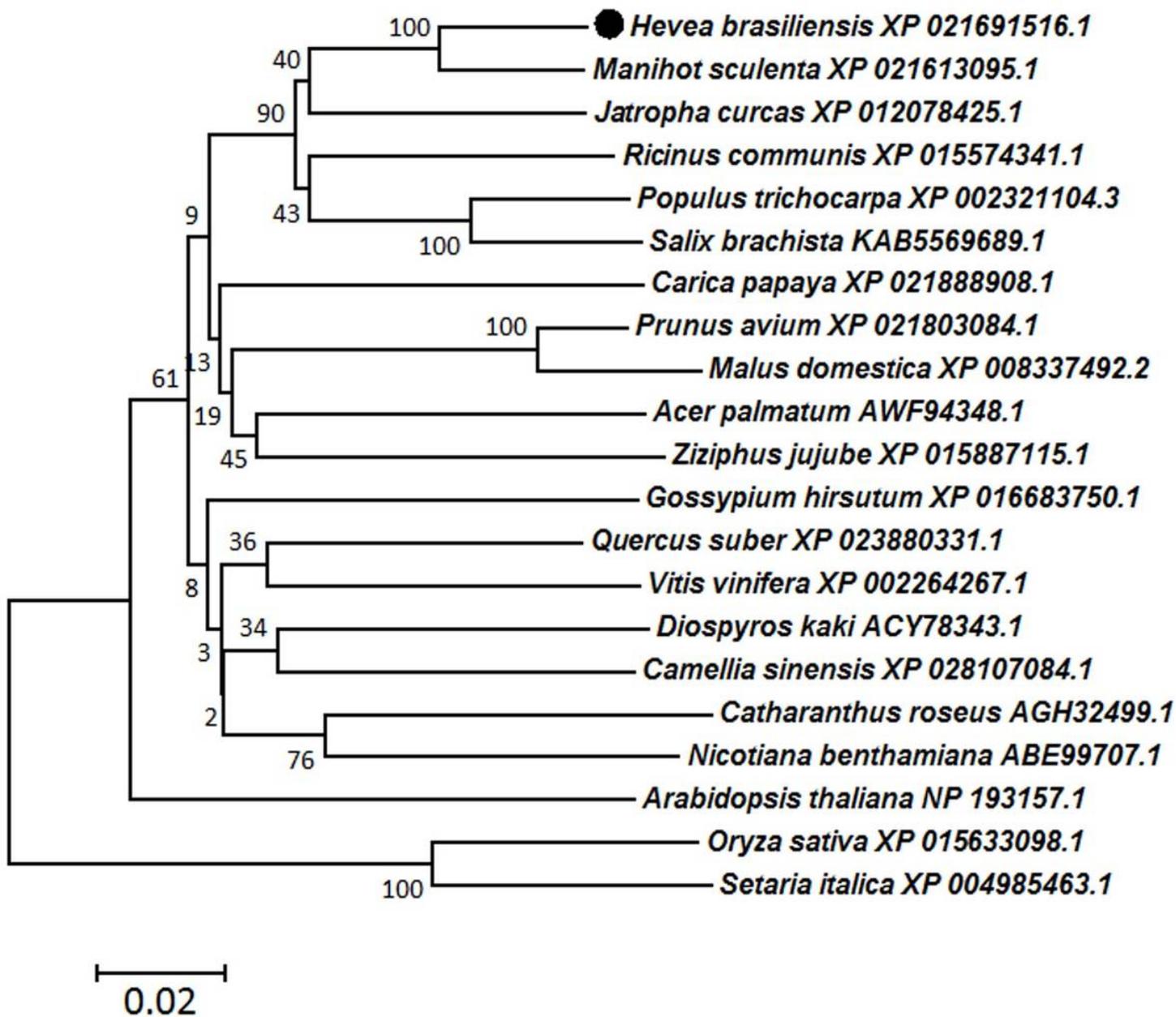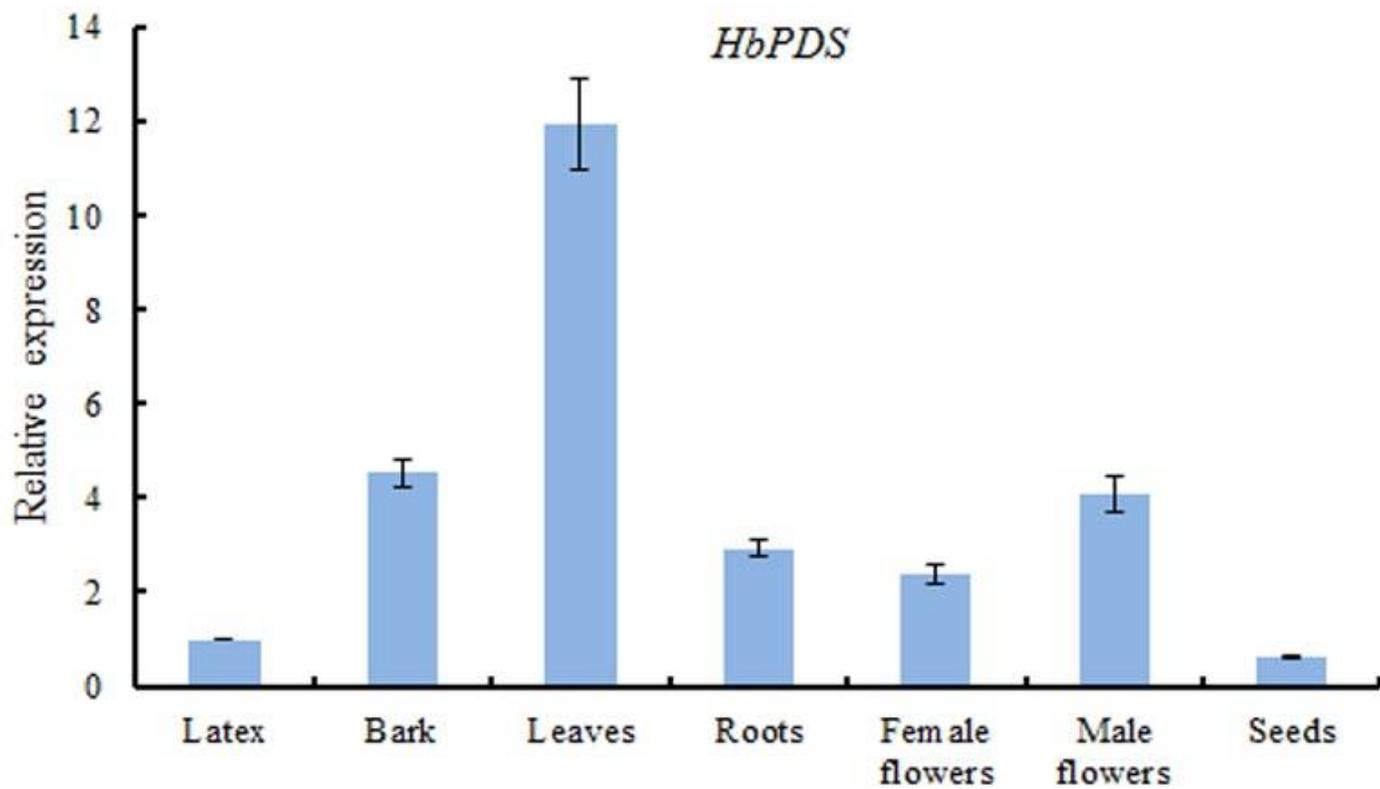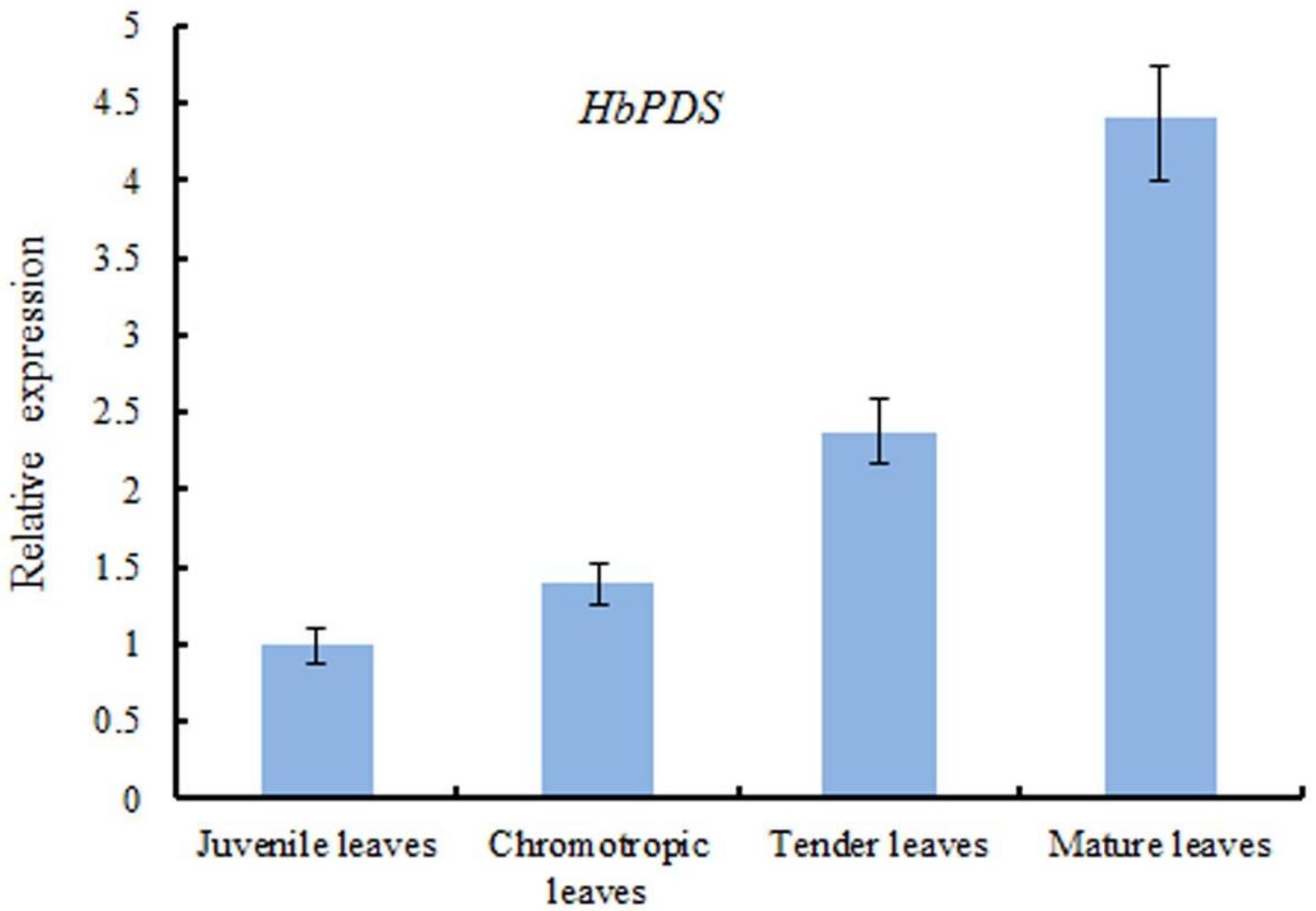
Figure 2

Phylogenetic analysis of HbPDS and PDSs from other plant species

Figure 3
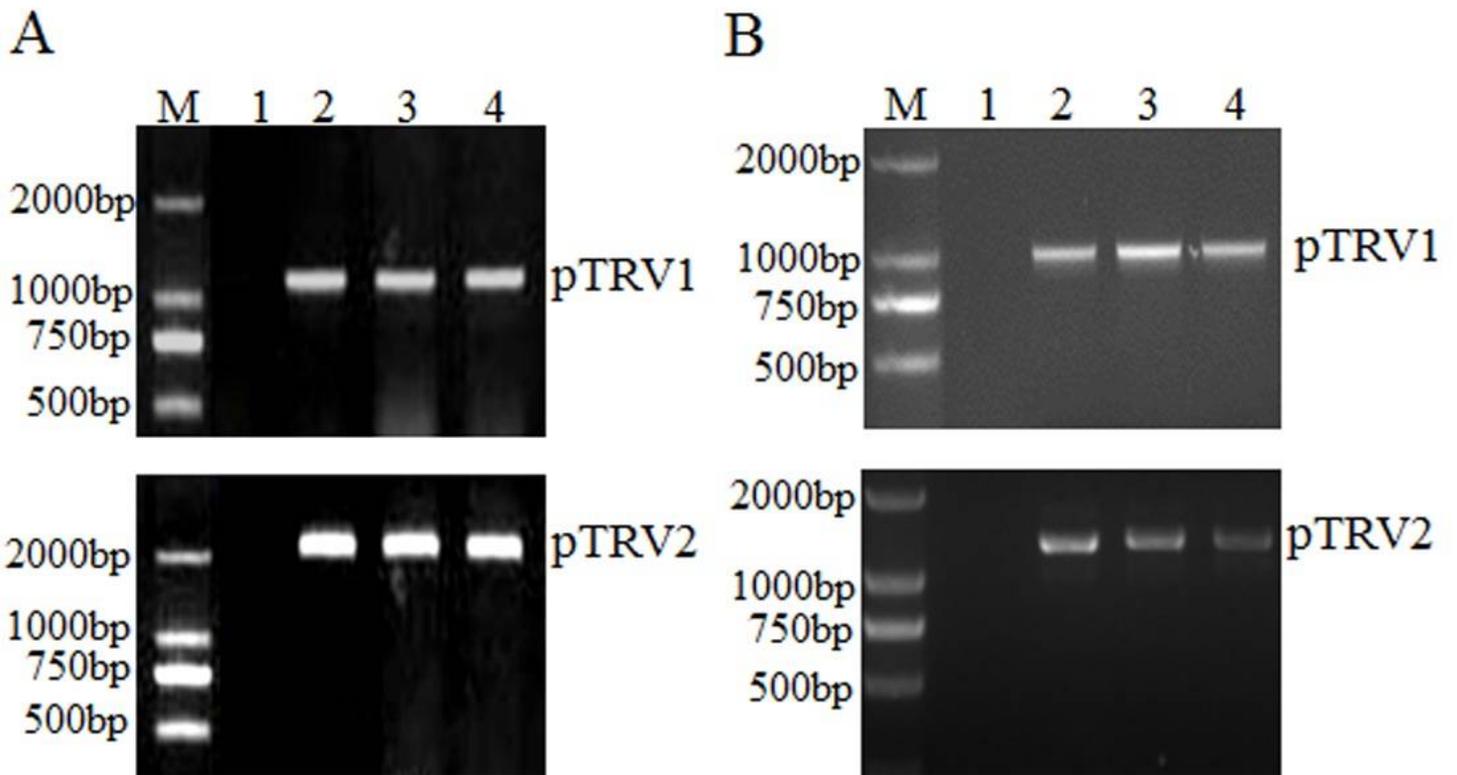
Expression analysis of HbPDS in rubber tree

Figure 4

Expression analysis of HbPDS in leaves during development
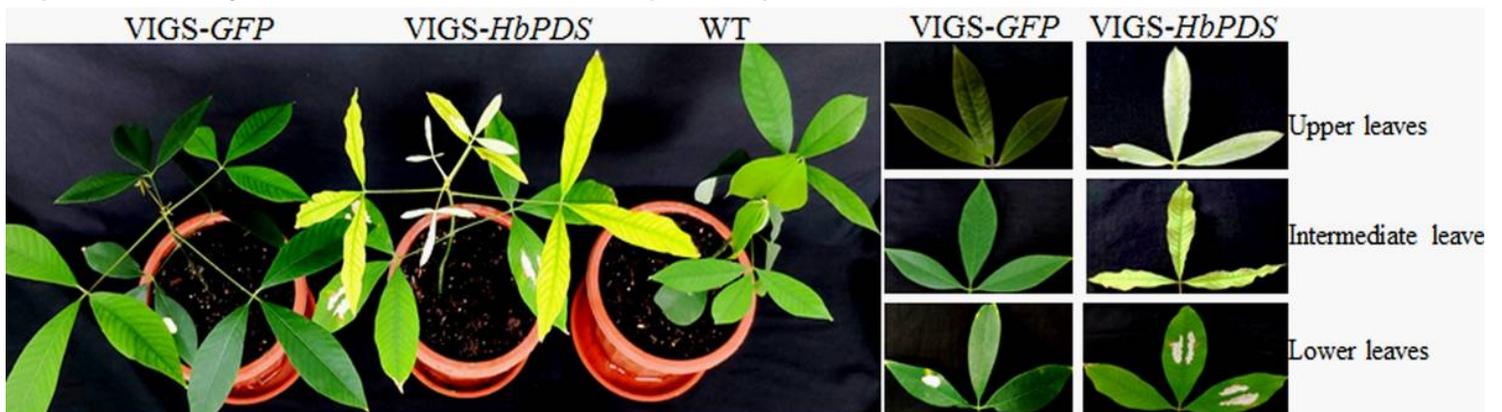
Figure 5

Phenotypes of silencing of HbPDS induced with TRV

Figure 6

Detection of VIGS rubber tree plants. (A) Detection of pTRV1 and pTRV2 sequences from the genomic DNA of VIGS-HbPDS plants by PCR. (B) Detection of pTRV1and pTRV2 sequences from the cDNA of VIGS-HbPDS plants by RT-PCR. M. DNA marker; 1. WT rubber tree plants; 2. VIGS-GFP plants; 3,4. VIGS-HbPDS plants
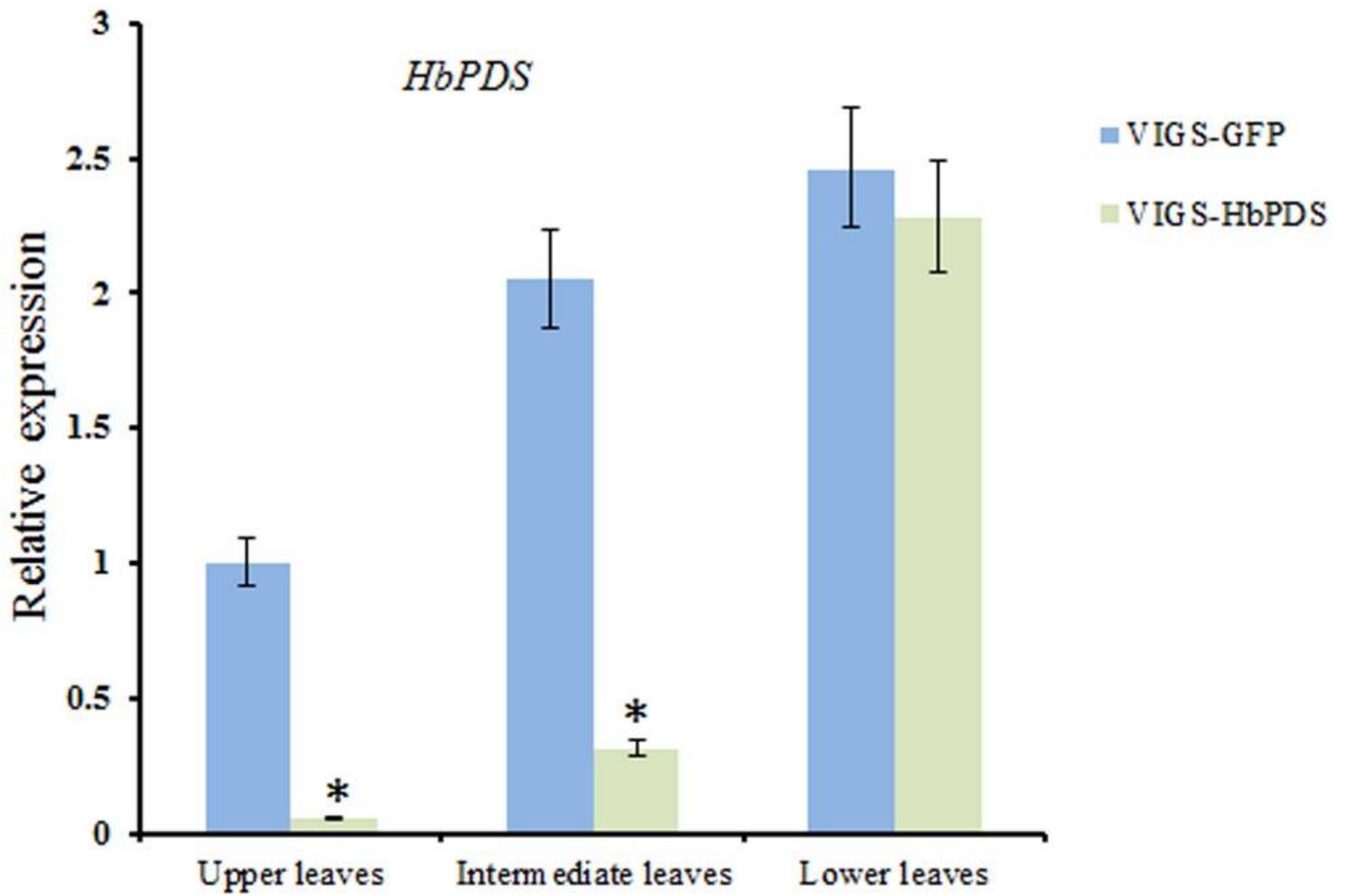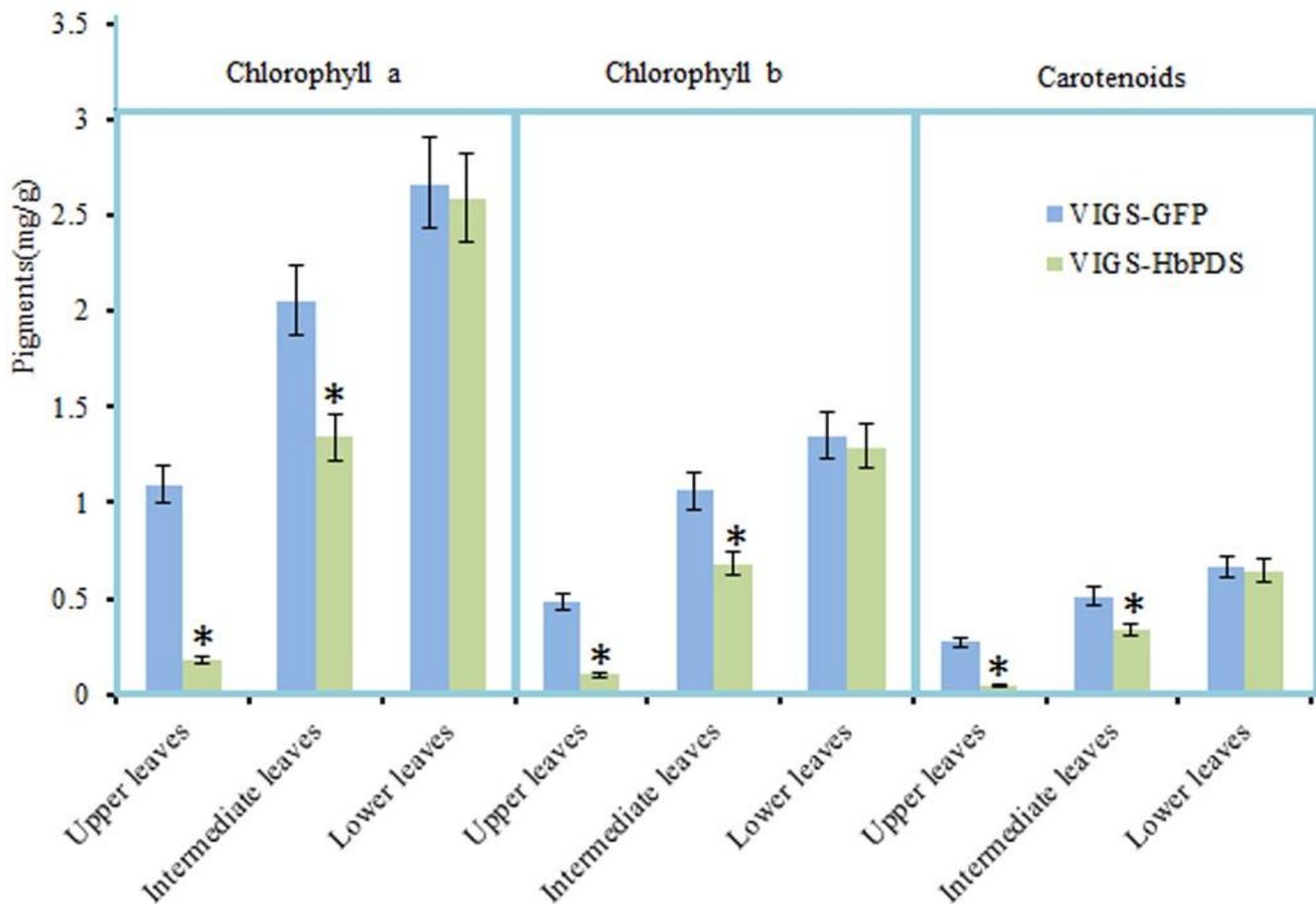
Figure 7

The expression level of HbPDS in VIGS-HbPDS plants. The error bars indicate the mean ± SE of triple independent experiments. The significant difference is indicated by an asterisk symbol (*) at $P < 0.05$

Figure 8

Quantification of pigment content in VIGS-HbPDS plants. The error bars indicate the mean ± SE of triple independent experiments. The significant difference is indicated by an asterisk symbol (*) at $P < 0.05$.