Computational Molecular Biology 2022, Vol.12, No.4, 1-8
http://bioscipublisher.com/index.php/cmb

Research Report Open Access

Cloning and Expression Analysis of Phytoene Desaturase (PDS) Gene from Mango (*Mangifera indica* L.)

Yu Kaili [1,2], Zhao Zhichang [2], Gao Aiping [2], Luo Ruixiong [2], Huang Jianfeng [2], Zhang Mengyun [2], Li Maofu [1]

1 College of Horticulture, Hainan University, Haikou, 570228, China

2 National Cultivar Improvement Center of Tropical Fruit Tree, Key Laboratory of Crop Gene Resources and Germplasm Enhancement in Southern China, Tropical Crops Genetic Resources Institute Chinese Academy of Tropical Agricultural Sciences, Haikou, 571701, China

Corresponding author email: zhaozhichang2001@163.com

Computational Molecular Biology, 2022, Vol.12, No.4 doi: 10.5376/cmb.2022.12.0004

Received: 14 Jul., 2022

Accepted: 15 Aug., 2022

Published: 28 Sep., 2022

Preferred citation for this article:

Yu K.L., Zhao Z.C, Gao A.P., Luo R.X., Huang J.F., Zhang M.Y., and Li M.F., 2022, Cloning and expression analysis of phytoene desaturase (PDS) gene from mango (*Mangifera indica* L.), Computational Molecular Biology, 12(4): 1-8 (doi: 10.5376/cmb.2022.12.0004)

Abstract Phytoene desaturase (PDS) affects the synthesis of carotenoids. It is a key gene in the carotenoids biosynthesis pathway. In order to study the function of *PDS* gene from mango fruits, the phytoene dehydrogenase (*PDS*) gene of ‘Guifei’ mango fruit was obtained with RACE methods. The full-length cDNA sequence of the gene is 1 820 bp, open reading frame is 1 650 bp, encoding 549 amino acids, the molecular weight is 61.34 KD, and the isoelectric point is 6.78. It was cluster analysis found that the mango PDS protein had a close relationship with grapefruit, cantaloupe, and papaya. Its amino acid composition is mainly alanine (ALa), leucine (Leu), valine (Val), and so on. Expression of *PDS* gene in different varieties by PCR showed: the high expression of the red ‘Guifei’ varieties and the expression of green mango varieties with low volume. Prediction of the domain, tertiary structure, and interaction protein of its protein, found to contain phytoene-desat, PLNO2487 superfamily, and other domains. The use of the STRING database found PDS interacted with proteins such as PSY, ZDS, and CRTISO. This research will be valuable for understanding the molecular mechanism of gene regulation in carotenoid biosynthesis and can be served as the basis for the metabolic engineering of mango.

Keywords Mango; PDS; Gene cloning

Mango (*Mangifera indica* L.) is known as the king of tropical fruits for its unique flavor. Its fruit has a variety of colors such as red, yellow, green, pink, yellow in red and green in yellow. Mango fruit color formation is related to metabolic pathways such as anthocyanin and carotenoids, of which the carotenoid synthesis pathway is involved in the color formation of fruits of various trees, including citrus (Peng, 2013; Zhu et al., 2017), persimmon (Zhao et al., 2011) and loquat (Hadjipieri et al., 2017). The analysis of different coloring mango varieties revealed that their fruits contained carotenoids, and it is speculated that carotenoid synthesis may be a key metabolic pathway for the coloring of mango fruits. Phytoene desaturase (PDS) catalyzes the dehydrogenation of Phytoene to generate 9,9'-dicis-ζ-carotene, followed by lycopene production via ζ-carotene desaturase (ZDS) and carotenoid isomerase (CRTISO), and PDS is involved in catalyzing the conversion of colorless Phytoene to colored carotenoids and the synthesis of other downstream carotenoids (Zhu et al., 2004). At the transcriptional level, the phytoenesynthase gene (psy), ζ-carotene dehydrogenase gene (zds) and phytoene desaturase (PDS) jointly regulate the accumulation of carotenoids in fruits (Kato et al., 2004).

Current studies have shown that PDS genes have been successfully isolated in many species, such as *Narcissus tazetta* (Chen et al., 2008, Molecular Plant Breeding, 6(3): 574-578), *Carica papaya* (Gao et al., 2009), *Fragaria×ananassa* (Zhu et al., 2011), *Strelitzia reginae* (Huang and Fan, 2013), *Cucumis melo* (Zhao, 2014), *Brassica alboglabra* (Sun et al., 2016), *Anthurium andraeanum* (Cui et al., 2016), *Cephalotaxus hainanensis* (Qiao et al., 2017), *Fructus Gardeniae* (Dong et al., 2018), *Prunus persica* (Liang et al., 2018), *Acer palmatum* (Lin et al., 2019), *Abelmoschus esculentus* (Li et al., 2019). However, the cloning of mango *PDS* gene has not been reported.

In this study, a *PDS* gene was cloned from mango fruit for the first time and its biological information was analyzed to further investigate the function of this gene and regulate carotenoid biomolecular synthesis in mango fruit.

1 Results and Analysis

1.1 Acquisition of *PDS* genes in mango

The full-length cDNA sequence of mango *PDS* gene was obtained by splicing the sequence information of 3' end and 5' end using RACE technique. Specific primers were designed to amplify the full-length cDNA sequence, and the electrophoretic strips were recovered and sequenced to produce a *PDS* gene cDNA sequence with length of 1 820 bp, which contains an open reading frame (ORF) with length of 1 650 bp and can encode 549 amino acids. The obtained gene sequences were compared with the NCBI-registered PDS protein sequences of pomelo (*Citrus maxima* (Burm) Merr.), tobacco (*Nicotiana tabacum* L.) and maize (*Zea mays* L.) (Figure 1), and the cloned gene was identified as PDS.

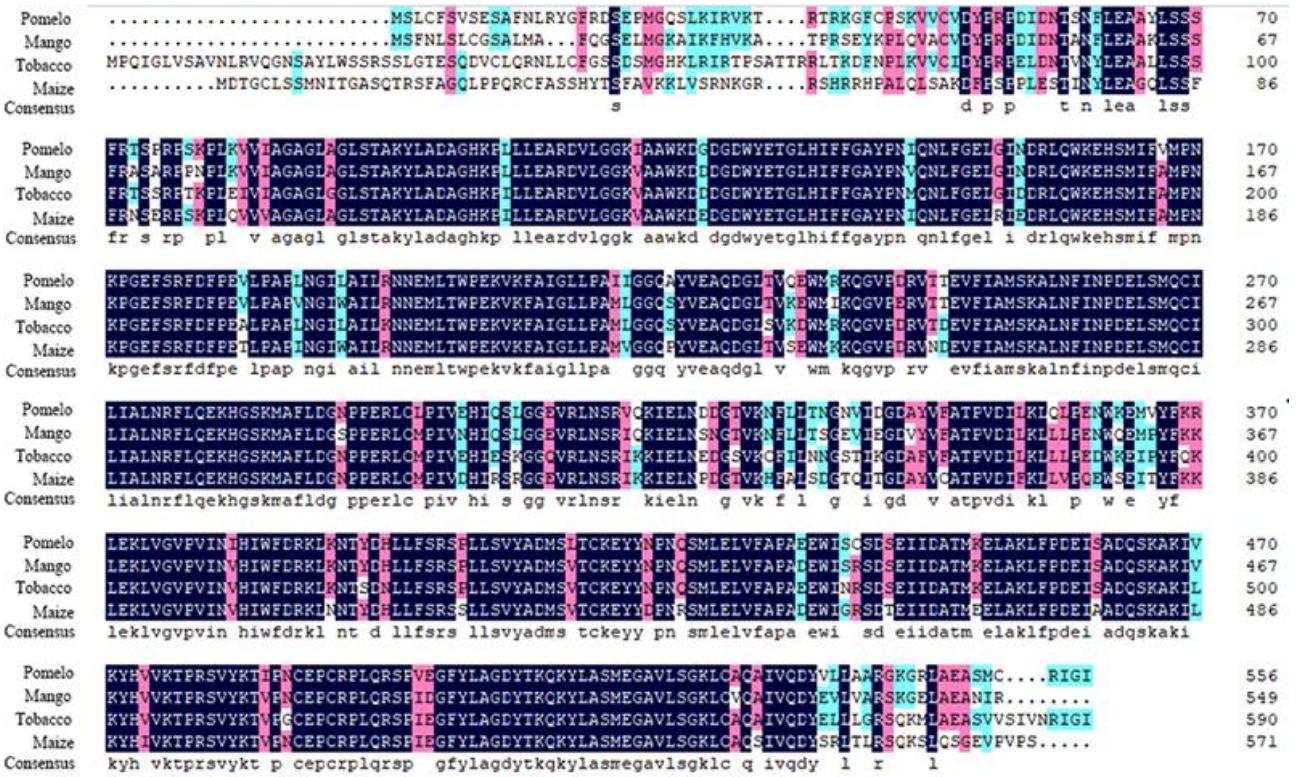

Figure 1 Sequence alignment of several PDS protein

1.2 Bioinformatics analysis of mango PDS protein

The amino acid content of mango PDS proteins was analyzed using bioinformatic Bioedit software. The results showed that the alanine, leucine and valine were found to be high in the protein sequence (Figure 2). By predicting the secondary structure of mango PDS protein with the help of DNAMAN software, we found that most of the secondary structures of mango PDS protein were irregularly coiles and β-turn, and there were also a few α-helices (Figure 3). The hydrophilicity/hydrophobicity of the PDS protein was analyzed using the Kyte and Doolittle algorithms of Bioedit software, and the amino acid hydrophobicity (positive value) of the PDS protein was the highest at 1.76 and the hydrophilicity (negative value) was the lowest at -1.68, with the amino acid content mainly between the two values (Figure 4). The Conserved domains (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) of NCBI were used to find the conserved domain of the sequence. It was found that PDS protein contained binding domains such as PLN02612, COG3349, Amino_oxidase, and phytoene_desat (Figure 5). PDS proteins were predicted to be localized in the nucleus with the help of WoLFPSORT online software (http://www.genscript.com/wolf-psort.html). The possible

conformations of the tertiary structure of the protein were predicted on the Swissmodel online software (http://swissmodel.expasy.org/) (Figure 6). Clustering analysis of the affinities by DNAMAN software showed that the PDS proteins of mango could be clustered into one group with closer affinity to the protein sequences of pomelo, muskmelon, and papaya (Figure 7). Its protein interactions were predicted according to STRING software and found to have interactions with PSY, ZDS, CRTISO and other proteins (Figure 8).

1.3 RT-PCR analysis of *PDS* gene in mango

RNA was extracted from the peels of different mango varieties, and the extracted RNA was used as a template for reverse transcription to cDNA, which was analyzed by RT-PCR. The results showed that the expression of *PDS* gene was higher in the red 'Guifei' mango peel, which was 1.51 times than the yellow 'Jinhuang' mango, and 1.88 times than the green 'Guiqi' mango, where the expression of *PDS* gene was less in the green 'Guiqi' mango peel (Figure 9). It is preliminarily speculated that the cloned *PDS* gene plays an important role in carotenoid synthesis in mango peel.

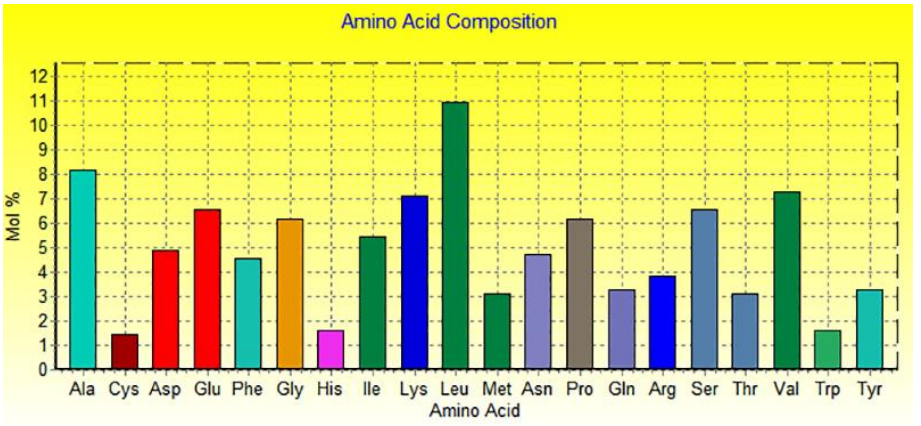

Figure 2 The contents of amino acids of PDS protein sequence from mango

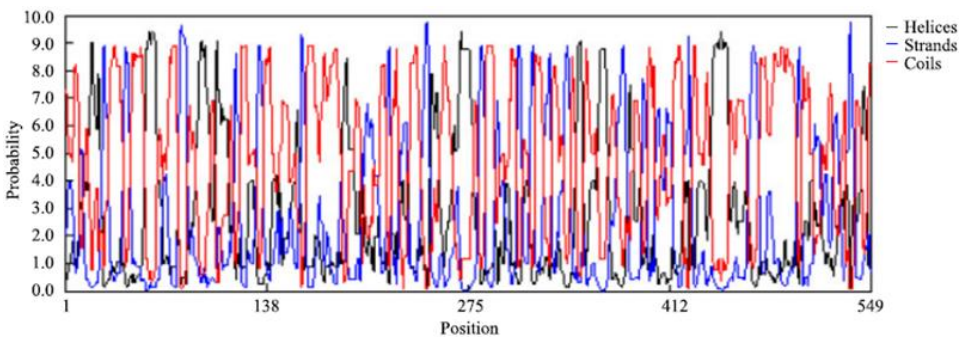

Figure 3 The prediction of PDS protein secondary structure from mango

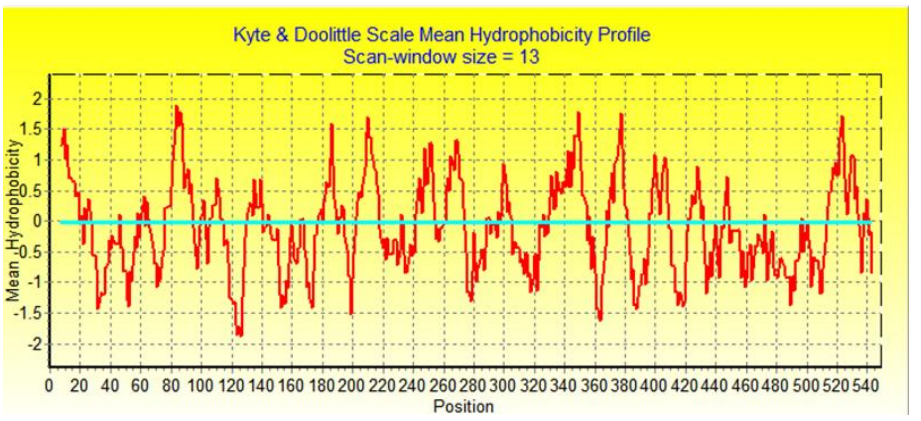

Figure 4 The hydrophobic region of PDS protein from mango

删除[Judy]:

设置格式[Judy]: 段落间距段前: 2 磅

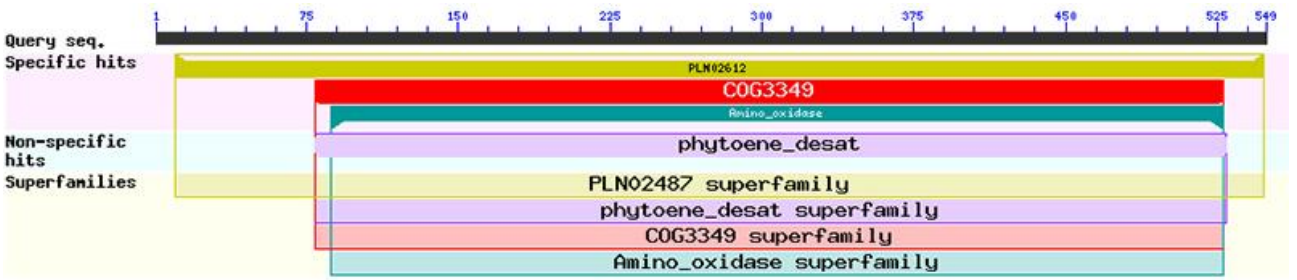

Figure5 Domain of protein coded by *PDS* gene

Figure 6 The model of tertiary structure of protein coded by *PDS* gene

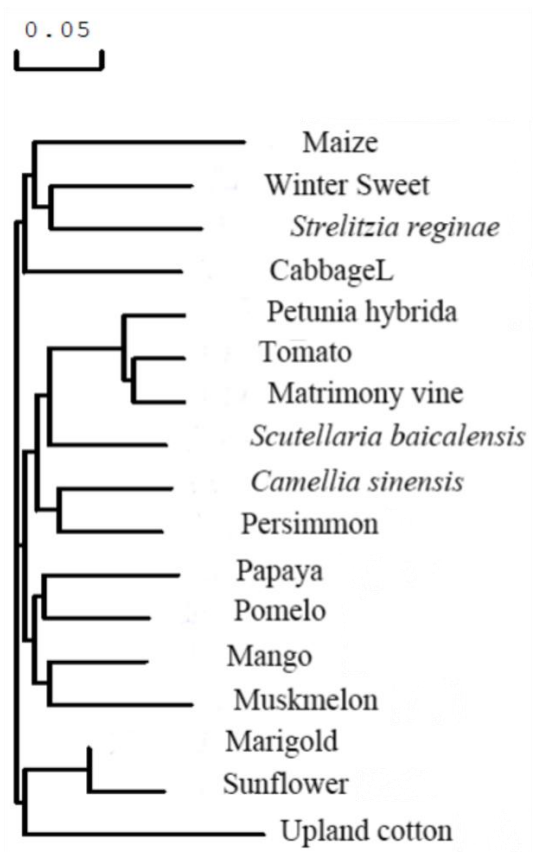

Figure 7 Phylogenetic relationship of some PDS protein sequences

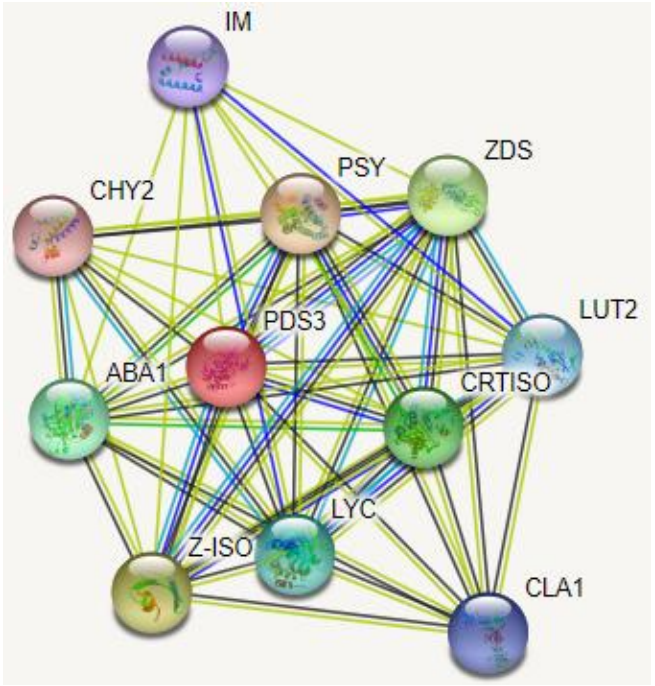

Figure 8 Prediction of PDS interacting protein from mango
Note: ZDS: ζ-carotene desaturase; PSY: Phytoene synthase; CRTISO: Carotenoid isomerase; ABA: Abscisic acid; LYC: Lycopene beta cyclase; IM: Ubiquinol oxidase 4; LUT2: Lycopene epsilon cyclase; CHY2: Beta-carotene 3-hydroxylase 2; Z-ISO: 15-cis-zeta-carotene isomerase; CLA1: 1-deoxy-D-xylulose-5-phosphate synthase

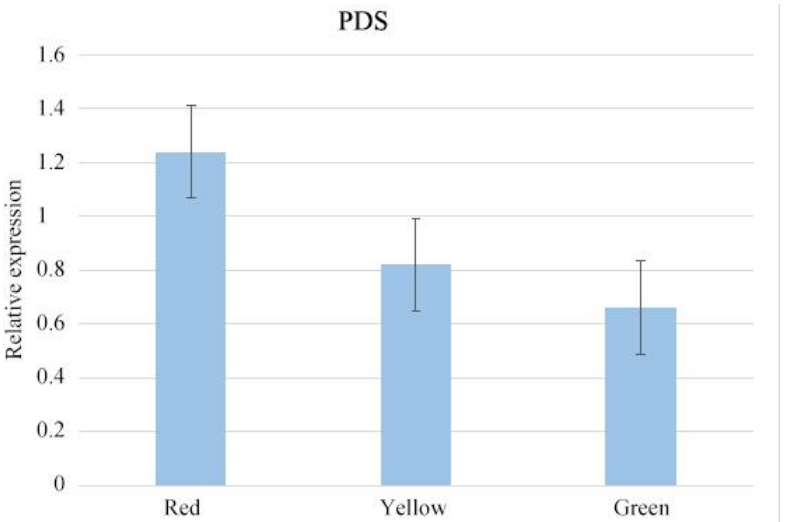

Figure 9 Expression of *PDS* gene in mango fruit

2 Discussion

Carotenoids are the main pigments in many flowers and fruits. Their species and content are important factors affecting fruit color and quality. Therefore, it is necessary to deeply study the key enzymes involved in carotenoid synthesis and analyze the regulation mechanism of carotenoid synthesis pathway. Studies have shown that phytoene desaturase (PDS) is an important branch in the carotenoid synthesis pathway and that inhibition of PDS expression leads to the accumulation of phytoene, which ultimately affects carotenoid synthesis. Therefore, *PDS* plays an important role in flower and fruit color development in plants (Bartley and Scolnik, 1995; Zhu et al., 2002; Kato et al., 2004). Disturbance of PDS affects the biosynthesis of chlorophyll, carotenoids and gibberellins, leading to albinism and dwarfism (Naim et al., 2018).

In this study, a phytoene desaturase (*PDS*) gene was successfully cloned from the fruit of mango. The full-length cDNA sequence of this gene is 1 820 bp, with an open reading frame of 1 650 bp, encoding 549 amino acids.

Analysis of the cloned gene-interacting proteins using the STRING database revealed that PDS proteins interacted with PSY, ZDS, and CRTISO, which is consistent with the results of Kato et al. (2004). PDS amino acid sequences of different species were searched on NCBI, and by predicting the conserved structural domains, it was found that the selected sequences all contain a conserved binding domain at the C-terminus of the PDS protein to bind either the NAD/NADP or FAD conserved structural domains (Xi et al., 2013). Prediction of the conserved structural domain of the mango PDS protein revealed that it also contains the FAD conserved structural domain, suggesting that this conserved structural domain may be related to the function of PDS and that the PDS code is highly conserved during evolution. The main form of the *PDS* gene in the genome exists as a single copy, and its encoded protein is in a cystoid on the chloroplast (Grunewald et al., 2000). Verification of kiwi AcPDS1 protein localization in the cell membrane and cytoplasm using laser confocal experiments (Chen, 2019). In this study, the software predicted that the mango PDS protein is localized in the nucleus, which is different from the former PDS protein localization, indicating that as organisms evolve, the PDS protein is not only restricted to the chloroplast, but the structure becomes more complex, thus requiring analysis of different species to better understand the function of this gene.

In red papaya and yellow papaya ripe fruits, the *PDS* expression differed within the two varieties, with significantly higher expression in red-fleshed fruits than in yellow-fleshed fruits (Gao et al., 2009). RT-PCR analysis revealed that *pds* gene expression was highest in mature red fruits of strawberry, followed by flowers, and the lowest expression was in leaves. It is speculated that *pds* may play an important role in carotenoid accumulation in strawberry fruits and color development of flowers (Zhu et al., 2011). The carotenoids in the peel of papaya are mainly lutein and β Carotenoids, while the carotenoids in the pulp are mainly lycopene. *PDS* gene expression is up-regulated during papaya fruit ripening, and the orange-red color of papaya may be due to the accumulation of lycopene, β-carotene and β-cryptoxanthin (Shen et al., 2019). Using the cloned kiwifruit *AcPDS1* gene, β-carotene content was significantly increased in transiently expressed tobacco leaves (Chen, 2019). In this study, *PDS* gene expression analysis was performed on three different mango varieties with large coloring differences, and it was found that the expression of *PDS* gene in different color mango peels had significant differences, with the highest expression in the red 'Guifei' variety, followed by yellow 'Jinhuang' variety, and the lowest expression in the green 'Guiqi' variety. This is the same as the former. *PDS* gene affects the accumulation of carotenoids, which plays a key role in the formation of red peel in mango. Further studies on the role of the *PDS* gene in mango fruit coloration require further validation of expression in transgenic plants.

3 Materials and Methods

3.1 Selection of materials and extraction of total RNA from mango

In this study, a total of 30 fully matured mango fruits (*Mangifera indica* L.) of ‘Guifei’, ‘Jinhuang’ and ‘Guiqi’ were selected from the Mango Resource Germplasm Nursery of the Ministry of Agriculture, Danzhou City, Hainan Province (20°02′45.97″ N, 110°11′38.39″ E). The mango peel was mixed and sampled with a razor blade, and the samples were immediately snap-frozen in liquid nitrogen and stored in a -80 °C refrigerator for backup. The RNA extraction kit of Tiangen was used to extract the total RNA from mango peel. In the extraction of RNA, the appropriate amount of RNase-free DNase I enzyme solution was added for the removal of residual DNA and dissolved in RNase-free water, and the integrity of RNA and whether DNA was completely removed were detected by 1.0% agarose gel electrophoresis.

3.2 Acquisition of full-length cDNA sequences of *PDS* genes in mango

The first strand cDNA of total mango RNA was synthesized by reverse transcription using the SMARTer™ RACE cDNA Amplification Kit (Cloontech) with the extracted total mango RNA as the template, and the cDNAs at the 3' and 5' ends were amplified, respectively. The PCR reaction program was set as follows: pre-denaturation at 94 °C for 4 min; denaturation at 95 °C for 50 s, denaturation at 50 °C for 50 s, extension at 72 °C for 2 min, 30 cycles, and final extension at 72 °C for 7 min. The reaction system was 25 μL, containing 10 PCR buffers (including Mg2+) 2.5 μL, 25 ng DNA template, 20 μmol primers, 1.0 U Taq DNA polymerase, and 5.0 mmol dNTPs. After electrophoretic analysis of the PCR products, the target gene fragments are recovered, ligated to the

vector, transformed into the sensory state, identified and the positive clones are sequenced. Based on the sequence results obtained for the 3' and 5' ends of the gene, the full-length gene of the gene was spliced and specific primers were designed to increase the full-length gene sequence.

3.3 Bioinformatics analysis and prediction of *PDS* gene in mango

ORF finder on NCBI was used to search for open reading frame of *PDS* gene. The amino acid content of PDS proteins was analyzed using Bioedit software and the hydrophilic/hydrophobic analysis of amino acids was performed with the help of Kyte and Doolittle algorithms. DNAMAN software was used to model protein secondary structure and perform homologous sequence alignment and establish a phylogenetic tree. The tertiary structure model of PDS protein was predicted and constructed using SWISS-MODEL online software (http://swissmodel.expasy.org/). BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) on NCBI was used to search for homologous sequences of PDS proteins. Conserved domains of the PDS protein sequence were analyzed using Conserved domains (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) in NCBI. Localization of PDS proteins was predicted on WoLFPSORT online software (http://www.genscript.com/wolf-psort.html); their protein interactions were predicted using STRING software. STRING software was used to predict their protein interactions.

3.4 Expression analysis of *PDS* genes in mango

RNA from three mango varieties with different fruit colors was reverse transcribed into the first strand of cDNA under the action of reverse transcriptase, and primers were designed for RT-PCR amplification using primer5.0. Design the internal reference primer sequences used for RT-PCR analysis and the primers used for target gene amplification (Table 1). The PCR products were subjected to 1.0% agarose gel electrophoresis and detected using ethidium bromide staining, and then analyzed by gel imaging system and Quantity one software to determine the relative expression.

Table 1 Information of PCR primer sequence

	Name of primers	Sequence of primers (5' race-3' race)
Internal reference primer	actin-F	5’-AATGGAACTGGAATGGTCAAGGC-3’
	actin-R	5’-TGCCAGATCTTCTCCATGTCATCCCA-3’
Target gene primers	PDS-F	5’-CAGCATAGCCTAGCACATACTC-3’
	PDS-R	5’-CAATGGAGCATATACGAAACTAT-3’

Author's Contributions

YKL and ZZC are the experimental designers and executors of this study. YKL, ZZC, GAP, and LMF completed the data analysis and the first draft of the paper. LRX, HJF, and ZMY participated in experimental design and analysis of experimental results. ZZC is the architect and person in charge of the project, guiding experimental design, data analysis and paper writing and revision. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by the National Key Research and Development Program of China (2018YFD1000500), the National Natural Science Foundation of China (31471850), the Special Fund for Basic Research Operations of the Chinese Academy of Tropical Agricultural Sciences (1630032017005; 1630032017004) and the Protection of Tropical Crop Germplasm Resources of the Ministry of Agriculture (15RZZY-07).

References

Bartley G.E., and Scolnik P.A., 1995, Plant carotenoids: pigments for photoprotection, visual attraction, and human health, Plant Cell, 7(7): 1027-1038. https://doi.org/10.1105/tpc.7.7.1027

Chen Q., 2019, Effect of *AcPDS1* gene on fruit quality of transgenic tomato, Thesis for M.S., School of Food and Biological Engineering, Hefei University of Technology, Supervisor: Huang S.X., pp.53.

Cui J.T., Tian N., Zhang K.Z., and Feng Y.R., 2016, Cloning and expression analysis of phytoene desaturase gene in *Anthurium andraeanum*, Zhongguo Nongxue Tongbao (Chinese Agricultural Science Bulletin), 32(34): 72-79.

Dong L.H., Wang P.L., and Wang X.Y., 2018, Cloning and expression analysis of phytoene desaturase gene from Gardenia fruit, Shengwu Jishu (Biotechnology), 28(4): 313-317.

Gao X.Z., Yan P., Shen W.T., and Zhou P., 2009, Temporal-spatial expression analysis of *pds* gene in papaya by real-time PCR, Guangxi Nongye Kexue (Guangxi Agricultural Sciences), 40(6): 610-613.

Grunewald K., Eckert M., Hirschberg J., and Hagen C., 2000, Phytoene desaturase is localized exclusively in the chloroplast and up-regulated at the mRNA level during accumulation of secondary carotenoids in *Haematococcus pluvialis* (Volvocales, Chlorophyceae), Plant Physiol., 122(4): 1261-1268.
https://doi.org/10.1104/pp.122.4.1261

Hadjipieri M., Georgiadou E.C., Marin A., Diaz-Mula H.M., Goulas V., Fotopoulos V., Tomás-Barberán F.A., Manganaris G.A., 2017, Metabolic and transcriptional elucidation of the carotenoid biosynthesis pathway in peel and flesh tissue of loquat fruit during on-tree development, BMC Plant Biol., 17(1): 102.
https://doi.org/10.1186/s12870-017-1041-3

Huang M.L., and Fan R.H., 2013, Cloning and expression analysis of phytoene desaturase in *Strelitzia reginae* Banks, Yuanyi Xuebao (Acta Horticulture Sinica), 40(2): 373-379.

Kato M., IKoma Y., Matsumoto H., Sugiura M., Hyodo H., and Yano M., 2004, Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit, Plant Physiol., 134(2): 824–837.
https://doi.org/10.1104/pp.103.031104

Li Y.P., Wang B., Chen M.D., Liu J.T., Zhu H.S., and Wen Q.F., 2019, Cloning and expression analysis of a phytoene desaturase gene in *Hibiscus esculentus* L., Xibei Zhiwu Xuebao (Acta Botanica Boreali-Occidentalia Sinica), 39(7): 1195-1202.

Liang M.H., Yang Z.F., Su X.G., and Song C.B., 2018, Cloning and expression of the phytoene desaturase gene, *PpPDS*, from pesches, Fujian Nongye Xuebao (Fujian Journal of Agricultural Sciences), 33(6): 608-613.

Lin R.Y., Fan R.H., Chen Y.D., Luo Y.H., Zhong H.Q., and Lin B., 2019, Cloning and expression analysis of *ApPSY* and *ApPDS* gene from *Acer palmatum*, Henong Xuebao (Journal of Nuclear Agricultural Science), 33(12): 2338-2346.

Naim F., Dugdale B., Kleidon J., Brinin A., Shand K., Waterhouse P., and Dale J., 2018, Gene editing the phytoene desaturase alleles of Cavendish banana using CRISPR/Cas9, Transgenic Res., 27(5): 451-460.
https://doi.org/10.1007/s11248-018-0083-0

Peng G., 2013, Chlorophyll and carotenoid metabolism based fruit color-break in citrus, Dissertation for Ph.D., College of Agriculture and Biotechnology, Zhejiang University, Supervisor: Xu C.J., pp.50.

Qiao F., Xu Z.J., Long Y.L., Cong H.Q., and Jiang X.F., 2017, Cloning of PDS synthase gene and expression characteristics induced by methyl jasmonate in *Cephalotaxus hainanensis*, Fenzi Zhiwu Yuzhong (Molecular Plant Breeding), 15(1): 129-135.

Shen Y.H., Yang F.Y., Lu B.G., Zhao W.W., Jiang T., Feng L., Chen X.J., and Ming R., 2019, Exploring the differential mechanisms of carotenoid biosynthesis in the yellow peel and red flesh of papaya, BMC Genom., 20(1): 49.
https://doi.org/10.1186/s12864-018-5388-0

Sun B., Zhang F., Xia X., Xue S.L., Yuan Q., Chen Q., and Tang H.R., 2016, Cloning and expression analysis of *BaPDS1* and *BaPDS2* in *Brassica alboglabra*, Yuanyi Xuebao (Acta Horticulture Sinica), 43(11): 2257-2265.

Xi H.X., Li H.Y., and Tong S.M., 2013, Conserved motif and phylogenetic analysis of phytoene desaturase (PDS) proteins in plants, Zhiwu Shengli Xuebao (Plant Physiology Communications), 49(12): 1407-1412.

Zhao D.Q., Zhou C.H., Kong F., and Tao J., 2011, Cloning of phytoene desaturase and expression analysis of carotenogenic genes in persimmon (*Diospyros kaki* L.) fruits, Mol. Biol. Rep., 38(6): 3935-3943.
https://doi.org/10.1007/s11033-010-0510-7

Zhao J.L., 2014, Cloning of melon's *PDS* gene and study of β-carotenoid accumulation molecular mechanism in muskmelon, Thesis for M.S., College of Horticulture Science and Engineering, Shandong Agricultural University, Supervisor: Wang X.F., pp.33-34.

Zhu C.F., Chen X., and Wang Y.D., 2004, Carotenoid biosynthesis in plants and application of its relative genes in gene engineering, Zhiwu Shengli yu Fenzi Shengwuxue Xuebao (Journal of Plant Physiology and Molecular Biology), 30(6): 609-618.

Zhu C.F., Yamamura S., Koiwa H., Nishihara M., and Sandmann G., 2002, cDNA cloning and expression of carotenogenic genes during flower development in *Gentiana lutea*, Plant Mol. Biol., 48(3): 277-285.
https://doi.org/10.1023/A:1013383120392

Zhu F., Luo T., Liu C.Y., Wang Y., Yang H.B., Yang W., Zheng L., Xiao X., Zhang M.F., Xu R.W., Xu J.G., Zeng Y.L., Xu J., Xu Q., Guo W.W., Larkin R.M., Deng X.X., and Cheng Y.J., 2017, An R2R3-MYB transcription factor represses the transformation of α- and β-branch carotenoids by negatively regulating expression of *CrBCH2* and *CrNCED5* in flavedo of *Citrus reticulate*, New phytol., 216(1): 178-192.
https://doi.org/10.1111/nph.14684

Zhu H.S., Li Y.P., Wen Q.F., and Lin H., 2011, Cloning and characterization of *pds* gene in *Fragaria* × *ananassa*, Yuanyi Xuebao (Acta Horticulturae Sinica), 38(1): 55-60.