Genomics and Applied Biology 2023, Vol.14, No.1, 1-11
http://bioscipublisher.com/index.php/gab

Research Article Open Access

Characteristics of *LDOX* Gene Structure Analysis and Construction of Protein Interaction Network in *Actinidia arguta*

Zhenpan Liu, Liyuan Lu, Yang Sun, Yue Zhang, Dongsheng Li, Wenzhong You
Liaoning Economic Forest Research Institute, Dalian, 116031, China
Corresponding author email: Wzhyou2002@163.com

Genomics and Applied Biology, 2023, Vol.14, No.1 doi: 10.5376/gab.2023.14.0001
Received: 08 Aug., 2022
Accepted: 15 Feb., 2023
Published: 13 Mar., 2023

Preferred citation for this article:
Liu Z.P., Lu L.Y., Sun Y., Zhang Y., Li D.S., and You W.Z., 2023, Characteristics of *LDOX* gene structure analysis and construction of protein interaction network in *Actinidia arguta*, Genomics and Applied Biology, 14(1): 1-11 (doi: 10.5376/gab.2023.14.0001)

Abstract The purpose of this study is to reveal the structure and function of the leucoanthocyanidin dioxygenase (*LDOX*) gene in *Actinidia arguta*. Bioinformatics methods were used to analyze the LDOX nucleotide and amino acid sequences of *Actinidia arguta*, construct the protein interaction network of LDOX gene, and conduct GO and KEGG functional annotation analysis. The results showed that there were 10 conserved motifs in *Actinidia arguta*, which was closely related to *Vaccinium corymbosum*, *Lonicera japonica* and *Daucus carota*. Cis-regulated elements were mainly involved in ABA signaling pathways, responding to drought response, anaerobic induction, gibberellic acid, auxin response factor, MeJA response, low temperature response, and photostimulus response. Physical and chemical properties and structural analysis of LDOX showed that it was hydrophilic protein, and no signal peptide and transmembrane structural regions were found, mainly including Ser phosphorylation sites (13), Thr phosphorylation sites (12) and Tyr phosphorylation sites (6). There were two O-glycosylation sites (Thr^4 and Thr^{323}) and $Asn^{107}$of N- glycosylation sites at residues ranging from 107 to 109. There was a domain structure conservative in 48-347 bp was 20G-FeII Oxy. The secondary structure main random coil (43.10%). There was a certain interaction between LDOX and proteins such as F3H, UFGT, FLS and PAL, which involved in flavonoid biosynthesis and metabolism process that plays an important role in plant-type vacuole membrane, and regulates the function of catalytic activity and redox active molecule. *LDOX* gene was highly conserved and plays an important regulatory role in progesterone pathway, which provides a theoretical basis for further research on the functional mechanism of *LDOX* gene in *Actinidia arguta*.

Keywords *Actinidia arguta*; *LDOX*; Structure function; Protein interaction

In recent years, the industry of *Actinidia arguta* has sprung up, and its fruit has become more and more popular in the market. At present, it has been widely cultivated in Liaoning, Zhejiang, Henan and other regions, and has become a new cultivated species of *Actinidia Lindl* plants with rapid development (Li, 2017, Graduate School of Chinese Academy of Agriculture Sciences, pp.14). *Actinidia arguta* is rich in resources, and its fruit is rich in flavonoid anthocyanins and antioxidants, which makes the fruit show a variety of colors (He and Giusti, 2010). Flavonoid anthocyanins play an important role in the anthocyanin synthesis pathway. As the main catalytic enzyme in the downstream region of the flavonoid synthesis pathway, colorless leucoanthocyanidin dioxygenase (*LDOX*) relies on 2-ketoglutarate ion and Fe^{2+} to catalyze the oxidation of colorless anthocyanins, thereby transforming colorless anthocyanins into colored anthocyanins (Appelhagen et al., 2011; Chung et al., 2010; Xie et al., 2004). The *LDOX* gene was cloned and identified from maize (*Zea mays* L.) A2 mutant according to transposon labeling technology (Menssen et al., 1990). At present, the *LDOX* gene has been successively isolated from *Arabidopsis thaliana* (Xie et al., 2004), *Chrysanthemum* × *morifolium cultivar Argus* (Chung et al., 2010), *Fragaria* × *ananassa* (Chen et al., 2018), *Vitis vinifera* (Li et al., 2016), *Musa nana* (Che et al., 2020). In addition, the study of *LDOX* in *Arabidopsis thaliana* showed that the seed coat color became lighter due to the reduction of anthocyanins and proanthocyanidins of *LDOX* allele mutants (Bowerman et al., 2010). The study found that the color difference of kiwifruit pulp color development into red, green and yellow is related to the initial color conversion process of red pulp involved in *LDOX* gene (Zhang et al., 2015). In grape research, it was found that *LDOX* gene in the peel of red varieties is higher than that of white varieties (Wen, 2005, College of Food Science

and Technology, China Agricultural University, pp.4-5). The above research showed that as an enzyme at the end of the anthocyanin synthesis pathway, *LDOX* gene plays a key role in the accumulation of anthocyanins.

LDOX not only plays an important role in the coloring of *Actinidia arguta*, but also plays an important role in improving plant stress resistance (Zhang, 2016). At present, although we have a certain understanding of the structure and function of *LDOX* gene, we have not deeply analyzed the details of *LDOX* gene. Therefore, based on the transcriptome (RNA-seq) data of *Actinidia arguta* in NCBI, we searched the cDNA sequence of *LDOX* gene of *Actinidia arguta*, by using the method of bioinformatics, we can further explore the physical and chemical properties, protein structure characteristics, homology and functional annotation of *LDOX* in *Actinidia arguta*, which lays a theoretical foundation for further exploring the functional mechanism of *LDOX* in *Actinidia arguta*.

1 Results and Analysis

1.1 Homology and phylogenetic analysis of *LDOX* gene sequence in *Actinidia arguta*

The nucleotide and amino acid sequences of *LDOX* gene of 29 species were downloaded from the online database of NCBI nucleotide blast and protein BLAST applications. The nucleotide and amino acid sequences of *Actinidia arguta* were compared and analyzed by MegAlign (ClustalW) in DNAStar Lasergene application. It was found that the nucleotide and amino acid sequences of *LDOX* gene of *Actinidia arguta* were similar to those of *Camellia Sinensis* (79.2%, 87.6%), *Vaccinium Ashei* (79.3%, 85.1%), *Durio zibethinus* (76.6%, 85.0%), *Morus notabilis* (76.60%, 83.1%), *Eucalyptus grandis* (75.50%, 81.7%), with 75%~80% or higher. It has low similarity with *Elaeis guineensis* (70.0%, 69.6%) and *Chrysanthemum × morifolium cultivar Argus* (68.6%, 77.3%).

The phylogenetic tree of *LDOX* of 28 species was constructed by using the Neighbor Joining (NJ) method in MEGA 7.0 after ClustalW comparison, with an average genetic distance of 0.294. In conclusion, the sequence can be used for phylogenetic analysis (Figure 1). *Actinidia arguta* is closely related to *Camellia sinensis*, *Vaccinium corymbosum*, *Vaccinium ashei*, *Lonicera japonica*, *Daucus carota* and *Nicotiana sylvestris*, which gathered into one branch. Ten conserved motifs were identified by MEME software. The conserved motifs of the LDOX protein of *Actinidia arguta* were analyzed, and the motifs were named Motif1, Motif2, Motif3, Motif4, Motif5, Motif6, Motif7, Motif8, Motif9 and Motif10 (Table 1). *Actinidia arguta* and other species except *Garcinia mangostana*, *Vigna angularis* and *Vigna radiata var. radiata* contained the same conserved motif at the same amino acid position, which showed that LDOX protein is highly conservative in various species.

1.2 The cis acting element of *LDOX* gene promoter

In order to further explore the main potential functions of *LDOX*, this study used the neural network promoter prediction and Plantcare online server to predict the cis response elements of *LDOX* gene in *Actinidia arguta* (Table 2), and further analyzed the type, number and location of *LDOX* promoter elements. The results showed that the functional elements were contained in transcription factors, mainly including CAATbox (55 bp), ABRE (719 bp), ARE (169 bp), AuxRR-core (279 bp), CGTCA-motif (704 bp), G-box (719 bp), LTR (172 bp and 502 bp) and TGA-element (348 bp). Among them, ABRE responds to drought response in ABA signaling pathway; ARE can play an important role in anaerobic-induced regulatory element; AuxRR-core and TGA-element are important regulatory factors conducive to the development of endosperm, cotyledon and lateral roots. In addition, many regulatory elements of light stimulus response have also been identified in this study. Therefore, it was judged that *LDOX* is an essential element in photosynthesis and flowering regulation of light responsive plants.

1.3 Analysis of *LDOX* gene protein characteristics

Using Protparam server to analyze the physical and chemical properties of the LDOX protein of *Actinidia arguta*, it can be seen that *LDOX* encodes 355 amino acids, with a relative molecular weight of 39 964.73 u, a theoretical isoelectric point of 5.61, and the number of alkaline being larger than that of acidic range. To sum up, this protein is an alkaline amino acid; The total average hydrophilic gravity is negative, so it is speculated that it is a hydrophilic protein with relatively high thermal stability. The total number of positive and negative charge residues is Asp + Glu=53 (positive), Arg+Lys=41 (positive), the molecular formula is $C_{1789}H_{2813}N_{477}O_{532}S_{14}$, the total number of atoms is 5 625, the instability index is 53.02, the fat coefficient is 87.07, and the average

hydrophilicity is -0.390. The highest content of LDOX is Glu (11.5%), followed by Leu (9.9%) and Gly (7.0%), and the lowest content is Trp (1.4%) and Cys (1.4%) (Figure 2A).

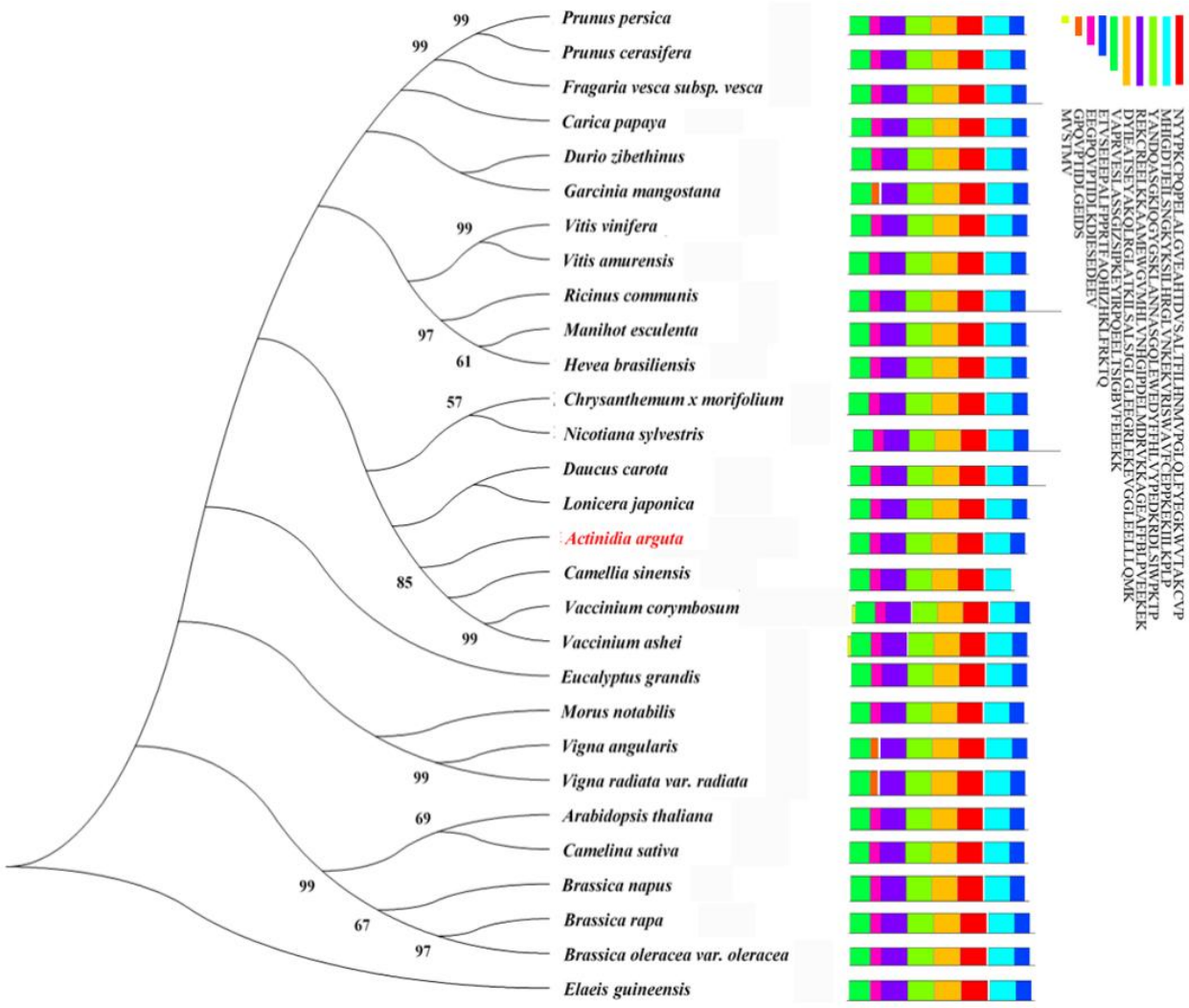

Figure 1 The LDOX proteins phylogenetic tree and the distribution of the conservative motifs
Note: The left side was the phylogenetic tree of LDOX protein; the color box on the right represented the conservative base sequence distribution of the gene

Table 1 The protein conserved motif of LDOX

Conserved region	Conserved Sequence	E Value	Position	Length
Motif 1		5.4e-1427	29	50
Motif 2		1.2e-1329	29	50
Motif 3		1.7e-1320	29	50
Motif 4		1.0e-1027	29	50
Motif 5		3.4e-1004	29	50
Motif 6		6.2e-724	29	39
Motif 7		7.0e-591	28	29
Motif 8		2.40E-284	26	21
Motif 9		4.20E-03	3	14
Motif 10		4.80E+01	2	6

Note: The ordinate indicated the conservative nature of amino acids, and the height of amino acid letters indicated the frequency of occurrence; The abscissa represented the position of the amino acid in the sequence

Table 2 The cis element analysis of *LDOX* gene promoter

Component Name	Plant Origin	Position/bp	+ / - Strand	Score	Sequence	Functional Annotation
ARE	*Zea mays L.*	169	-	6	AAACCA	Anaerobic-induced regulatory element
CAAT-box	*Nicotiana tabacum L.*	55	+	4	CAAT	
	Arabidopsis thaliana	389	-	5	CCAAT	Cis-types common in promoter and enhancer subregions
	Arabidopsis thaliana	394	+	5	CCAAT	Cis-types common in promoter and enhancer subregions
	Nicotiana tabacum L.	395	+	4	CAAT	
	Pisum sativum L.	474	-	5	CAAAT	Cis-types common in promoter and enhancer subregions
Sp1	*Oryza sativa L.*	220	+	6	GGGCGG	Optical response element
	Oryza sativa L.	301	+	6	GGGCGG	Optical response element
Gap-box	*Arabidopsis thaliana*	636	+	9.5	CAAATGAA(A/G)A	Optical response element
TGACG-motif	*Hordeum vulgare L.*	704	+	5	TGACG	MeJA response response element
TCCC-motif	*Spinacia oleracea L.*	192	-	7	TCTCCCT	Optical response element
ABRE	*Arabidopsis thaliana*	719	-	5	ACGTG	Abscisic acid response element
P-box	*Oryza sativa L.*	458	-	7	CCTTTTG	Gibberellin response element
G-Box	*Pisum sativum L.*	719	+	6	CACGTT	Optical response element
AuxRR-core	*Nicotiana tabacum L.*	279	-	7	GGTCCAT	Auxin response element
TGA-element	*Brassica oleracea L.*	348	+	6	AACGAC	Auxin response element
GC-motif	*Zea mays L.*	298	-	6	CCCCCG	Enhancers are involved in specific induction of anaerobic
CGTCA-motif	*Hordeum vulgare L.*	704	-	5	CGTCA	MeJA response response element
LTR	*Hordeum vulgare L.*	172	-	6	CCGAAA	Low temperature response element
	Hordeum vulgare L.	502	-	6	CCGAAA	Low temperature response element
TCT-motif	*Arabidopsis thaliana*	516	+	6	TCTTAC	Optical response element

ProtScale online server predicted and analyzed that the strongest hydrophilic site of LDOX was located at position 64 (-3.511) of glutamic acid Glu (E), while methionine Met (M) was the strongest hydrophobic site (2.167), which was located at position 190 (Figure 2B). The hydrophilic region of LDOX protein was larger than the hydrophobic region, which further verified the prediction results of the total average hydrophilic protein that LDOX protein was a hydrophilic protein. Moreover, it can be seen (Figure 3) that no signal peptide region and transmembrane structure were found in LDOX protein.

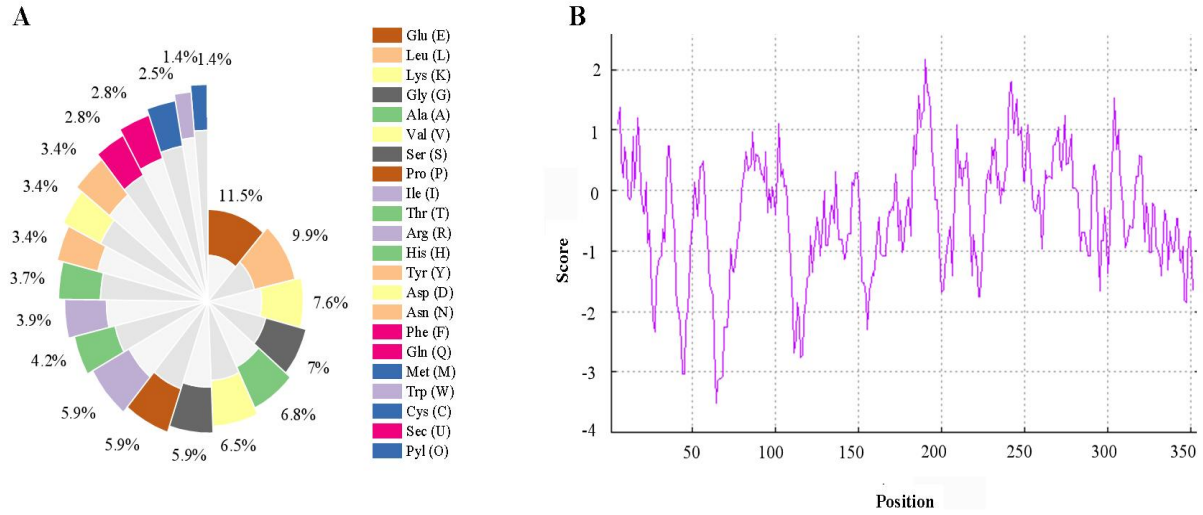

Figure 2 Analysis of physical and chemical properties of LDOX protein
Note: A: Amino acid compositions of protein encoded by the LDOX; B: Hydrophilicity /hydrophobicity analysis of LDOX

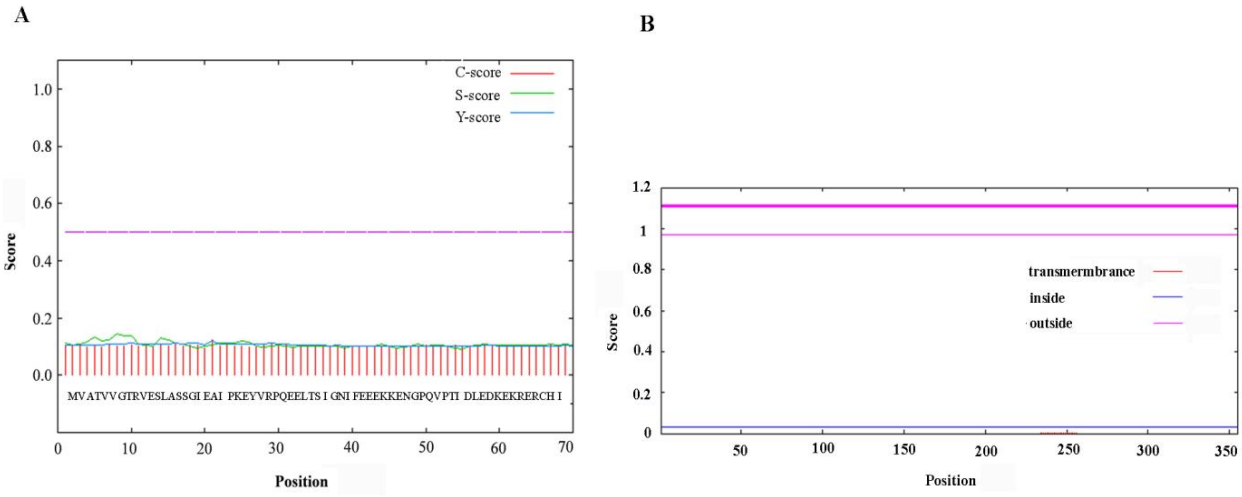

Figure 3 Analysis of physical and chemical properties of LDOX protein
Note: A: LDOX protein signal peptide prediction; B: Prediction of transmembrane structure of LDOX protein; C value: The value of the original shear site; S value: The value of signal peptide; Y value: The value of the integrated shear site

1.4 Phosphorylation and glycosylation sites of LDOX protein

Protein phosphorylation regulates its activity and function. NetPhos 3.0 Server was used to predict the phosphorylation modification sites of the LDOX protein of *Actinidia arguta*. The prediction results were shown in Figure 4. The LDOX protein sequence of *Actinidia arguta* contains 13 Ser phosphorylation sites (12, 16, 60, 109, 130, 159, 166, 170, 173, 174, 191, 239, 269), 12 Thr phosphorylation sites (4, 8, 52, 78, 164, 172, 184, 236, 242, 262, 323, 349) and 6 Tyr phosphorylation sites (25, 116, 145, 168, 175, 286).

NetOGlyc 3.1 Server predicted that there were two O-glycosylation sites in LDOX protein, Thr^{4} and Thr^{323} respectively. The N-glycosylation site Asn^{107} was predicted by NetNGlyc 1.0 Server that it exists at the LDOX residues (107~109) of *Actinidia arguta*. And it was predicted that Thr^{4} exists in both phosphorylation and glycosylation modification, but its glycosylation score (0.539) is less than the phosphorylation score (0.799), so it is considered that it is more likely to be phosphorylated.

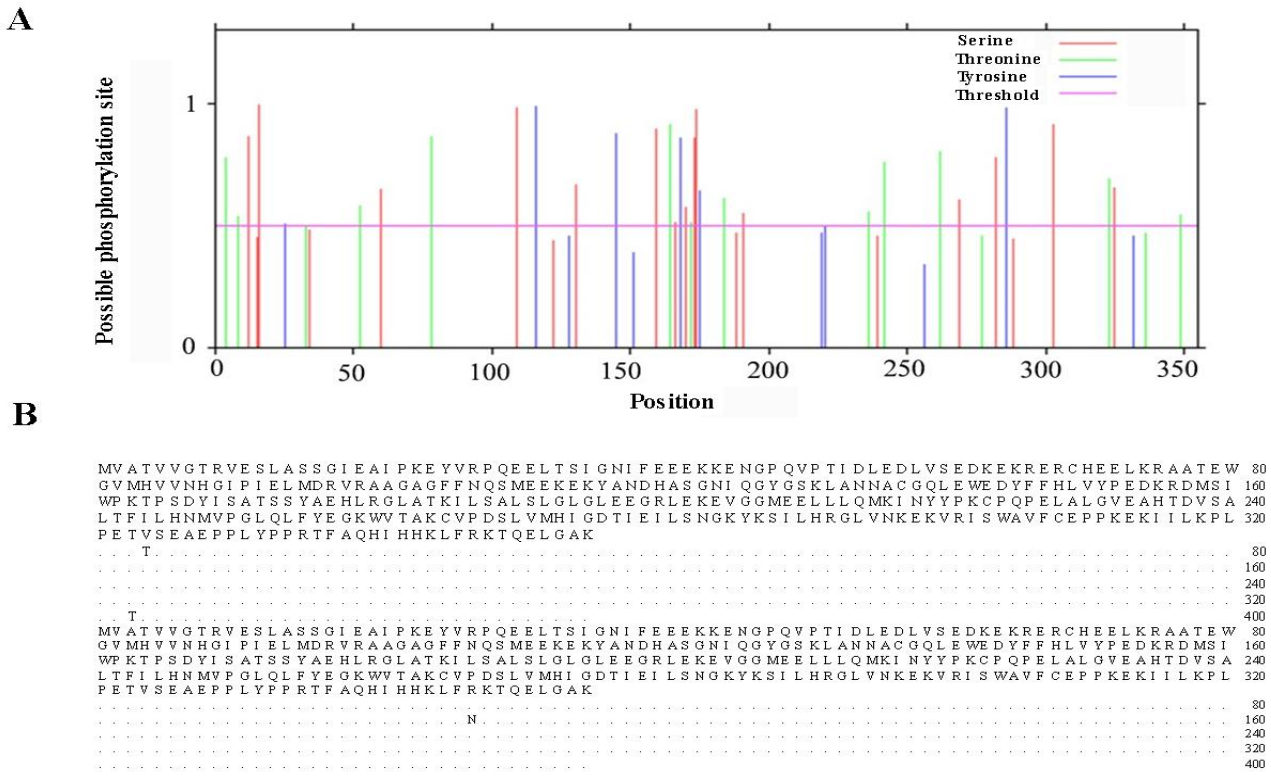

Figure 4 Prediction of phosphorylation sites of LDOX

1.5 Domain and advanced structure prediction of LDOX protein

The LDOX protein was analyzed and predicted by the "Conserved Domains" tool in NCBI. It was found that the LDOX protein of *Actinidia arguta* includes the 2-ketoglutarate-Fe^{2+}-dioxygenase family structure (positions 48-347) (Figure 5).

By analyzing and predicting the advanced structure of the LDOX protein in *Actinidia arguta*, it was found that the proportions of α-helix, extended-chain, β-turn and random coil were 34.65% (123), 16.06% (57), 6.20% (22) and 43.10% (153) respectively. In conclusion, the structure of LDOX protein mainly contains random coil, while α-helix and β-turn structure accounts were less (Figure 6).

The tertiary structure prediction model of SWISS-MODEL for LDOX protein of *Actinidia arguta* found that (Figure 7) the similarity between LDOX protein sequence of *Actinidia arguta* and 2brt.1.A template sequence in Blast database was as high as 78.96%. At the same time, the prediction results showed that QMEAN was 0.92, QMEAN was -0.26, and the coverage was 0.98%, indicating that the structural model was reasonable.

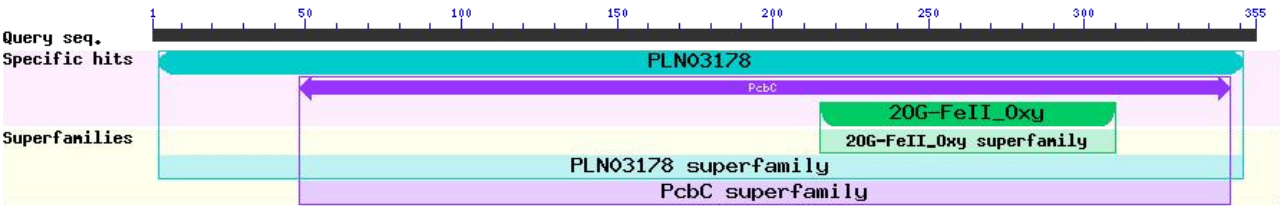

Figure 5 LDOX protein conserved domain prediction

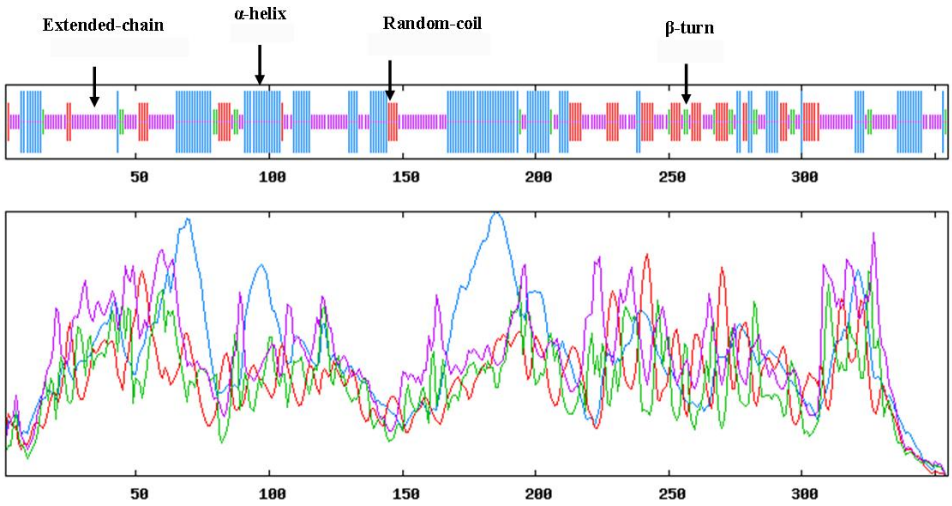

Figure 6 The secondary structure prediction of LDOX
Note: Blue: α-helix; Red: β-sheet; Green: β-turn; Purple: Random coil

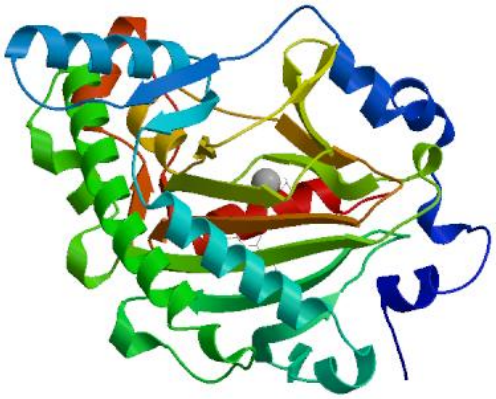

Figure 7 The tertiary structure prediction of LDOX protein

1.6 Interaction network construction and function enrichment analysis of LDOX enriched protein of *Actinidia arguta*

The database String was used to predict the functional relationship of LDOX protein in *Actinidia arguta* and the interconnected genes in the network diagram of protein interaction (*Arabidopsis thaliana* was selected as species mode). The results showed that there was a certain interaction relationship between LDOX and flavonol synthase (FLS) and other genes, and it was likely that they interacted with each other in completing a certain item or a certain physiological process (Figure 8). GO and KEGG enrichment analysis showed (Table 3) that LDOX interaction network genes participate in flavonoid biosynthesis and metabolism, mainly play a role in plant-type vacuolar membrane, and have the function of regulating catalytic activity and redox active molecules. KEGG enrichment function pathway is mainly flavonoid biosynthetic process and biosynthesis of secondary metabolites.

2 Discussion

Color plays an important role in the commodity value of fruits and is also one of the important indicators to evaluate the quality of fruits (Cui et al., 2020). Water soluble flavonoids mainly contain anthocyanins. The downstream synthetic gene *LDOX* gene plays an important role in the anthocyanin synthesis pathway. As an important regulatory hub in the regulation of color and metabolic genes, it is essential in the process of color appearance and change (Nakajima et al., 2001). This study used bioinformatics methods to predict and analyze the physical and chemical properties, structure and function of LDOX in *Actinidia arguta*, which can provide an important theoretical basis for further understanding the main functions of *LDOX* gene. Through the construction of phylogenetic tree, it was found that *LDOX* of *Actinidia arguta* is closely related to *Camellia sinensis*,

Vaccinium corymbosum, *Vaccinium ashei*, *Lonicera japonica* and *Daucus carota*, indicating that *LDOX* is highly conservative in different species and conforms to the characteristics of biological evolution. No signal peptide region fragment was found in the sequence of *Actinidia arguta*. It was inferred that LDOX protein did not undergo protein transport. After initial synthesis on free ribosomes, it was retained in the cytoplasmic matrix as a catalyst to catalyze the formation of colorless anthocyanins to colored anthocyanins (Dong et al., 2010). Promoter prediction and results showed that a large number of acting elements were involved in the promoter sequence of *LDOX* gene of *Actinidia arguta* in response to different biological and abiotic stresses, indicating that the biological function of *LDOX* gene was related to stress induction, plant hormone induction and fruit maturation.

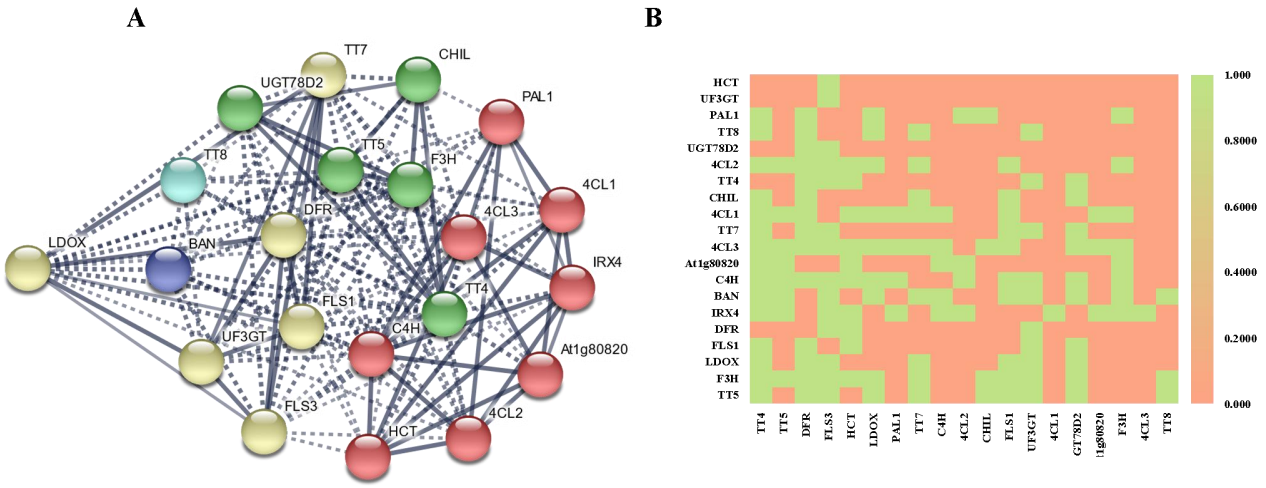

Figure 8 Function interaction network and co-expression of LDOX protein

Branches of the flavonoid pathway in plants have a unified upstream pathway with the synthesis of proanthocyanidins and flavonols, which controls the biosynthesis of anthocyanins (Li, 2017, Graduate School of Chinese Academy of Agricultural Sciences, pp.14). All pathways are jointly participated by a series of catalytic enzymes. The first step of chalcone synthase catalyzed flavonoid pathway is that 4-coumarinyl-CoA and malonyl-CoA are catalyzed to synthesize chalcone. Then, chalcone isomerase catalyzes the synthesis of trihydroxyflavanone. Under the catalysis of F3H, DFR and other enzymes, trihydroxyflavanone synthesizes three colorless anthocyanins. Finally, Colorless anthocyanins form various anthocyanins under the catalytic modification of LDOX and UDP-flavonoid glycosyltransferases (UFGT). LDOX enzyme has not only sequence similarity with F3H and FLS, but also functional complementarity. Current studies have found that LDOX mainly replaces FLS enzyme catalytic activity in vitro. For example, *Medicago Sativa* (Pang et al., 2007), *Oryza sativa* (Xu et al., 2012) and *Petunia hybrida* (Nakajima et al., 2001) LDOX recombinant protein can replace FLS enzyme to catalyze diaminoflavonol to flavonol in vitro. LDOX protein also plays a bifunctional catalytic activity function in *Reaumuria trigyna* (Zhang, 2016, Inner Mongolia University, pp.23-26), but unlike other plant LDOX catalytic properties, LDOX recombinant protein cannot replace the catalytic function of FLS in vitro. Instead, it replaces F3H to catalyze the production of triamcinolone acetonide (Xu et al., 2012). In vivo, LDOX catalyzes the modification of the central carbon ring of flavonoids, which can catalyze the formation of procyanidins and anthocyanins from colorless anthocyanins (Welford et al., 2005). In addition, PAL protein, as an important hub of plant primary metabolism and phenylpropane secondary metabolism, plays a key role in regulating the synthesis of secondary metabolites such as flavonol, anthocyanin and lignin in plants. In this study, the interaction protein network of *LDOX* was constructed, and the enrichment analysis of GO and KEGG showed that the interaction between *LDOX* and *F3H*, *UFGT*, *FLS* as well as *PAL*, and the biosynthesis and metabolism of flavonoids were significant enrichment functional pathways. This indicates that *LDOX*, together with F3H, UFGT, FLS and PAL, plays a role in the regulation of *Actinidia arguta* color. The construction of LDOX protein network lays a solid foundation for further exploring the pathway and functional mechanism of LDOX involving in flavonoids.

Table 3 Functional enrichment analysis of *LDOX* genes

GO Enrichment		Term description	False discovery rate (FDR)
	Biology Process	Flavonoid biosynthetic process	2.38E-27
		Flavonoid metabolic process	8.09E-27
		Phenylpropanoid metabolic process	4.83E-17
		Secondary metabolic process	5.29E-14
		Organic substance biosynthetic process	5.56E-11
		Organic substance metabolic process	1.03E-09
		Response to karrikin	3.61E-09
		Phenylpropanoid biosynthetic process	8.93E-08
		Regulation of flavonoid biosynthetic process	1.33E-07
		Response to abiotic stimulus	2.11E-07
	Cell component	Extrinsic component of endoplasmic reticulum membrane	0.00038
		Endoplasmic reticulum	0.00041
		Vacuolar membrane	0.0322
		Endoplasmic reticulum membrane	0.0322
		Plant-type vacuole membrane	0.0322
		Intracellular part	0.0322
		Endoplasmic reticulum subcompartment	0.0322
		Cytoplasm	0.0372
		Organelle membrane	0.0413
	Molecular Function	Catalytic activity	3.93E-09
		4-coumarate-CoA ligase activity	2.75E-07
		Oxidoreductase activity	2.75E-07
		Cofactor binding	2.75E-07
		Coenzyme binding	2.75E-07
		2-oxoglutarate-dependent dioxygenase activity	6.08E-07
KEGG Enrichment	Enrichment of LDOX related pathways	Flavonoid biosynthesis	4.23E-30
		Biosynthesis of secondary metabolites	1.76E-24
		Metabolic pathways	5.30E-18
		Phenylpropanoid biosynthesis	1.11E-12
		Phenylalanine metabolism	4.73E-10
		Ubiquinone and other terpenoid-quinone biosynthesis	3.07E-08
		Flavone and flavonol biosynthesis	1.19E-05
		Stilbenoid, diarylheptanoid and gingerol biosynthesis	2.50E-05

3 Materials and Methods

3.1 Acquisition of *LDOX* gene sequence and construction of phylogenetic tree of different species

Take "Actinidia arguta leucoanthocyanidin dioxygenase (*LDOX*)+*Actinidia arguta*" as the keyword, and use NCBI (https://www.ncbi.nlm.nih.gov/) database to search and download the nucleotide and protein sequences of the *LDOX* gene of *Actinidia arguta*. The nucleotide (MK896863.1) and protein sequence (QGN03641.1) of the *LDOX* gene of *Actinidia arguta* were obtained, and the similarity was compared and analyzed by using the BLAST online server tool in NCBI. The nucleotide and protein sequences of the *LDOX* gene of species with high homology were downloaded one by one. The NJ method in MEGA7.0 was used to carry out the phylogenetic tree between *Actinidia arguta* and other species, and the Bootstrap value was set to 1 000.

3.2 Prediction and analysis of *LDOX* gene promoter in *Actinidia arguta*

Neural Network Promoter Predictio and PlantCARE online server were used to predict and analyze the transcription start sites and potential cis elements of the promoter of *LDOX* gene in *Actinidia arguta*.

3.3 Physicochemical properties and subcellular localization of *LDOX* gene in *Actinidia arguta*

The molecular formula, molecular weight, acidity and alkalinity, isoelectric point, stability and hydrophobicity of LDOX were predicted and analyzed by using ProtParam tool and ProtScale tool in ExPASy data analysis system. SignalP 4.0 server, TMHMM v. 2.0 server, NetPhos 3.1 server, NetNGlyc 1.0 server and NetOGlyc 3.1 server software were used to predict and analyze the signal peptide and transmembrane region of the LDOX in *Actinidia arguta* species, the serine, threonine or amino acid phosphorylation sites, and the O-glycosylation and N-glycosylation sites of LDOX in *Actinidia arguta*. The conserved region of LDOX protein was analyzed using MEME database.

3.4 Prediction of the advanced structure of LDOX protein in *Actinidia arguta*

The secondary structure prediction and analysis software SOPMA and the tertiary structure model prediction and analysis software SWISS-MODEL were used to predict and analyze the advanced structure of LDOX protein.

3.5 Prediction of LDOX protein interaction network and functional enrichment analysis of *Actinidia arguta*

STRING database was used to construct the interaction network of proteins closely related to LDOX. *Arabidopsis thaliana* was selected as a model species with a reliability value of 0.7, and the number of interacting proteins was within 10. Go and KEGG pathway enrichment analysis of different proteins in the predicted protein interaction network was carried out through the functional annotation software DAVID.

Authors' contributions

LZP completed the experimental design, experimental research and the writing of the first draft of this study; LLY, SY, ZY and LDS participated in the completion of data and manuscript proofreading; YWZ was the designer and person in charge of the project, guiding the experimental design, data analysis, manuscript writing and revision. All authors read and approved the final manuscript.

Acknowledgments

This study was jointly funded by "Xing-Liao Talents Plan" Project of Liaoning Province (NO.XLYC1902015) and Special Project of Guiding Local Scientific and Technological Development by the Central Government of Liaoning Province (NO.2019JH6/10200005).

References

Appelhagen I., Jahns O., Bartelniewoehner L., Sagasser M., Weisshaar B., and Stracke R., 2011, Leucoanthocyanidin dioxygenase in Arabidopsis thaliana: characterization of mutant alleles and regulation by MYB-BHLH-TTG1 transcription factor complexes, Gene, 484(1): 61-68
https://doi.org/10.1016/j.gene.2011.05.031
PMid:21683773

Bowerman P.A., Ramirez M.V., Price M.B., Helm R.F., and Winkel B.S.J., 2012, Analysis of T-DNA alleles of flavonoid biosynthesis genes in Arabidopsis Ecotype Columbia, BMC Research Notes, 5(1): 485
https://doi.org/10.1186/1756-0500-5-485
PMid:22947320 PMCid:PMC3526476

Chen R.X., Peng L.T., Ye J.L., and Yang S.Z., 2018, Cloning of the LDOX of'Falandi'Strawberry and construction of its overexpression and silencing vector, Fenzi Zhiwu Yuzhong (Mol Plant Breed), 16(22): 7329-7335

Chung S.J., Lee G.J., Lee H.J., Kim J., Kim D.S., and Kang S., 2010, Isolation of a leucoanthocyanidin dioxygenase (LDOX) gene from a spray-type Chrysanthemum (Dendranthema×grandiflorum) and its colored mutants, Korean Journal of Horticultural Science and Technology, 28(5): 595-600

Cui W., Li Y.K., Fang J.B., Qiao C.K., Hang H.L., and Qi X.J., 2020, Screening of anthocyanin-related key light response transcription factor AaMYB1 in Actinidia arguta, Guoshu Xuebao (Journal of Fruit Science), 37(3): 301-312

Che J.R., Sun X.L., Wang S.J., Yi X.Y., Tian N., Liu F., Deng S.F., Lia Z.X., and Chen C.Z., 2020, Cloning and expression characteristics of the LDOX gene in Musa itinerans, Yingyong Yu Huangjing Shengwu Xuebao (Chinese Journal of Applied and Environmental Biology), 26(2): 246-254

Dong J., Zhou J., Xin P.Y., Xu Y.L., Liu Y., Wei Y.J., and Fu H.H., 2010, Bioinformatics analysis of LDOX/ANS gene in different plants, Jiyingzuxue Yu Yingyong Shengwuxue (Genomics and Applied Biology), 29(5): 815-822

He J, and Giusti M.M., 2010, Anthocyanins: Natural colorants with health promoting properties, Annual Review of Food Science & Technology, 1(1): 163-187
https://doi.org/10.1146/annurev.food.080708.100754
PMid:22129334

Li J., Liu H.F., and Cao F.F., 2016, Cloning and analysis of leucoanthocyanidin dioxygenase (LDOX) in Vitis Amurensis Rupr., Xibei Nongye Xuebao (Acta. Agric. Boreali-occident Sin.), 25(1): 103-108

Menssen A., Höhmann S., Martin W., Schnable P.S., Peterson P.A., Saedler H., and Gierl A., 1990, The En/Spm transposable element of Zea mays contains splice sites at the termini generating a novel intron from a dSpm element in the A2 gene, EMBO Journal, 9(10): 3051-3057
https://doi.org/10.1002/j.1460-2075.1990.tb07501.x
PMid:2170105 PMCid:PMC552029

Nakajima J., Tanaka Y., Yamazaki M., and Saito K., 2001, Reaction mechanism from leucoanthocyanidin to anthocyanidin 3-glucoside, a key reaction for coloring in anthocyanin biosynthesis, J. Biol. Chem., 276(28): 25797-25803
https://doi.org/10.1074/jbc.M100744200
PMid:11316805

Pang Y.Z., Gregory J.P., Wright E., Wang Z.Y., and Richard A.D., 2007, Early steps in proanthocyanidin biosynthesis in the model legume medicago truncatula, American Society of Plant Biologists,145(3): 601-615
https://doi.org/10.1104/pp.107.107326
PMid:17885080 PMCid:PMC2048810

Xie D.Y., Sharma S.B., and Dixon R.A., 2004, Anthocyanidin reductases from Medicago truncatula and Arabidopsis thaliana, Archives of Biochemistry and Biophysics, 422(1): 91-102
https://doi.org/10.1016/j.abb.2003.12.011
PMid:14725861

Xu F., Li L.L., Zhang W.W., Cheng H., Sun N.N, Cheng S.Y., and Wang Y., 2012, Isolation, characterization, and function analysis of a flavonol synthase gene from ginkgo biloba, Molecular Biology Reports, 39(3): 2285-2296
https://doi.org/10.1007/s11033-011-0978-9
PMid:21643949

Welford R.W.D., Clifton I.J., Turnbull J.J., Wilson S.C., and Schofield C.J., 2005, Structural and mechanistic studies on anthocyanidin synthase catalysed oxidation of flavanone substrates:the effect of C-2 stereochemistry on product selectivity and mechanism, Organic Biomolecular Chemistry, 3(17): 3117
https://doi.org/10.1039/b507153d
PMid:16106293

Zhang L., Man Y.P., Jiang Z.W., and Wang Y.C., 2012, Cloning and expression of anthocyanin pathway genes, AcCHS and AcLDOX, in Actinidia chinensis, Yuanyi Xuebao (Acta Horticul-turae Sinica), 39(11): 2124-2132