Cloning and expression analysis of pistillata-like gene in *Sedirea japonica*

Suhua Jiang[1], Suyan Niu[2], Xiuyun Yuan[1], Fang Liang[1], Shenping Xu[1], Mofei Wang[1], Bo Cui[1*]

[1]Institute of Bioengineering, Zhengzhou Normal University, 450044 Zhengzhou, Henan, P.R. China

[2]College of Forestry, Henan Agricultural University, 450011 Zhengzhou, Henan, P.R. China

Corresponding author: Bo Cui

E-mail: laocuibo@163.com

ABSTRACT. Members of the pistillata-like (PI-like) subfamily of plant MADS-box genes play multiple roles in regulation of reproductive meristems and the specification of sepal and petal identities. However, the function of *PI-like* genes in the *Sedirea japonica* is largely unknown. In this study, a full-length of *PI-like* gene in *Sedirea japonica* (designated PI-like, GenBank No. KM975642) was cloned using 5'/3'-RACE. The *PI-like* gene were 921 bp length with an open reading frame (ORF) of 633 bp, which encodes a protein of 210 amino acids, with a putative molecular weight of 24.5 kDa and a theoretical isoelectric point (pI) of 9.3. It also contains a 96-bp 5′ untranslated region (UTR), and a 192-bp 3′ UTR. The result of multiple sequence alignment indicated that *PI-like* belongs to a Class-B MADS-box gene. In addition, the phylogenetic tree analysis revealed that *PI-like* gene was highly like *PI-like* in *Phalaenopsis*. The qRT-PCR results revealed that the expression of *PI-like* gene was higher in the tissues on florescence compared to vegetative phase, suggesting that the *PI-like* gene of *S. japonica* performed important physiological functions on florescence.

Key words: *Sedirea japonica;* RACE kit; *Pistillata*-like; Gene cloning; qRT-PCR

INTRODUCTION

The ABCDE model was commonly used to explain floral organ development in many plants. It was applied in diversity of plants range from dicotyledonous plants such as *Arabidopsis thaliana* and *Antirrhinum* (Purugganan et al. 1995; Theissen et al. 2000; Weigel and Meyerowitz 1994) to the monocot plants including the rice and

orchid species (Chung et al. 1995; Tsai and Chen 2006). Generally, the function of A gene regulates the formation of the sepal, while A and B genes cooperate to determine the formation of the petal, and B and C genes together to regulate the stamen development. In addition, gene C determines the carpel formation, gene D is necessary for ovule development, and gene E is indispensable for the determination of petal, stamen, carpel, and ovule identity (Kang et al. 1998; Kramer et al. 1998; Schneitz 1999; Theissen and Saedler 2001; Zahn et al. 2005). Members of the MADS domain family of transcriptional regulators participate in a variety of developmental processes in plants, including reproductive (flower, seed, and fruit) and vegetative (root and leaf) development (Ng and Yanofsky 2001). Most of classes A, B, C, D, and E homeotic genes belong to the MADS-box transcription factor family (Favaro et al. 2003). Pistillata (PI) is B-functional genes, and Class-B MADS-box genes are classified into two subclasses, PI-type and AP3-type (Theissen et al. 2000).

Sedirea japonica (Orchidaceae) is an economically-important orchid species indigenous to sub-tropical regions of southern China, Japan, and Korea. It was different from the flowers in dicotyledonous plants that all orchid flowers have colored sepals. The sepals and petals of orchid are called tepals because of their similar color and shape. The median tepal in the inner whorl generally differs from the rest of the tepals and is therefore named as labellum or lip. In addition, the stigma and stamens are always fused to form another specialized structure, a highly modified column. Although the unique floral morphology has made orchid an attractive model for botanists, only few functional studies of MADS-box genes of had been reported in *S. japonica* species.

In this study, we cloned a *PI-like* cDNA from *S. japonica* and analyzed its expression profiles and biological function. Therefore, the role of *PI-like* in the development of *S. japonica* flowers could be discussed. Furthermore, these findings might enrich the knowledge of how MADS-box genes are involved in defining the different highly specialized structures in *S. japonica* flowers.

MATERIALS AND METHODS

Plant materials

Sedirea japonica plants were grown in greenhouse at 25°C to 28°C under a 16/8 h (light/dark) photoperiod. At least three parallel samples were prepared in Zhenzhou Normal University (Zhengzhou, Henan Province, China). The organs/ tissues (root, leaf, pedicel, sepal, petal, labellum, column (fused structure of stamen and pistil) were collected from three parallel plant samples, respectively, and subsequently mixed, frozen immediately in liquid nitrogen, and stored at −86°C until further use.

RNA extraction and cDNA synthesis

Total RNA was extracted from different tissues of *S. japonica* using RNA isolation kit (RNAiso for Polysaccharide-rich Plant Tissue, Takara Biotechnology Dalian CO., LTD.) followed the manufacturer's instructions. Then, the concentrations of each total RNA sample were measured by spectrophotometry (Quawell 5000, San Jose, CA, USA). PrimScript TM first-strand cDNA synthesis kit (Takara Biotechnology DALIAN CO., LTD.) was used for first-strand cDNA synthesis through reverse transcription (RT) to transcribe poly (A) +mRNA with oligo-dT primers following the manufacturer's instructions. Then the cDNA was stored at -20°C for further analysis (qRT-PCR).

Amplification of the *PI-like* gene using RACE

Firstly, a partial sequence of *PI-like* gene was obtained through RT-PCR, using primers based upon the cloned homolog of *PI-like* gene (logined in GenBank) from *Phalaenopsis*, Dendrobium thyrsiflorum, *Cymbidium goeringii, Cymbidium faberi, Cymbidium ensifolium* and *Dendrobium* hybrid cultivar. The forward primer (F) PI-F and the reverse primer(R) PI-R were listed in Table 1.

Table 1. Primers for RACE and qRT-PCR assay

Names	Purpose	Sequences (5'-3')
PI-F	Partial cDNA	5'-CTTGTYATCTTYTCMAGCCTTG-3'
PI-R	Partial cDNA	5'TCTCTCATRCTCCCTTCCATTG- 3' (R=A/G, M=A/C,Y=C/T)
PI-like-3F1	3'RACE	GAGAATTTGAGCGCGGAGATT
PI-like-3F2	3'RACE	GCAATTGGCAATGGAAGGGAGC

oligo dT-anchor	3'RACE	TACCGTCGTTCCACTAGTGATTT
anchor	3'RACE	CGCGGATCCTCCACTAGTGATTTCACTATAGG
PI-like-5R1	5'RACE	TGTTTATCCCGAACGCTAGTGAGAC
PI-like-5R2	5'RACE	CCTTTCAAATGCCTGAGTTCG
UPM	5'RACE	Long:CTAATACGACTCACTATAGGGCAAGCAGTG GTATCAACGC AGAGT
		Short: CTAATACGACTCACTATAGG
		GCAAGCAGTG GTATCAACGC AGAGT
NUP	5'RACE	
PI-like-F	qRT-PCR	CAAATGGCAATGGAAGGGAG
PI-like-R	qRT-PCR	GCTGAATGGGTTGGACACGA
EF1α-F	qRT-PCR	CAGATTGTGCCGTTCTCATTAT
EF1α-R	qRT-PCR	GGTGTGGTAGCATCCATCTTGT

The first cDNA synthesized from poly (A) + mRNA was used as a template to amplify the partial sequence of PI-like. PCR reactions was performed as follows: 95°C (pre-denaturation) for 5 min, followed by 95°C for 30s, 58°C for 45s and 72°C for 2 min for 33 cycles. The duration of the 72°C elongation step was 10 min. PCR products were ligated into the pGEM-T Easy vector (Promega, USA) for identification and sequencing. Secondly, TaKaRa 3′ RACE and 5' RACE kit was used to amplify the 5′-ends and 3′-ends of the cDNA, respectively. The primers for 3′-RACE were as follows: oligo dT-anchor primer and the internal gene specific primer PI-like-3F1 (Table 1) for the first round PCR, anchor primer and the internal gene specific primer PI-like-3F2 for the second round PCR. Two gene-specific primers PI-like-5R1 and PI-like-5R2 were used for 5' RACE. The primer UPM was used as the first primer and NUP was used as the second amplification primer. All of the PCR amplification products were cloned into pGEM-T Easy vector and transformed into *Escherichia coli* strain DH5α. Recombinant bacteria was selected by blue/white screening and verified by PCR.

Nucleotide sequence and bioinformatics analysis

The full-length cDNAs of *PI-like* gene were obtained using DNAMAN software to splice the cloned gene fragments, which were then analyzed using a program available on the NCBI website (http://www.ncbi.nlm.nih.gov/). Phylogenetic relationships were deduced using the MEGA4.0 software. Searching for ORFs was performed using the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). PI/Mw (http://web.expasy.org/compute_pi/) was used to analyze the PI/Mw. ProtScale (http://web.expasy.org/protscale/) was used to analyze the hydrophilicity of *PI-like* amino acid sequences. Protfun (http://www.cbs.dtu.dk/services/ProtFun/) was used to take a function prediction analysis of *PI-like* coding products. Hopfield neural network (HNN) (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html) was used to predict secondary structure of *PI-like* protein. Swiss-model (http://swissmodel.expasy.org) was used to predict the tertiary structure of *PI-like* protein.

## Analysis of the expression of the *PI-like* gene using qRT-PCR

The specific expression of *PI-like* at different stages and different tissues was analyzed by qRT-PCR (a real-time fluorescence quantitative PCR). The sequences for all the primers used in this study were shown in Table 1. Diluted cDNA was used as a template in 20 μL PCR reaction containing 10 μL 2×SYBRR Premix Ex TaqTM II（TaKaRa, 0.8 μL (10 μmol/L) of sense and antisense primers, 2 μL of the diluted template, and 6.4 μL of RNA-free water. The qRT-PCR was performed using an Eppendorf Master Cycler Ep Realplex2 Real-time PCR Detection system. The EF1α gene was used as an internal control gene. The thermal profile for qRT-PCR was performed as follows: 95°C for 30s, followed by 40 cycles of 95°C for 15s, 60°C for 15 s and 72°C for 30s. Dissociation curve analysis of the amplicons was performed at the end of each PCR reaction to confirm that only one specific PCR product was amplified and detected. The qRT-PCR was performed in triplicate for each sample. Finally, relative transcripte abundance of different samples was calculated using the formula Rel. Exp= 2-(ΔΔCt), where ΔCt =Ct (PI-like) - Ct (EF1α rRNA),   Ct = (The plant tissue ΔCt) - (pedicel ΔCt).

RESULTS

Clone, sequence analysis and characterization of *PI-like* gene

The partial cDNA was cloned successfully using the specific primers that designed according to the homologous sequence (Figure 1). The length of partial gene was 398 bp. A fragment of 228 bp at the 5' end and a 295-bp fragment at the 3'end with a short polyA tail cDNA were obtained using RACE method. The full-length cDNAs were obtained by splicing three cloned gene fragments using DNAMAN software and designated *PI-like* gene. Sequence analysis confirmed that the clone in this study belongs to a *PI-like* gene. The full-length cDNA of *PI-like* was 921 bp. The sequence was deposited in GenBank under accession number KM975642. The complete nucleotide sequence of *PI-like* and the deduced amino acids are shown in Figure 2. ORF finder analysis showed

that the complete sequence of *PI-like* cDNA consisted of 96-bp 5'UTR, a 192-bp 3'UTR, and an ORF of 633 bp. The ORF encoded a 211 amino acids' protein with a predicted molecular weight of 24.5 kDa and a theoretical pI of 9.3. Domain analysis showed that the protein was consisted of 118 Alpha helix, 29 extended strands and 63 Random coils (Figure 3). The sequence of the *S. japonica PI-like* was more closely to *Phalaenopsis* hybrid cultivar and *Cymbidium faberi* with the similarity of 99% and 96%, respectively, than to any other *PI-like* family member (Figure 4).
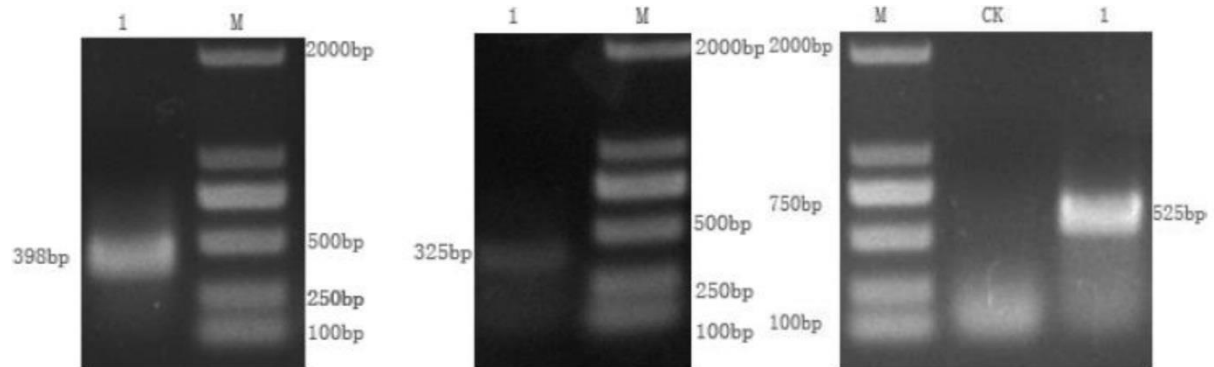

Figure 1. 1% agarose gel electrophoresis of *PI*-like. M DL2000 Marker; A, CDS product; B 5' RACE product; C 3' RACE product



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1     ACATGGGGGCTGCGCTTCTTTTGGGATTTTTCGCTTCTGTTCCTGAGATCACTTGCAGTC
61    TCTTTTATTTTTCATCCTGCTTGGTTTTGGGTGGAG ATG GGTCGGGGGAAGATAGAGATC
1                                          M   G  R  G  K  I  E  I
121   AAGAGAATCGAGAACTCAACTAACCGGCAAGTGACCTTCTCGAAGAGGCGGAATGGAATC
21     K  R  I  E  N  S  T  N  R  Q  V  T  F  S  K  R  R  N  G  I
181   ATGAAGAAGGCGAAGGAGATCAGCGTGCTCTGCGACGCCCAGGTTTCGCTTGTTATCTTT
41     M  K  K  A  K  E  I  S  V  L  C  D  A  Q  V  S  L  V  I  F
241   TCAAGCCTTGGAAAGATGTTTGAGTATTGTAGCCCATCCACCACGCTGTCGAAGATGCTG
61     S  S  L  G  K  M  F  E  Y  C  S  P  S  T  T  L  S  K  M  L
301   GAGAAATACCAGCAAAACTCGGGCAAGAAGCTCTGGGACGCCAAGCACGAGAACTTGAGC
81     E  K  Y  Q  Q  N  S  G  K  K  L  W  D  A  K  H  E  N  L  S
361   GCGGAGATTGATCGGATCAAGAAGGAAAATGATAATATGCAGATCGAACTCAGGCATTTG
101    A  E  I  D  R  I  K  K  E  N  D  N  M  Q  I  E  L  R  H  L
421   AAAGGGGAGGATCTGAACTCTCTTAACCCAAAAGAGCTTATTCCGATTGAGGAAGCCCTG
121    K  G  E  D  L  N  S  L  N  P  K  E  L  I  P  I  E  E  A  L
481   CAGAATGGTCTCACTAGCGTTCGGGATAAACAAATGGACTACTTGAAGATGCTAAAAAAG
141    Q  N  G  L  T  S  V  R  D  K  Q  M  D  Y  L  K  M  L  K  K
541   AATGAAAGAATGCTGGAAGATGAAAATAAAAGGCTAACATACCTTTTGCACCAACAACAA
161    N  E  R  M  L  E  D  E  N  K  R  L  T  Y  L  L  H  Q  Q  Q
601   ATGGCAATGGAAGGGAGTATGAGAGAACTCGACATCGGCTTTCATCATAAAGATCGTGAG
181    M  A  M  E  G  S  M  R  E  L  D  I  G  F  H  H  K  D  R  E
661   TATGCAGCTCAGATGCCAATGACCTTTCGTGTCCAACCCATTCAGCCCAACTTGCAGGGA
201    Y  A  A  Q  M  P  M  T  F  R  V  Q  P  I  Q  P  N  L  Q  G
721   AATAAG TAA AAGTGTTAGCCTACTGCTTTCCTGTTGTTTAAATGAATTATTATATTAACT
221    N  K  *
781   TTTGGCAGTTATGTGAGATTATGAAAACTTACATTGCTAATTATGATATATGTGTTTACT
841   AGTGATATTCATATTGTAACTCTCGCAACTCATTAGTAACTATGGTTAAATATTTTTATG
901   TTCTAATCGAAAAAAAAAAAA
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Figure 2. Nucleotide and deduced amino acid sequence of *PI*-like.

Start codon and stop codon were indicated by the boxes, respectively; numbers in the left were represented the location of nucleotide and deduced amino acid sequences.
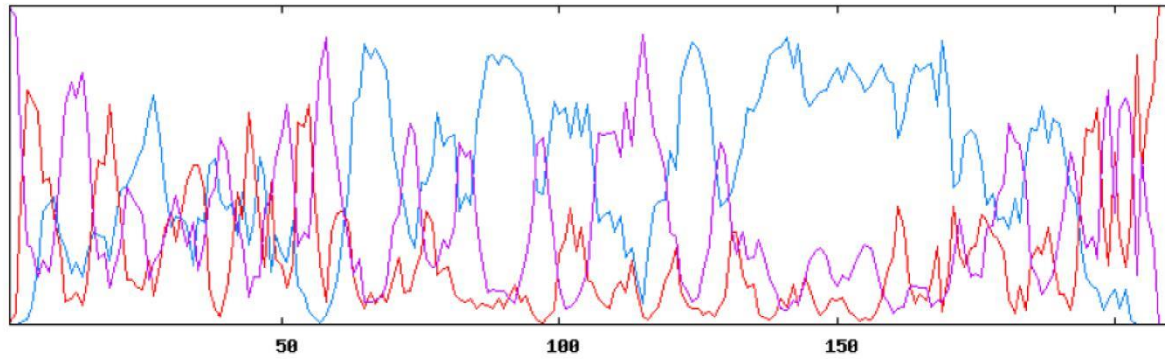

Figure 3. The secondary structure was predicted for *PI-like* protein. Blue, alpha helix; Roseo, random coil; red, extended strand.
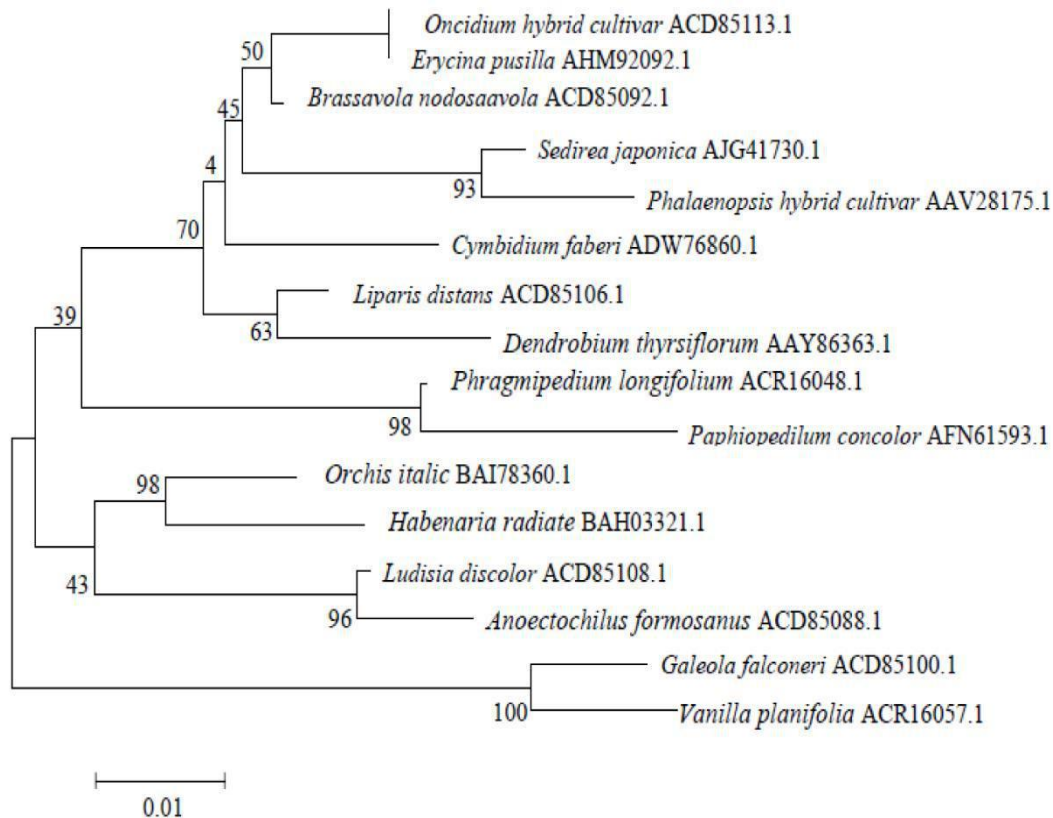
Figure 4. Phylogenetic tree illustrated the genetic relationships between *S. japonica PI-like* and other plant PIs.

Hydrophobicity and hydrophilicity analysis of PI-like

ProtScale is an application in ExPASy for analysing hydrophobicity of *PI-like* protein. The maximum and minimum hydrophobicity values of the protein were 2.322 and -2.811, respectively. *PI-like* protein was water-soluble protein cause most amino acid residues to be hydrophilic (Figure 5).
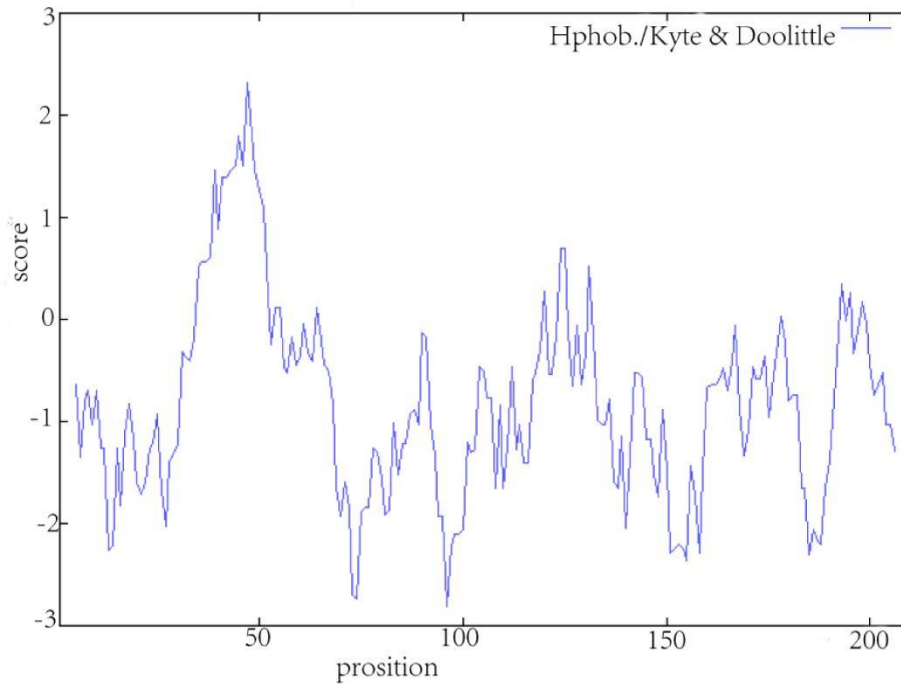
Figure 5. The hydropathy profile of *PI-like* amino acids. The horizontal scale indicated the number of amino acid residues and the vertical one was the relative hydropathy scale. Points above the zero-horizontal line corresponded to hydrophobic region, and points below the line were hydrophilic.

Prediction and analysis of encoding products function

Protfun software of CBS was used to analyze and predict the structure functional domains and functional classification of PI-like. The possibility values of *PI-like* function, including biosynthesis of cofactors, fatty acid metabolism, purines and pyrimidines, translation, transport, and binding, were 2.917, 1.308, 1.362, 1.614 and 1.885, respectively (Table 2). It suggested that *PI-like* has a similar function to that of other *PI-like* proteins which play an important role in biosynthesis of cofactors, transport and binding. It was also important to translation, fatty acid metabolism and purines and pyrimidines.

Table 2. The prediction of PI-like function	
Functional category	**odds**
Amino_Acid_Biosynthesis	0.500
Biosynthesis_of_Cofactors	2.917
Cell_Envelope	0.541
Cellular_Processes	0.411
Central_Intermediary_Metabolism	0.762
Energy_Metabolism	0.389
Fatty_Acid_Metabolism	1.308
Purines_And_Pyrimidines	1.362
Regulatory_Functions	0.211
Replication_And_Transcription	0.075
Translation	1.614
Transport and Binding	1.885

Tertiary structure prediction of *PI-like* domain

By selecting related structures after Blast analysis (Figure 6), tertiary structure of *PI-like* domain was predicted by SWISS-MODEL (http://swissmodel.expasy.org/). The structure of the *PI-like* domain of *S. japonica* was like that of MADS-box transcription enhancer factor 2 (with a similarity level reaching 55.71%). The results showed that *PI-like* domain constituted curved spiral structure.
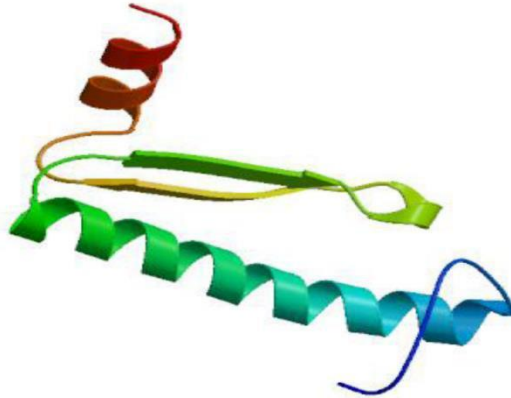
Figure 6. The domain area of *PI-like* protein's tertiary structure prediction.

Expression patterns of *PI-like* gene

The relative expressions of the *PI-like* gene in different phase of the life cycle and different tissues were analyzed by qRT-PCR assay (Figure 7). The results showed that the expression levels in the leaves were higher than that in the roots during these three development stages. Moreover, the expressions exhibited the high levels in the plantlet stage, decreased in the bud stages and then increased in the full-bloom stage in both two vegetative organs. Furthermore, in the reproductive organs, the highest expression level was in sepal and the lowest expression level was in pedicel. The expression level in petal was higher than that in labellum. The expression level in stigma was significantly higher than that in pedicel. In full-bloom stage, the expression levels in the reproductive organs were significantly higher than that in the vegetative organs. In bud stage, the expression level in pedicel was higher than that in leaf and root. While in plantlet stage, the expression level in root and leaf were near to zero.
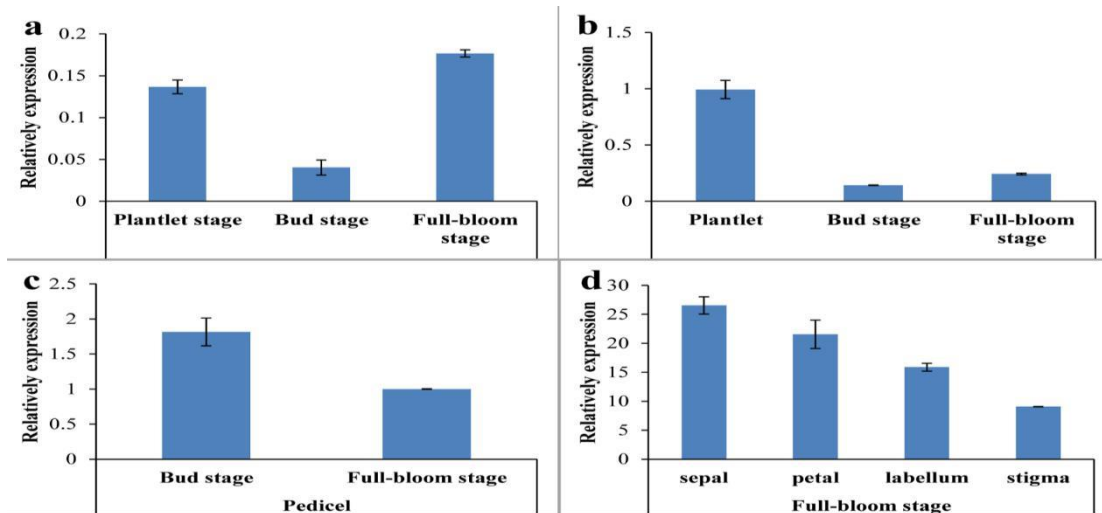
Figure 7. Relative expression levels of *PI-like* gene detected in different tissues by real-time quantitative PCR (a and b represent the expression levels of gene from the root and leaf at three development stages, respectively; c represents the expression levels of gene from pedicel at bud and full-bloom stages; d represents the expression levels of gene from four tissues of flower at the full-bloom stage), EF1α was used as the endogenous reference gene Standard error of the mean for three replicated is represented by the error bars.

DISCUSSION

MADS-box genes encode transcription factors which play critical roles in diverse developmental process including floral organ identity, flowering time control, meristem identity, formation of dehiscence zone, fruit ripening, embryo, and vegetative organs development (Alvarez-Buylla et al. 2000; Arora et al. 2007). It was well-known that members of MADS-box gene contributing to the modified ABCDE model of floral organ morphogenesis have dominated theory. Thus, many of genes controlling the flower development had been well-characterized in model plant species such as *Arabidopsis* and rice. For instance, 107 and 75 MADS-box genes had been identified in *Arabidopsis* and rice by the phylogenetic analysis, respectively (Arora et al. 2007; Parenicova et al. 2003). Although the relatively few MADS-box genes had been identified in the monocot species, several reports about MADS-box genes in many species of orchid had been published (Yu and Goh 2000; Hsu and Yang 2002; Tsai et al. 2004; Tsai et al. 2005; Tsai and Chen 2006; Xu et al. 2006).

The orchid flowers possess a typical petaloid perianth arrangement that provided the opportunity for researches to discover the new variant genes or orthologs and understand the complexity of the ABCDE model (Tsai and Chen 2006). The class B genes of MADS-box are further divided into two categories of PI-type and AP3-type, which determine the specify petal (lodicule in monocots) and stamen identity (Theissen et al. 2000). It was reported that various numbers of B genes had been identified and cloned from several orchid species. Among them, one AP3-like gene had been discovered in Oncidium Gower Ramsey (Yu and Goh 2000), two AP3-like genes identified in Dendrobium crumenatum (Xu et al. 2006), and four AP3-like genes obtained in *Phalaenopsis* equestris (Tsai et al. 2004). Moreover, since the *PI-like* gene had been also isolated from P. equestris (Tsai et al. 2005), the *PI-like* gene from the *Phalaenopsis* hybrid cultivar, Cymbidium faberi, Orchis italica and other *Phalaenopsis* species had also been published (Guo et al. 2007; Salemme et al. 2011; Yuan et al. 2017). Although the class B genes play the critical roles on the orchid floral development and identify in many orchids, the report of *PI-like* genes or orthologs in *S. japonica* species had not been published till now. Therefore, to clone and discuss the dynamic of *PI-like* gene from the different organ during the different developmental stages in *S. japonica* species is meaningful.

In this study, to dissect the potential role of *PI-like* in floral development, *S. japonica PI-like* full length cDNA sequence (921bp), including 633 bp ORF, was cloned according to the conservation of DNA sequence. The gene encoded protein which contained 211amino acids (24.5kDa) and the theoretical pI was 9.3. The deduced amino acid sequence showed that it has a typical MIKC domain structure. Multiple sequence alignment indicated that the protein belonged to a Class-B MADS-box gene, and only one Amino acid is difference in the *PI-like* gene was found between P. equestris and S. japonica. Furthermore, it showed a high homology to other PI-type MADS-box genes of other monocot plant species (Schilling et al. 2015; Yamaguchi et al. 2006; Yang et al. 2012; Yao et al. 2008), especially in the *Phalaenopsis* hybrid cultivar (99%) and *Cymbidium faberi* (96%). These findings suggested that *PI-like* might be a putative PI homologue from *S. japonica*, and the *PI-like* gene is highly conserved in the monocot plant species.

It was reported that the B function genes in dicotyledonous plants such as *Arabidopsis thaliana* and *Antirrhinum* is specifically expressed in flower (Jack et al. 1992; Tröbner et al). The same expression pattern of B function genes was reported later in the monocot plant species (Guo et al. 2007; Tsai et al. 2005). For instance, Tsai et al. (2005) had confirmed that the gene PeMADS6 (a member of *PI-like* gene) is also a flower specific gene in P. equestris species by used the Northern blot analysis. Guo et al. (2007) had found that PhPI15 was expressed in all the flower organs of *Phalaenopsis* hybrid cultivar, whereas the expression of it was not detected in the root and leaf. In this present study, the total RNA was extracted from vegetative organs (root and leaf) and floral organs (pedicel, sepal, petal, labellum, and column) at different developmental stages for qRT-PCR assay to verify whether the orchid cultivar *S. japonica* also has the similar expression trends of flower specific. It should be noted that the *PI-like* gene was expressed in both vegetative and floral organs, although the expression in the root and leaf at very low level (near-zero). These results suggested that the expression profile of *PI-like* gene from *S. japonica* is likely inconsistent with the findings of previous reports from the P. equestris and *Phalaenopsis* hybrid cultivar (Guo et al. 2007; Tsai et al. 2005). These findings indicated that the expression of *PI-like* gene in the *S. japonica* species is not flower specific. However, the similar phenomena that the expression of B function gene detected in the vegetative organs were also observed in Oncidium Gower Ramsey and *Phalaenopsis* 'Neishanguniang' (Hsu et al. 2002; Yuan et al. 2017), which indicated that the B function genes are not all the flower specific in all plant species and implied that this group genes is highly conserved in orchids.

The B group MADS-box genes in dicotyledonous species is known to be only expressed in the second, third and fourth whorls of immature flower organs (Goto and Meyerowitz, 1994), while the expression of *PI-like* gene in our study had been detected in all the whorls of S.japonica flower. Moreover, Guo et al. (2007) suggested that

the B function genes PhPI15 were expressed in four whorls of flower in *Phalaenopsis* hybrid cultivar, while Yuan et al. (2017) found that the same expression pattern of PhalPI gene was observed in P. 'Neishanguniang' and *Phalaenopsis* mutant plants. This abnormal phenomenon in orchids is probably attributed to that the structures of sepals and petals in orchid flowers are usually very similar and cannot be easily distinguished. Meanwhile, the similar structures between sepals and petals could be also to explain the higher expression of *PI-like* gene in sepals and petals when compared to the other floral organs in S. japonica. In addition, we found that the expression levels in the reproductive organs were significantly higher than that in the vegetative organs; and the highest expression was observed in the sepal, while the lowest expression was hunted in pedicel. All together, these findings strongly suggested that *PI-like* gene performs the critical role in floral organ morphogenesis. Furthermore, these findings indicated that the *PI-like* gene involved in multiple physiological functions in S. japonica.

CONCLUSION

In summary, we cloned and analyzed the expression patterns of *PI-like* from different tissues in *S. japonica* at three developmental stages. The *PI-like* gene constitutively expressed in both reproductive and vegetative organs, indicated that *PI-like* gene participate in a variety of developmental processes in S. japonica. Our results provide information that will be useful for further analysis of regulatory mechanism of MADS-box gene for controlling of the floral organ morphogenesis in orchids. Further studies are required to clarify the biological functions of *PI-like* gene regulation in *S. japonica* and will be applied to breed new *S. japonica* cultivars in future.

ACKNOWLEDGMENT

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