

In Silico Analysis of the Phalaemopsis amabilis FLOWERING TIME (PaFT) Gene Function

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Abstract. *Phalaenopsis amabilis* (L.) Blume, as one of the charms of Indonesia, this orchid is very popular with the public because of the beauty of its flower shape and the long duration of flowering. In plant, the flowering period begins when the vegetative stage switched to reproductive stage. Some genes play such role in the flowering plant, i.e.: FT (FLOWERING LOCUS T), FTL (FLOWERING LOCUS T-Like), and FT1 (FLOWERING TIME 1). The characterization of FT- flowering genes in *P. amabilis* orchids is still limited. The objective of this research is to know difference and similarity between FT genes of *P. amabilis* and other orchids and Angiospermae. Phylogenetic characterization was carried out using MEGA 11 software. The analysis was carried out using the maximum likelihood parameter with a total sample of 24 types of monocot and dicot plants. The results showed that the flowering genes in *P. amabilis* orchids were closely related to *P. equestris* and had a high degree of similarity with Dendrobium orchids.

Keywords: P. amabilis · FLOWERING TIME · Phylogenetic · Monocots · Eudicots

1 Introduction

Orchid is one of the most popular ornamental plants in Indonesia. Its flower has unique characters in shape and colors that make this plant attracted many enthusiasts. One of the most famous orchid species is *Phalaenopsis amabilis*. As an epiphytic orchid, P. amabilis has a monopodial stem growth system, with an indeterminate type of inflorescence [11]. *P. amabilis* has a specific flowering time affected by light and temperature conditions available in its environment, making them end up with a relatively long vegetative stage [19]. On average, this orchid takes about three years to produce flowers [15]. The flowering stage starts from shoot apical meristem (SAM) become flower meristem (FM) maturation to the formation of flowers.

513



Fig. 1. Growth and development process of *P. amabilis* seedlings (3–36 WASP), adult plants (130 WASP) and a flower. Scale bars: 5 mm WASP = Week After Seed Plantation

Figure 1 shows partial fragment of *PaFT* flowering gene has been isolated and characterized [15]. This gene plays a key role in flowering time regulation in orchids. To find out more about the characteristics of the PaFT1 gene, a full characterization of the PaFT1 gene was carried out. Previous research on *P. aphrodite*, a close relative of *P. amabilis*, showed that flowering could be induced on low ambient temperature (25°C) and inhibited on a relatively high ambient temperature (28°C) [7]. Meanwhile, photoperiodicity has no significant effect influence on flowering in *P. aphrodite* [7]. It is possible that PaFT has a similar structure in these two orchids.

2 Methods

This study use nucleotide sequence data from NCBI database. The ft gene sequence criteria that used in this research were ft genes of plants from members of angiosperm, as listed in Table 1.

The sequences obtained was aligned and visualized with MULTALIN [2]. Protein sequence samples were obtained from the protein translation process using ESX-PASY translate-tool (web.expasy.org/translate). Then the analysis of protein motifs using BLAST-tools from UNIPROT and analysis of nucleotide and protein motifs using MEME suite [18]. Phylogenetic analysis was performed using Maximum Likelihood approach with bootstrapping 1000 repeats, JTT (Jones Taylor Thornton) with 5 gamma distribution model using MEGA 11. [17] Physiochemical properties i.e. grand average of hydropathicity, molecular weight, aliphatic index, instability index and isoelectric point analyses using Expasy-Protparam server [4], Sub-cellular protein location determination using CELLO v2.5 and PSORT [6]. Molecular modelling was analyzed using SOPMA secondary structure prediction tool for two dimensional protein modelling, then three dimensional structure were analyzed with CHIMERA X [5, 12], AutoDock 4.2 [9], PyRx docking tools [3], PyMol visualization tools [14].

514 I. S. Prasojo et al.

No	Species	Accession Number	Gene	Sources
1	Arabidopsis thaliana	AB027504.1	FT	Kobayashi et al., 1999
2	Arachis hypogaea	XM_025802743.2	FT	NCBI-REFSEQ
3	Asparagus officinalis	XM_020397853.1	FT-like	NCBI-REFSEQ
4	Coffea eugenioides	XM_027326819.1	FT-like	NCBI-REFSEQ
5	Coffea arabica	XM_027224031.1	Hd3a-like	NCBI-REFSEQ
6	Coffea eugenioides	XM_027326838.1	Hd3a-like	NCBI-REFSEQ
7	Chenopodium ficifolium	MK212025.1	FT-like	NCBI
8	Cattleya trianae	MN968819.1	FT	Ospina-Zapata et al., 2020
9	Dendrobium catenatum	XM_020846691.2	FT	NCBI-REFSEQ
10	Elleanthus aurantiacus	MN968825.1	FT	Ospina-Zapata et al., 2020
11	Ananas comosus	XM_020232740.1	FT-like	NCBI-REFSEQ
12	Masdevallia coccinea	MN968852.1	FT	Ospina-Zapata et al., 2020
13	Masdevallia wendlandiana	MN968856.1	FT	Ospina-Zapata et al., 2020
14	Durio zibethinus	XM_022864452.1	FT	NCBI-REFSEQ
15	Maxillaria aurea	MN968868.1	FT	Ospina-Zapata et al., 2020
16	Miltoniopsis roezlii	MN968881.1	FT	Ospina-Zapata et al., 2020
17	Phalaenopsis amabilis	-	FT	DNA Sequencing result
18	Phalaenopsis equestris	XM_020736438.1	Hd3a-like	NCBI-REFSEQ
19	Tolumnia hybrid	MN968899.1	FT	Ospina-Zapata et al., 2020
20	Hevea brasiliensis	XM_021794815.1	Hd3a-like	NCBI-REFSEQ
21	Hevea brasiliensis	MH590658.1	FT1	Li et al., 2018
22	Musa acuminata	XM_009392515.2	FT-like	NCBI-REFSEQ
23	Phalaenopsis equestris	XM_020721263.1	FT-like	NCBI-REFSEQ
24	Solanum stenotomum	XM_049548199.1	Hd3a-like	NCBI-REFSEQ
25	Solanum tuberosum	KC348397.1	FT	Sundaresha et al., 2012
26	Vigna umbellata	XM_047315996.1	Hd3a-like	NCBI
27	Zingiber officinale	XM 042606100.1	FT-like	NCBI

Table 1. Nucleotide sequences that related to Flowering Time Genes in plants

3 Results and Discussions

3.1 Polymorphism Analysis of FT Gene in Plants

The *PaFT* gene sequence analyses show a polymorphism in nucleotide level. It was found that many mutations, deletion, and gaps appeared in this alignment. In nucleotide levels, variation among different gene family because of high variation. Below nucleotide



Fig. 2. Sequence Alignment of *P. amabilis FT* gene with *FT* from various plants.

data alignment shows conserved nucleotide part among FT gene are highlighted with red color, few conserved regions in this alignment showed with blue color. A suspected conserved nucleotide sequences pattern is observed on sequence 189 to 695 with several minor variations. Figure 2 shows pattern that is suspected to be a conservative nucleotide part. As for the pattern formed from the nucleotide sequence to 189 to 695. Several gaps showed in this alignment. Some of them are caused by the absence of a suitable nucleotide arrangement in Fig. 2 with other sequences. Variations at the nucleotide level have the potential to cause changes in the makeup of amino acids. A more in-depth analysis was carried out using DNA SP [13], to find out the polymorphism contained in the aligned sequences.

Figure 3 shows sequences processed in DNASP software to determine the position and location of the polymorphism. The nucleotide sample used as a reference for polymorphism analysis is P. amabilis. These polymorphism data show the many differences between nucleotide sequences in P. amabilis and other plants. This is because in this analysis different plant DNA sequences are used starting from the class taxon level, so that the polymorphisms found are very numerous. The similarity in the sequence is marked by a period, while there is a difference in the sequence, characterized by the different writing contained in the sample below. There are 283 polymorphism points in the data, with a total mutations number of 517 points.

I. S. Prasojo et al.

Nucleotide numbers	33333333333333333333333333333333333333
'Phalaenopsis amabilis'	79123589014570345678901235789145670346790124 CTAAATCAACCCTTTGCCGAGCTCATATCCTTCGACGAGCGTCA
'Arabidopsis thaliana'	.C.G.CT.CGTATCCCTTCG.C.ATT
'P.equestris_FTlike'	
'P.equestris_Hd3alike'	G.CT.AACCACT.TTC.CCGG.TACG.
'Cattleya trianae'	G.CT.AACAACGCATC.C.TGC.A.G.TA.G.
'Dendrobium catenatum'	CTGAACAC.ATATTCGC.TGC.A.G.TA.G.
'Elleantnus auranticus'	CTAAGCA.CAG.TTCTA.T.TTAG.
Masdevallia coccinea	
Masuevalla wenulanulan	C CTTCT AACCA AC T ATC C TCC A C T ACA
'Miltoniopsis roezlii'	CTAAAA.CAG.TTC.C.TAATTAT.
'Tolumnia hybrid'	CTAAAA.CAG.TTCTAATTAT.
'Ananas comosus'	.C.G.CTGAGCCT.A.CAT.TTAG.ACA.
'Arachis hypogaea'	CT.AATTGCAATGTC TT.C.AT
'Asparagus officinale'	.A.G.CT.AACA.CATTC.GAC.ATT.TC.G.
'C.eugenoides_FTlike'	.C.GGAGCTGTCAC.T.TTAT.
'C.arabica_Hd3a'	.C.GGAGCTGTCAC.T.TTAT.
'C.eugenoides_Hd3alike'	.C.GGAGCTGTCTAC.T.TTAT.
'Chenopodium ficifolium	.CCTAACTAGTC.CC.T.CT.TT
'Durio zibethinus'	GAACTTTCTA.T.GG.T.TT.
'H.brasiliensis_Hd3a'	
'H. Drasiliensis_FTI'	
'Musa acuminata'	AACG.AAGA.CAG.IIC.C.AGGAIIA.I.GA.GI
'Solanum tuberosum'	
'Vigna umbellata'	CAATAG.TTC.C.TA.TAGT.C.T
'Zingiber officinale'	AAGCCAAGCA.CTGCGT.GCGATTG.TA.G
Nucleotide numbers	33333333333334444444
	7778888999999900000011
IPhalaananaia amahilial	77788889999999900000011 5781478013456901258912
'Phalaenopsis amabilis' 'Arabidopsis thaliana'	7778888999999900000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA
'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris FTlike'	777888899999990000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA .ACTTG.GAGTG.A T.CTTGACATT.GT.C
'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris Hd3alike'	777888899999990000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA .ACTTG.GAGTG.A T.CTTGACATT.GT.C G.G.AAATGTAC
'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae'	777888899999990000011 5781478013456901258912 CTTCCAAAGGGCCCTCCTAAAA .ACTTG.GAGTG.A T.CTTGACATT.GT.C G.G.AAAT.GTAC G.G.AAAT.GTAT
'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum'	777888899999990000011 5781478013456901258912 CTTCCAAAGGGCCCTCCTAAAA .ACTTG.GAGTG.A T.CTTGACATT.GT.C G.G.AAAT.GTAC G.G.AAATT.GTAT
'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus'	7778888999999900000011 5781478013456901258912 CTTCCAAAGGGCCCTCCTAAAA ACTTG.GAGT.G.A T.CTTGACATT.GT.C G.G.AAATT.GTAC G.AAATT.GTAT T.CTTGCATT.GTAT
'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus' 'Masdevallia coccinea'	777888899999990000011 5781478013456901258912 CTTCCAAAGGGCCTCCTAAAA .CTTG.GAGT.G.A T.CTTGACATT.GT.C G.G.AAAT.GTAC G.AAATT.GTAT T.CTTGCAATT.GTAT T.CTTGCATT.GT.C CT.GACATT.GT.CC
'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus' 'Masdevallia coccinea'	777888899999990000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA .ACTTGACAT.GT.C G.G.AAAT.GTAC G.AAAT.GTAC G.AAATT.GTAT T.CTTGCATT.GT.C CT.GACATT.GT.CC 'G.G.AAATT.GT.T
'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus' 'Masdevallia coccinea' 'Masdevalia wendlandian 'Maxillaria_aurea'	777888899999990000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA .ACTTGACAT.GT.C G.AAAT.GTAC G.AAAT.GTAC G.AAATT.GTAT T.CTTGCATT.GT.C G.AAATT.GT.C G.AAATT.GT.C G.AAATT.GT.C
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<pre>'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Lelleanthus auranticus' 'Masdevallia coccinea' 'Masdevalia wendlandian 'Maxillaria_aurea' 'Masdevalia wendlandian 'Maxillaria_aurea' 'Masdevalia vendlandian 'Maxillaria_aurea' 'Masdevalia vendlandian 'Maxillaria_aurea' 'Masdevalia vendlandian 'Maxillaria_aurea' 'Masdevalia vendlandian 'Maxillaria_aurea' 'Asparagus officinale' 'C.eugenoides_FTlike' 'C.eugenoides_FTlike' 'C.eugenoides_HDA3like' 'Chenopodium ficifolium 'Durio zibethinus' 'H.brasiliensis_FT1' 'Musa acuminata'</pre>	777888899999990000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA .ACTTG.G.AGT.G.A T.CTTGACATT.GT.C G.G.AAAT.GTAC G.AAATT.GTAT T.CTTGCATT.GT.C AAAT.GT.T T.CTTGACATT.GT.T T.CTTGACATT.GT.T T.CTTGACATT.GT.T T.CTTGACATT.GT.T T.CTTGACAT.GT.T T.CTTGACAT.GT.T T.CT.G.C.AGT.GT.T
<pre>'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus' 'Masdevallia coccinea' 'Masdevalia wendlandian 'Maxillaria aurea' 'Miltoniopsis_roezlii' 'Iolumnia hybrid' 'Ananas comosus' 'Arachis hypogaea' 'Asparagus officinale' 'C.eugenoides_FTlike' 'C.eugenoides_FTlike' 'C.eugenoides_HDA3like' 'Chenopodium ficifolium 'Durio zibethinus' 'H.brasiliensis_Hd3a' 'H.brasiliensis_FT1' 'Musa acuminata' 'Solanum stenototum'</pre>	777888899999990000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA .ACTTG.G.AGT.G.A T.CTTGACATT.GT.C G.G.AAAT.GTAC G.AAATT.GTAT T.CTTGCATT.GT.C ACATT.GT.C AAAT.GT.T T.CTTGACATT.GT.T T.CTTGACATT.GT.T T.CTTGACATT.GT.T T.CTTGACAT.GT.T T.CTTGACAT.GT.T AAAT.GT.T AAT.GT.T ACAT.GT.T
<pre>'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus' 'Masdevalia coccinea' 'Masdevalia vendlandian 'Maxillaria_aurea' 'Miltoniopsis_roezlii' 'Tolumnia hybrid' 'Ananas comosus' 'Arachis hypogaea' 'Asparagus officinale' 'C.eugenoides_FTlike' 'C.eugenoides_FTlike' 'C.eugenoides_FTlike' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.henopodium ficifolium 'Durio zibethinus' 'H.brasiliensis_FT1' 'Musa acuminata' 'Solanum stenototum' 'Solanum tuberosum'</pre>	777888899999990000011 5781478013456901258912 CTTCCAAAGGGCCCTCCTAAAA ACTTG.G.AGT.GTAC G.G.AAAT.GTAC G.G.AAAT.GTAC G.G.AAAT.GTAT G.G.AAAT.GTAT G.G.AAAT.GTAT G.G.AAAT.GT.T AAAT.GT.T AAAT.GT.T ACTT.GT.C ACT.G.T.G
<pre>'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus' 'Masdevalia coccinea' 'Masdevalia vendlandian 'Maxillaria_aurea' 'Masdevalia vendlandian 'Maxdevalia vendlandian 'Masdevalia vendlandian 'Masdevalia vendlandian 'Masdevalia vendlandian 'Masdevalia vendlandian 'Masdevalia vendlandian 'Masdevalia vendlandian 'Masdevalia vendlandian 'Masdevalia vendlandian 'Masdevalia vendlandian 'Arachis hypogaea' 'Arachis hypogaea' 'Arachis hypogaea' 'Asparagus officinale' 'C.eugenoides_FTlike' 'C.eugenoides_FTL' 'Musa acuminata' 'Solanum stenototum' 'Solanum tuberosum' 'Vigna umbellata'</pre>	7778888999999900000011 5781478013456901258912 CTTCCAAAGGGCCCTCCTAAAA ACTTG.G.AGT.G.A T.CTTGACATT.GT.C G.G.AAAT.GTAC G.AAATT.GTAT T.CTTGCATT.GT.C G.G.AAAT.GT.T T.CTTGACATT.GT.C AAAT.GT.T T.CTTGACA.T.GT.T T.CTTGACA.T.GT.T ACT.G.T.G.T
<pre>'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus' 'Masdevalia coccinea' 'Masdevalia wendlandian 'Maxillaria_aurea' 'Masdevalia wendlandian 'Maxillaria_aurea' 'Masdevalia wendlandian 'Maxillaria_aurea' 'Masdevalia wendlandian 'Masdevalia wendlandian 'Masdevalia wendlandian 'Masdevalia wendlandian 'Masdevalia wendlandian 'Masdevalia wendlandian 'Masdevalia wendlandian 'Asparagus officinale' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_HDA3like' 'C.eugenoides_FT1' 'Musa acuminata' 'Solanum stenototum' 'Solanum tuberosum' 'Vigna umbellata' 'Zingiber of polymorphic fereigenetics 'Sumber of polymorphic fereigenetics' 'Solanum tuberosum'</pre>	777888899999990000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA ACTTGACATT.GT.C G.G.AAAT.GTAC G.G.AAAT.GTAC G.AAATT.GTAT T.CTTGCATT.GT.C AAAT.GT.C AAAT.GT.T T.CTTGACATT.GT.T T.CTTGACATT.GT.T T.CTTGACAT.GT.T AAT.GT.T T.CTTGACAT.GT.T ACATT.GT.T ACAT.GT.T
<pre>'Phalaenopsis amabilis' 'Arabidopsis thaliana' 'P.equestris_FTlike' 'P.equestris_Hd3alike' 'Cattleya trianae' 'Dendrobium catenatum' 'Elleanthus auranticus' 'Masdevalia coccinea' 'Masdevalia wendlandian 'Maxillaria_aurea' 'Masdevalia wendlandian 'Maxillaria_aurea' 'Masdevalia wendlandian 'Maxillaria_aurea' 'Masdevalia wendlandian 'Masdevalia wendlandian 'Masdevalia wendlandian 'Masdevalia wendlandian 'Masdevalia wendlandian 'Arachis hypogaea' 'Arachis hypogaea' 'C.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTlike' 'S.eugenoides_FTli</pre>	777888899999990000011 5781478013456901258912 CTTCCAAAGGCCCTCCTAAAA ACTTG.G. AGT.G.A T.CTTGACATT.GT.C G.G.AAAT.GTAC G.AAATT.GTAT T.CTTGCATT.GT.C G.G.AAATT.GT.T T.CTTGACATT.GT.C AAAT.GT.T T.CTTGACATT.GT.T T.CTTGACATT.GT.T AAAT.GT.T T.CTTGACAT.GT.T AG.T.G.T

Fig. 3. Polymorphism analysis of nucleotide sequences using DNASP Software

516



Fig. 4. Amino acid sequences Alignment of FT protein from *P. amabilis* and other plants

3.2 Protein Motifs Analysis of FT Gene in Plants

The PaFT Protein sequence analysis show conservative amino acid. Below protein motifs analysis show conserved region on plant. Highly conserved amino acid showed with red colors, others in blue color, and non-conserved amino acid showed with black colors.

Figure 4 shows comparison between P. amabilis protein and reference shows the presence of a conserve motif, namely the PEBP (Phosphatidylethanolamine Binding-Protein) region. Black lines show PEBP superfamily region, red line show exon number 1, orange line show exon number 2, violet line show exon number 3, then green line show exon number 4. There are 5 motifs found based on MEME-suite protein alignment motifs discovery, colored in red, sea blue, purple, green, and yellow.

Figure 4 shows there are several gaps formed in the alignment above, indicating the presence of some amino acid that are not present in some of the sequences above. Differences in the arrangement of amino acids affect the function of proteins.

Figure 5 shows the presence of several patterns of protein arrangement in each protein sequence with the FT protein and its homologous. Using tools from the MEME suite, it was found that there were 9 protein motifs that compiled the FT protein sequence. Of the 9 motifs present in the FT protein sequence and other homologous protein, it was found that there is similarity in protein regions between the member of Orchidaceae family and other plant families. There are 6 high conserved motifs discovered in the protein



Fig. 5. FT Protein motifs in *P. amabilis* and its homologous gene in various plants, by MEMEsuites

alignment and its function, i.e. GIHR, TRDF, YTLVMV, DPDAP, DIP and GGRR shown in Table 1.

The conserved amino acid arrangement motif found in the alignment of sequences is divided into several functions, including meristem determinancy, regulation of flower development, and anion binding activity. Some of the important functions of this amino acid arrangement are closely related to the function of proteins encoded by the *FT* flowering gene in plants, which are found in the PEBP Superfamily conserved region [21].

Table 2 shows physico-chemical data, from the highest *Arachis hypogaea_*FT (**294**), *Zingiber officinale_*FTlike (**285**), *Hevea brasiliensis_*Hd3a (**281**), *Dendrobium catena-tum_*FT (**278**), *Arabidopsis thaliana_*FT (**276**) with an average length value of protein

No.	Motifs	Function	References
1	MMHGRDPLV	Meristem determinancy	Nakamura et al. [10]
2	VVGDVLDPF	Meristem determinancy	Nakamura et al. [10]
3	LKVTY	Meristem determinancy	Nakamura et al. [10]
4	REV	Regulation of flower development	Watanabe et al. [20]
5	YTLVMV	Meristem determinancy	Nakamura et al. [10]
6	DPDAP	Anion binding activity	Zhang et al. [21]
7	DIP	Meristem determinancy	Nakamura et al. [10]
8	GNEIV	Meristem determinancy	Nakamura et al. [10]
9	GIHR	Anion binding activity	Zhang et al. [21]

Table 2. Comparison of protein motifs from FT protein and its homologs in plants

Table 3. Physico-chemical characteristic of FT protein and its homologous in plants

Protein	AA	MW	IP	Ins	AI	GRAVY	Loc	SP	
Phalaenopsis amabilis_FT	208	23773,2	8,64	44,21	80,91	-0,14	nucleus	0	0
Arabidopsis thaliana_FT	276	31766,6	9,17	49,08	83,95	-0,329	nucleus	0	0
Phalaenopsis equestris_FTlike	227	25501,1	5,77	40,3	90,93	-0,111	nucleus	0	0
Phalaenopsis equestris_Hd3alike	266	29643,9	8,51	47,93	87,14	-0,104	nucleus	0	0
Cattleya trianae_FT	178	19813,4	6,72	42,86	75,51	-0,321	nucleus	0	0
Dendrobium catenatum_FT	278	31741,5	7,68	51,2	90,07	-0,047	nucleus	0	0
Elleanthus auranticus_FT	178	19883,6	5,92	41,49	80,96	-0,234	nucleus	0	0
Masdevallia coccinea_FT	178	20014,7	5,32	49,77	80,45	-0,279	nucleus	0	0
Masdevalia wendlandiana_FT	178	19815,5	5,68	42,14	81,52	-0,231	nucleus	0	0
Maxillaria aurea_FT	178	19960,6	6,73	49,2	82,08	-0,301	nucleus	0	0
Miltoniopsis roezlii_FT	178	19815,5	5,68	42,14	81,52	-0,231	nucleus	0	0
Tolumnia hybrid_FT	178	19964,7	5,47	49,26	81,52	-0,239	nucleus	0	0
Ananas comosus_FTlike	247	27554,3	6,47	37,14	90,32	-0,126	nucleus	0	0
Arachis hypogaea_FT	294	33669,8	9,33	37,76	91,43	-0,085	nucleus	0	0
Asparagus officinalis FTlike	243	27687,0	9,15	42,44	88,97	0,002	nucleus	0	0
Coffea eugenoides_FTlike	174	19401,1	6,58	52,17	88,39	-0,14	nucleus	0	0
Coffea arabica_Hd3alike	174	19401,1	6,58	52,17	88,39	-0,14	nucleus	0	0
Coffea eugenoides_Hd3alike	174	19401,1	6,58	52,17	88,39	-0,14	nucleus	0	0
Chenopodium ficifolium_FT1like	264	30504,9	9,66	44,64	80	-0,362	nucleus	0	0
Durio zibethinus_Hd3a	175	19777,5	6,73	45,89	83,94	-0,25	nucleus	0	0
Hevea brasiliensis_Hd3a	281	32295,6	9,51	54,02	95,3	-0,14	nucleus	0	0
Hevea brasiliensis_FT1	252	28590,9	9,41	51,18	85,79	-0,255	nucleus	0	0
Musaacuminatasubsp.malaccensis_FTlike	267	30461,9	9,17	39,42	79,89	-0,133	nucleus	0	0
Solanum stenototum_Hd3alike	192	21940,1	8,81	49,92	90,78	-0,243	nucleus	0	0
Solanum tuberosum_cult.Kufri_FT	173	19693,5	7,75	41,37	85,49	-0,309	nucleus	0	0
Vigna umbellata_Hd3alike	180	20531,7	9,94	49,98	90,89	-0,303	nucleus	0	0
Zingiber officinale_FTlike	285	31952,8	9,35	42,92	88,25	-0,076	nucleus	0	0

Description: Peptide length (AA), protein molecular weight (MW) in kDa, isoelectric point (IP), instability index (Ins), aliphatic index (AI), grand average of hydropathy (GRAVY), localization (Loc), signal Peptide (SP), transmembrane domain (TMD).

sequences of 217.6 sequences. *P. amabilis* has FT protein with 207 amino acid contains. It is below the average value protein sequence length (217.6 aa) Likewise with the results obtained from the molecule weight. *P. amabilis* FT protein has molecular weight of 23773,2 kDa. Its below average Molecular weight value (24613,23). All sequence were located in nucleus.

520 I. S. Prasojo et al.

Phalaenopsis amabilis_FT		Asparagus officinalis_FTlike	
Arabidopsis thaliana_FT	······	Coffea eugenoides_FTlike	
Phalaenopsis equestris_FTlike		Coffea eugenoides_HDA3like	
Phalaenopsis equestris_HDA3like	[]-]]-0-0-0-0-0-0-0-0-0-0-0-0-0-0	Coffea arabica_HDA3like	
Cattleya trianae_FT		Chenopodium ficifolium_FT1like	
Dendrobium catenatum_FT		Durio zibethinus_HDA3	
Elleanthus auranticus_FT	[+([]+])])+(++++)]==[]++++]][++++]]==(]]+++(]]++(]]++(]]+	Hevea brasiliensis_HDA3	
			56 AMI 158 PW
Masdevallia coccinea_FT		Hevea brasiliensis_FT1	
Masdevallia wendlandiana_FT	all and dense for the set of the	Musa acuminata_FTlike	
Maxillaria_aurea_FT		Solanum stenototum_HDA3like	
Miltoniopsis roezlii_FT		Solanum tuberosum_FT	
Tolumnia hybrid_FT		Vigna umbellata_HDA3like	· []-]]] - (
Ananas comosus_FTlike		Zingiber officinale_FTlike	
Arachis hypogaea_FT		= Random coils	= Beta-turn = Alpha-helix

Fig. 6. Flowering Time Protein Structure in plants. Some flowering time protein: FT, FT1, FT-like, FT1-like, Hd3a, Hd3a-like Protein have similar structures related to folding configuration of protein.



Fig. 7. a) FT, b) Hd3a protein 3D structures, c) Predicted GA3 position on FT protein

3.3 Protein Structure Analysis of FT Protein in Plants

To understand the similarities and differences in the structure of ft protein arrangement in plants, a 2D analysis of protein sequences was carried out using SOPMA software. From these results, it can be known what kind of arrangement and folding of proteins occurs in each FT protein sequence in plants.

Figure 6 shows two dimensional protein structure of *P. amabilis, A. thaliana, P. equestris, C. trianae, D. catenatum, E. auranticus, Masdevallia coccinea, Masdevallia wenlandiana, Maxillaria aurea, Miltoniopsis roezlii, T. hybrid, Ananas comosus, Arachis hypogaea, Asparagus officinalis, Coffea eugenoides, Coffea arabica, Chenopodium ficifolium, Durio zibethinus, H. brasiliensis, Musa acuminata, S. stenototum, S. tuberosum, V. umbellata, Z. officinale.*

There are two main structures in FT protein and its homologs, i.e. Alpha helix and Beta-turn. Alpha helix protein is red color, then Beta-turn protein structure is blue color. Another structure consisted in protein structure is random coil, in violet color. *P. amabilis* tends to have enormous amount of random coil structure than Alpha-helix and Beta-turn structures.

Figure 7 shows three dimensional protein structures of FT and Hd3a. FT and Hd3a have big similarity of sequence motif and structure. The main difference located in



Fig. 8. Phylogenetic analysis of FT gene protein sequences

sequence length and its motif. FT protein has active binding site with GA3, showed in Fig. 7c and 7d, that GA3 binds in position with FT protein with binding affinity -7.9 kcal/mol.

Figure 8 shows phylogenetic tree protein sequence data. It also shows *clade* differences between plant species. The two largest *clades* are divided from Orchidaceae family and other family. *P. amabilis* sequence included in the clade of Orchidaceae family, closest to *P. equestris* FTlike protein. With genetic distance of 0,01. There is far genetic distance compared with *A. thaliana*, and other plant families, above 0,01 genetic distance.

4 Conclusion

Flowering Time Gene of P. amabilis has a specific amino acid sequence that difference to other higher plants, namely PEBP-region, such as meristem determinacy, regulation of flower development, and anion binding activity function. Based on phylogenetic trees, *P. amabilis* has close genetic distance with other orchid species. Based on two dimensional protein structure analysis, *P. amabilis* FT has huge amount of random coils motifs in its protein sequence. Based on three dimensional protein structures analysis, *P. amabilis* FT has close similarity with Hd3a Protein. FT with GA3 ligands has -7,9 mol/kcal binding affinity.

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