



In Silico Analysis of the *Phalaenopsis 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.

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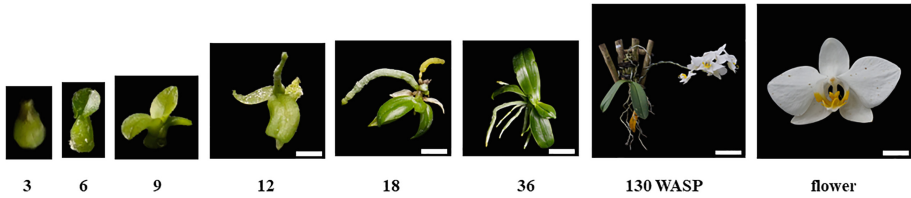


Fig. 1. Growth and development process of *P. amabilis* seedlings (3–36 WASP), adult plants (130 WASP) and a flower. Scale bars: 5 mm WASP = Week After Seed Plantation

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 EXPASY 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].

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

No	Species	Accession Number	Gene	Sources
1	<i>Arabidopsis thaliana</i>	AB027504.1	<i>FT</i>	Kobayashi et al., 1999
2	<i>Arachis hypogaea</i>	XM_025802743.2	<i>FT</i>	NCBI-REFSEQ
3	<i>Asparagus officinalis</i>	XM_020397853.1	<i>FT-like</i>	NCBI-REFSEQ
4	<i>Coffea eugenioides</i>	XM_027326819.1	<i>FT-like</i>	NCBI-REFSEQ
5	<i>Coffea arabica</i>	XM_027224031.1	<i>Hd3a-like</i>	NCBI-REFSEQ
6	<i>Coffea eugenioides</i>	XM_027326838.1	<i>Hd3a-like</i>	NCBI-REFSEQ
7	<i>Chenopodium ficifolium</i>	MK212025.1	<i>FT-like</i>	NCBI
8	<i>Cattleya trianae</i>	MN968819.1	<i>FT</i>	Ospina-Zapata et al., 2020
9	<i>Dendrobium catenatum</i>	XM_020846691.2	<i>FT</i>	NCBI-REFSEQ
10	<i>Elleanthus aurantiacus</i>	MN968825.1	<i>FT</i>	Ospina-Zapata et al., 2020
11	<i>Ananas comosus</i>	XM_020232740.1	<i>FT-like</i>	NCBI-REFSEQ
12	<i>Masdevallia coccinea</i>	MN968852.1	<i>FT</i>	Ospina-Zapata et al., 2020
13	<i>Masdevallia wendlandiana</i>	MN968856.1	<i>FT</i>	Ospina-Zapata et al., 2020
14	<i>Durio zibethinus</i>	XM_022864452.1	<i>FT</i>	NCBI-REFSEQ
15	<i>Maxillaria aurea</i>	MN968868.1	<i>FT</i>	Ospina-Zapata et al., 2020
16	<i>Miltoniopsis roezlii</i>	MN968881.1	<i>FT</i>	Ospina-Zapata et al., 2020
17	<i>Phalaenopsis amabilis</i>	-	<i>FT</i>	DNA Sequencing result
18	<i>Phalaenopsis equestris</i>	XM_020736438.1	<i>Hd3a-like</i>	NCBI-REFSEQ
19	<i>Tolumnia hybrid</i>	MN968899.1	<i>FT</i>	Ospina-Zapata et al., 2020
20	<i>Hevea brasiliensis</i>	XM_021794815.1	<i>Hd3a-like</i>	NCBI-REFSEQ
21	<i>Hevea brasiliensis</i>	MH590658.1	<i>FTI</i>	Li et al., 2018
22	<i>Musa acuminata</i>	XM_009392515.2	<i>FT-like</i>	NCBI-REFSEQ
23	<i>Phalaenopsis equestris</i>	XM_020721263.1	<i>FT-like</i>	NCBI-REFSEQ
24	<i>Solanum stenotomum</i>	XM_049548199.1	<i>Hd3a-like</i>	NCBI-REFSEQ
25	<i>Solanum tuberosum</i>	KC348397.1	<i>FT</i>	Sundaresha et al., 2012
26	<i>Vigna umbellata</i>	XM_047315996.1	<i>Hd3a-like</i>	NCBI
27	<i>Zingiber officinale</i>	XM_042606100.1	<i>FT-like</i>	NCBI

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

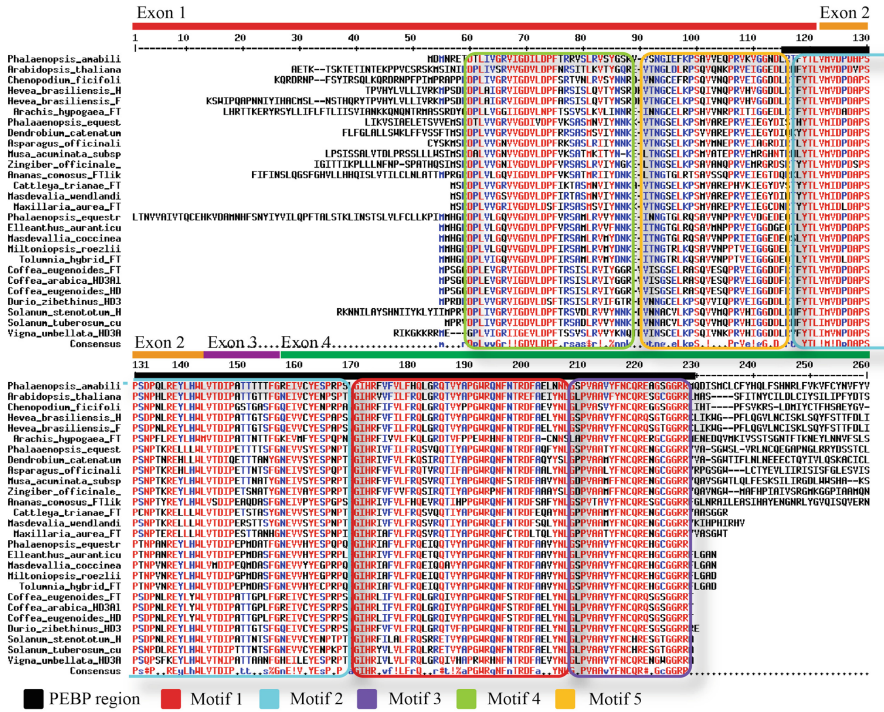


Fig. 4. Amino acid sequences Alignment of FT protein from *P. amabilis* and other plants

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). 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

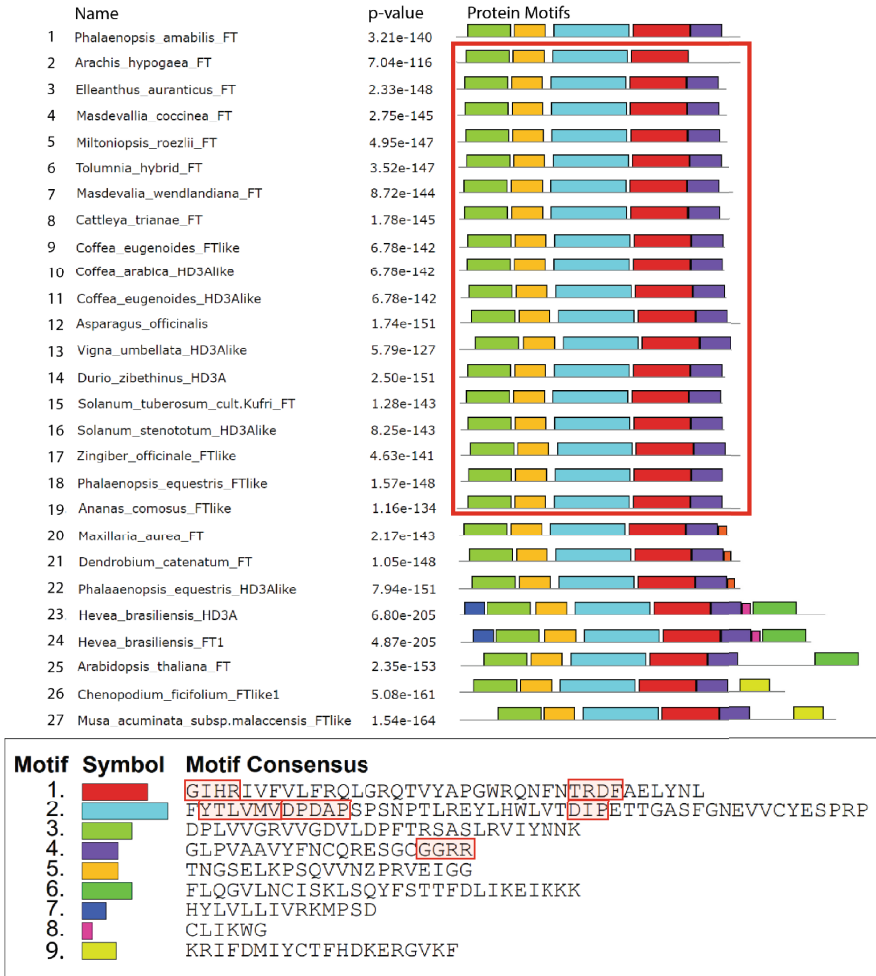


Fig. 5. FT Protein motifs in *P. amabilis* and its homologous gene in various plants, by MEME-suites

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 catenatum_FT* (278), *Arabidopsis thaliana_FT* (276) with an average length value of protein

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

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

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

Protein	AA	MW	IP	Ins	AI	GRAVY	Loc	SP	TH
<i>Phalaenopsis amabilis</i> _FT	208	23773,2	8,64	44,21	80,91	-0,14	nucleus	0	0
<i>Arabidopsis thaliana</i> _FT	276	31766,6	9,17	49,08	83,95	-0,329	nucleus	0	0
<i>Phalaenopsis equestris</i> _FTlike	227	25501,1	5,77	40,3	90,93	-0,111	nucleus	0	0
<i>Phalaenopsis equestris</i> _Hd3alike	266	29643,9	8,51	47,93	87,14	-0,104	nucleus	0	0
<i>Cattleya trianae</i> _FT	178	19813,4	6,72	42,86	75,51	-0,321	nucleus	0	0
<i>Dendrobium catenatum</i> _FT	278	31741,5	7,68	51,2	90,07	-0,047	nucleus	0	0
<i>Elleanthus auranticus</i> _FT	178	19883,6	5,92	41,49	80,96	-0,234	nucleus	0	0
<i>Masdevallia coccinea</i> _FT	178	20014,7	5,32	49,77	80,45	-0,279	nucleus	0	0
<i>Masdevallia wendlandiana</i> _FT	178	19815,5	5,68	42,14	81,52	-0,231	nucleus	0	0
<i>Maxillaria aurea</i> _FT	178	19960,6	6,73	49,2	82,08	-0,301	nucleus	0	0
<i>Miltoniopsis roezlii</i> _FT	178	19815,5	5,68	42,14	81,52	-0,231	nucleus	0	0
<i>Tolumnia hybrid</i> _FT	178	19964,7	5,47	49,26	81,52	-0,239	nucleus	0	0
<i>Ananas comosus</i> _FTlike	247	27554,3	6,47	37,14	90,32	-0,126	nucleus	0	0
<i>Arachis hypogaea</i> _FT	294	33669,8	9,33	37,76	91,43	-0,085	nucleus	0	0
<i>Asparagus officinalis</i> _FTlike	243	27687,0	9,15	42,44	88,97	0,002	nucleus	0	0
<i>Coffea eugenoides</i> _FTlike	174	19401,1	6,58	52,17	88,39	-0,14	nucleus	0	0
<i>Coffea arabica</i> _Hd3alike	174	19401,1	6,58	52,17	88,39	-0,14	nucleus	0	0
<i>Coffea eugenoides</i> _Hd3alike	174	19401,1	6,58	52,17	88,39	-0,14	nucleus	0	0
<i>Chenopodium ficifolium</i> _FT1like	264	30504,9	9,66	44,64	80	-0,362	nucleus	0	0
<i>Durio zibethinus</i> _Hd3a	175	19777,5	6,73	45,89	83,94	-0,25	nucleus	0	0
<i>Hevea brasiliensis</i> _Hd3a	281	32295,6	9,51	54,02	95,3	-0,14	nucleus	0	0
<i>Hevea brasiliensis</i> _FT1	252	28590,9	9,41	51,18	85,79	-0,255	nucleus	0	0
<i>Musaacuminatasubsp.malaccensis</i> _FTlike	267	30461,9	9,17	39,42	79,89	-0,133	nucleus	0	0
<i>Solanum stenototum</i> _Hd3alike	192	21940,1	8,81	49,92	90,78	-0,243	nucleus	0	0
<i>Solanum tuberosum</i> _cult.Kufri_FT	173	19693,5	7,75	41,37	85,49	-0,309	nucleus	0	0
<i>Vigna umbellata</i> _Hd3alike	180	20531,7	9,94	49,98	90,89	-0,303	nucleus	0	0
<i>Zingiber officinale</i> _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 hydrophathy (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.

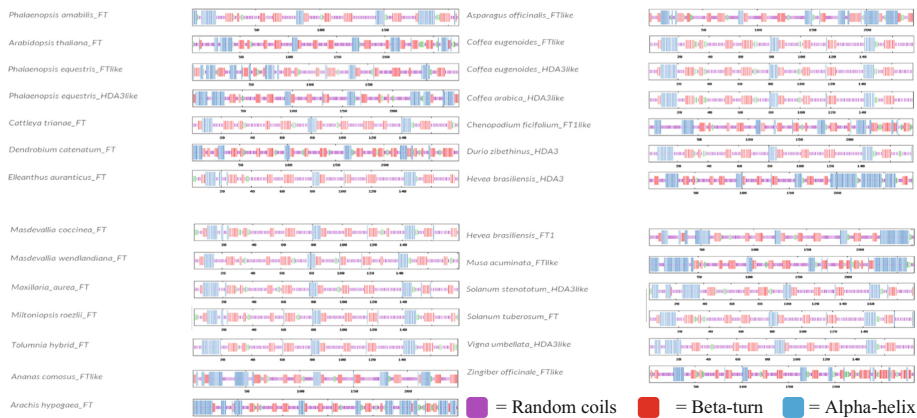


Fig. 6. Flowering Time Protein Structure in plants. Some flowering time protein: FT, FT1, FT-like, FT1-like, Hd3a, Hd3a-like Protein have similar structures related to folding configuration of protein.

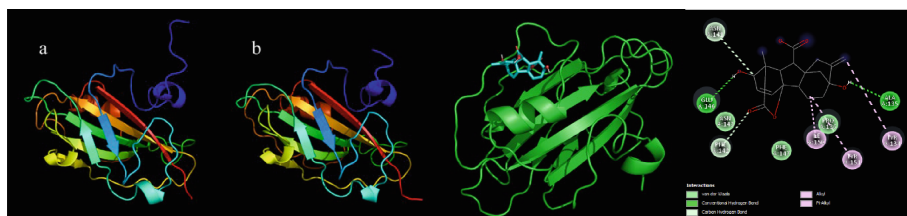


Fig. 7. a) FT, b) Hd3a protein 3D structures, c) Predicted GA3 position on FT protein

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

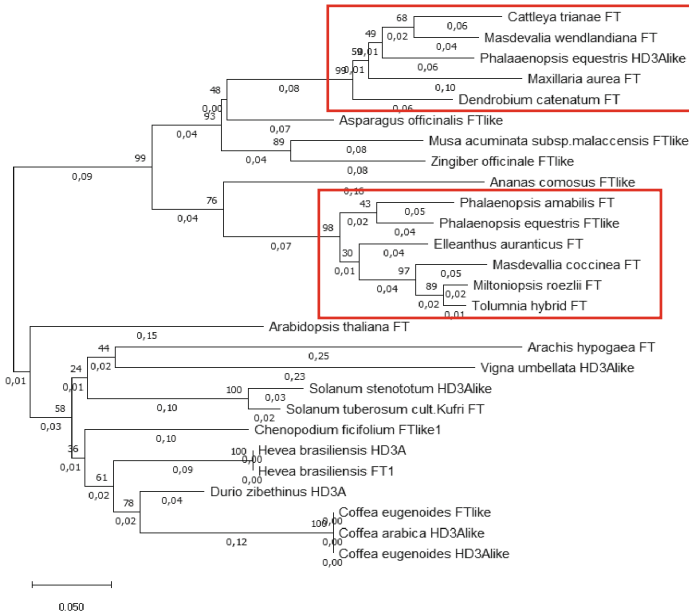


Fig. 8. Phylogenetic analysis of FT gene protein sequences

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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