Conference Paper

Isolation of Flower Development Regulator Gene SEPALLATA 1 in *Phalaenopsis amabilis* (L.) Blume

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Abstract

Phalaenopsis amabilis (L.) Blume is an indigenous orchid species in Indonesia. This orchid has a white large flower. The large flower is caused by the existence of gene that has an important role in flower development. One of the genes is *SEPALLATA 1.* This gene is a member of superfamily MADS-Box gene. *SEPALLATA 1* gene is a marker of primordial flower organ. This study aimed to isolate *SEPALLATA1* gene from *Phalaenopsis amabilis* (L.) Blume by PCR using forward primer 5'-GCT-GGA-GCG-GAT-CGA-GAA-CA-3'and reverse primer 5'-TCA-TGC-AAG-CCA-ACC-AGG-TG-3'. This study successfully amplified 691 bp lengths of *SEPPALATA1* fragment, lacking 20 bp upstream which consist its start codon.

Keywords: Flower development regulation; *Phalaenopsis amabilis* (L.) Blume; SEPALLATA 1 gene.

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1. Introduction

Phalaenopsis amabilis (L.) Blume is a member of the family Orchidaceae. This orchid has unique flower form [1, 2]. The sepal is white-colored like the petal, but it has a different form. The sepals are ellipse to acute shaped while the petals are widened circular with a small base and dull top, one of petal modified to be labellum form. The labellum has a pale yellow color to dark yellow with red stripes on the inside [3, 4]. There are stamen and pistil in the gynostemium [5].

The flowers begin to develop from primordial organ on apical and axillary shoots [6]. In the initial primordial stage, the sepals are smaller than the leaves and petals and stamen is even smaller. Size reduction of the primordial form goes along with its formation change. Primordial on the top will conduct the phyllotaxis. There are three flower circles with 120° difference between flower sections in monocotyledons [7].

Flower development encoded by morphogenesis genes. One of the morphogenesis encoding genes is *SEPALLATA (SEP)*. *SEP* is a member of superfamily MADS-box [8, 9].

SEP gene consists of *SEP1, SEP2, SEP3,* and *SEP4* [10–14]. This gene plays role in flower primordial organ regulation, determining flower sections from the meristem of flower candidate [15, 16]. *SEP1, SEP2* and *SEP4* are expressed in meristem primordial flower; *SEP1, SEP2* are expressed in all of flower primordial organ; *SEP3* is expressed in third circle (stamen) and *SEP4* expressed in center of flower circle (carpel) [13].

In *Arabidopsis thaliana* (L.) Heynh. *SEP* gene plays determining role on petal, stamen and carpel candidate [17]. In *Dendrobium crumenatum* Sw. it is called *DcSEP1* [18] and in *Phalaenopsis equestris* (Schauer) Rchb.f it is called *PeSEP1* [9]. The genes play the role of sepal, petal, and labellum forming [9, 18, 19]. This study aimed to isolate *SEPALLATA1* gene from *Phalaenopsis amabilis* (L.) Blume.

2. Material and Methods

2.1. Plant material

Phalaenopsis amabilis (L.) Blume samples were collected from DD' Orchid Nursery, Dadaprejo, Batu. Indonesia.

2.2. DNA extraction

Total DNA was extracted from young leaves using Geneaid Genomic DNA Mini Kit (Plant) protocol with some modification in incubation duration (about 4 h) and Proteinase-K addition.

2.3. SEP1 gene amplification

The primer was designed based on the conserve region in *P. equestris.* The pair of oligonucleotides was *Forward* F1: (5'ATG GGA AGA GGG AGA GTG GA-3'), *Forward* F2: (5'GCT GAA GCG GAT CGA GAA CA-3') and *Reverse* R1: (5'TCA TGC AAG CCA ACC AGG TG-3'). PCR reactions were carried out in a total volume of 50 μL. *SEP1* gene was amplified using Qiagen Rotor-Gene Q. DFR gene amplification was done with 40 cycles of PCR which was initiated by template DNA initial denaturation at 94°C for 5 min, then followed by denaturation 94°C for 20 s, annealing at 56°C for 20 s, extension at 72°C for 50 s, and final extension at 72°C for 5 min. PCR product was examined using 1% agarose gel electrophoresis then checked using UV Transilluminator.

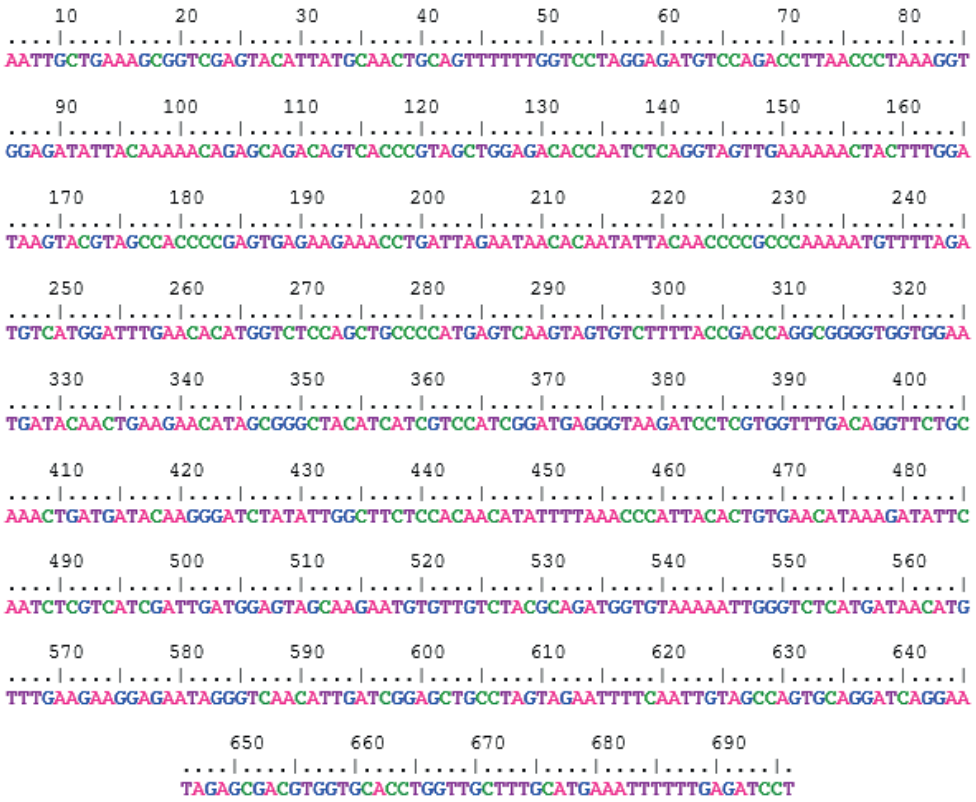

Figure 1: *SEP1 sequence from Phalaenopsis amabilis* (L.) Blume using F2/R1 oligonucleotides pair.

2.4. DNA sequence analysis

The sequencing of *SEP1* gene was carried out in *First* BASE *Laboratories,* Malaysia. DNA sequence were analyzed with *FinchTV* to read the chromatogram of sequencing product, *DNA Baser* to make a consensus sequence, *Basic Local Alignment Search Tool* (BLAST) to check the compatibility between target gene and query from *Gene* Bank, ClustalX to make multiple alignment between *SEP1* gene in *P. amabilis*, *P. equestris* and other species.

3. Result and Discussion

The targeted *SEP1* sequence from *P. amabilis* could not be amplified using F1/R1 oligonucleotides pair. Nevertheless, this study acquired *SEP1* sequence of 691 bp length using F2/R1 pair (Figure 1).

The acquired DNA sequence then compared to *SEP1* gene from *P. equestris* to determine their similarity index. BLAST analysis showed no similarity between *P. amabilis* and *P. equestris*. Mega 6 analysis showed 22.45% similarity between *P. amabilis* and *P. equestris*. *SEP1* encodes of the sepals, petals and labellum. The low similarity index caused the different shape of sepals, petals and labellum between *P. amabilis* and *P.*

(a) (b)

Figure 2: The differences between *P. amabilis* with *P. equestris*. (A) Flower of *P. amabilis* (B) Flower of *P. equestris*.

Species 1	Species 2	Dist	Std. Err	Similarity index (%)
P. amabilis	*P. equestris*	0.775	0.051	22.45326558
E. grandis	*P. amabilis*	0.885	0.062	11.48317698
N. nucifera	*P. amabilis*	0.922	0.066	7.772061
A. thaliana	*P. amabilis*	0.926	0.065	7.39735856
M. domestica	*P. amabilis*	0.935	0.066	6.540036
T. hassleriania	*P. amabilis*	0.937	0.067	6.275825
C. sativa	*P. amabilis*	1.076	0.080	−7.563754132

Table 1: Levels of gene similarity *SEPALLATA 1 Phalaenopsis amabilis (L.) Blume* with various gene *SEPALLATA 1* of several species.

equestris (Figure 2). The similarity index of *SEP1* sequences between *P. amabilis* and *P. equestris* is shown in Figure 3.

Alignment results showed that there are many differences between *SEP1* bases from *P. amabilis* and *SEP1* bases from *P. equestris*, including some discovered gaps. There are some gaps in 19th to 21st, 65th and 99th to 104th base of *SEP1* sequences between *P. amabilis* and *P. equestris*, and another gaps in 45th, 85th to 88th and 130th base. This study could not amplify the start codon of *P. amabilis* due to failed F2/ R1 amplification of which the start codon be.

SEP1 in *P. amabilis* was also compared to various species taken from Gene Bank databases, namely *Arabidopsis thaliana*, *Camelina sativa* (L.) Heynh., *Eucalyptus grandis* W. Hill ex Maiden, *Malus domestica* Mill, *Nelumbo nucifera* Gaertn. and *Tarenaya hassleriana* (Chodat) Iltis to analyze their similarity (Tabel 1).

4. Conclusion

The 691 bp length of *SEPALLATA 1* gene was isolated from *Phalaenopsis amabilis* (L.) Blume F2/R1 oligonucleotide pair. The start codon of the sequence could not be obtained. The similarity index between *SEPALLATA 1* gene in *Phalaenopsis amabilis* (L.) Blume and *Phalaenopsis equestris* (Schauer) Rchb.f is 22.45%.

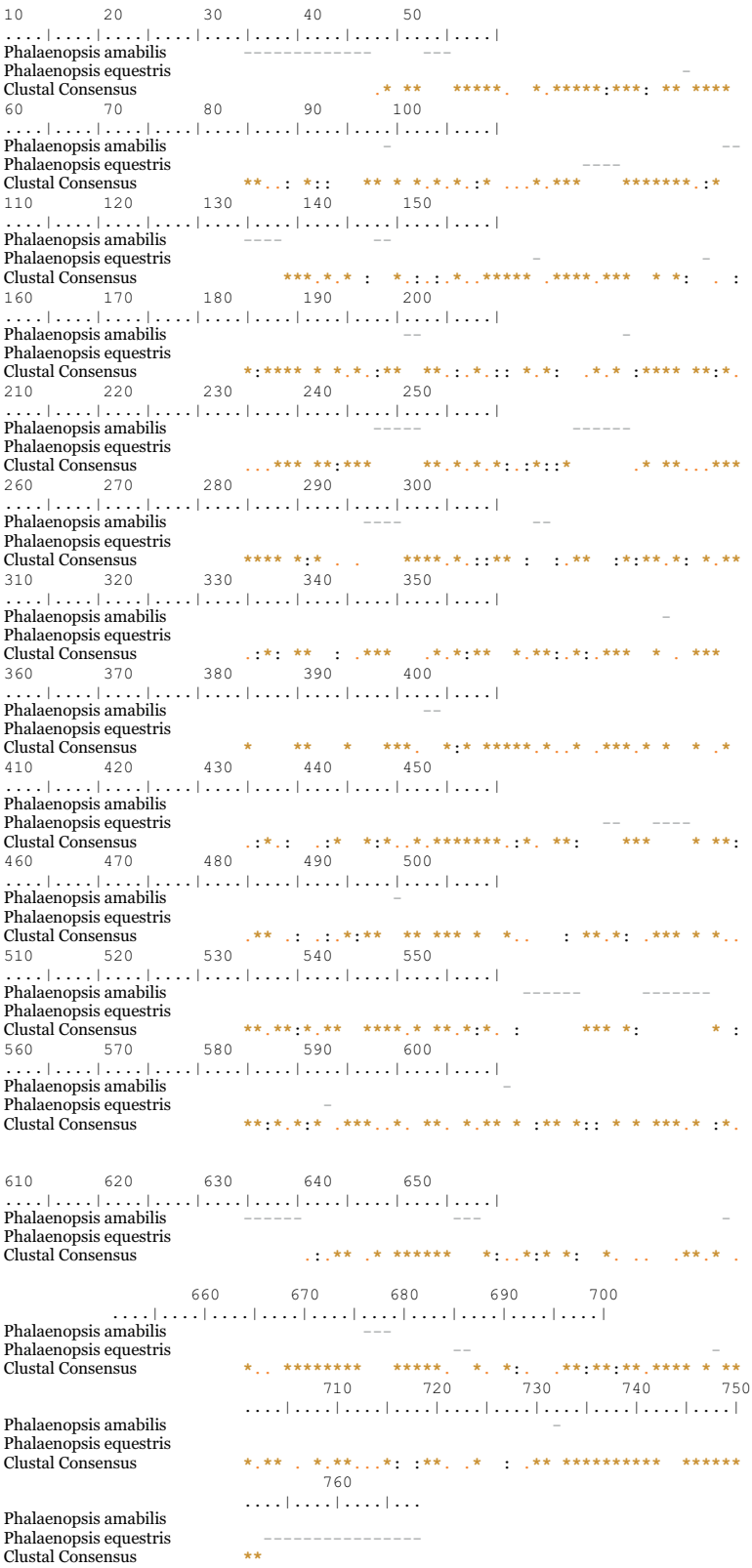

Figure 3: Sequence alignment of *SEPALLATA 1* from *P. amabilis* and *SEPALLATA 1* from *P. equestris;* (*): *conserved* gene sequence; (:): different gene sequence; (–): unknown gene sequence.

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References

[1] H. Yu and C. J. Goh, "Identification and characterization of three orchid MADS-box genes of the AP1/AGL9 subfamily during floral transition," *Plant Physiology*, vol. 123, no. 4, pp. 1325–1336, 2000.

[2] R. Peakall, "Speciation in the Orchidaceae: Confronting the challenges," *Molecular Ecology*, vol. 16, no. 14, pp. 2834–2837, 2007.

[3] T. Gunadi, *Anggrek dari bibit hingga berbunga. [Orchid from seed to flowering]*, PAI Bandung, Bandung, 1979, in Bahasa Indonesia.

[4] HL. Valmayor, *Orchidiana Philippiniana*, vol. 1, 1983, Los Banos: University of the Philippines.

[5] CA. Backer and VDB. Bachuizen, *Flora of Java (Vol III*, The, Netherland: Noodhoof. N.V.P, 1968.

[6] EB. Hidajat, "Morfologi tumbuhan. [Plant morphology]., Bandung," *Departemen Pendidikan dan Kebudayaan*, 1994.

[7] D. F. Ip, "Book Reviews : THE SOCIOLOGY OF MODERNIZATION AND DEVELOPMENT. David Harrison. London, Unwin Hyman Academic, 1988. 196 pp. $29.95 (paper)," *Journal of Sociology*, vol. 26, no. 2, pp. 244–246, 1990.

[8] R. Cui, J. Han, S. Zhao et al., "Functional conservation and diversification of class e floral homeotic genes in rice (Oryza sativa)," *Plant Journal*, vol. 61, no. 5, pp. 767–781, 2010.

[9] Z.-J. Pan, Y.-Y. Chen, J.-S. Du et al., "Flower development of Phalaenopsis orchid involves functionally divergent SEPALLATA-like genes," *New Phytologist*, vol. 202, no. 3, pp. 1024–1042, 2014.

[10] H. Ma, M. F. Yanofsky, and E. M. Meyerowitz, "AGL1-AGL6, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes," *Genes and Development*, vol. 5, no. 3, pp. 484–495, 1991.

[11] H. Huang, M. Tudor, C. A. Weiss, Y. Hu, and H. Ma, "The Arabidopsis MADS-box gene AGL3 is widely expressed and encodes a sequence-specific DNA-binding protein," *Plant Molecular Biology*, vol. 28, no. 3, pp. 549–567, 1995.

[12] M. A. Mandel and M. F. Yanofsky, "The Arabidopsis AGL9 MADS box gene is expressed in young flower primordia," *Sexual Plant Reproduction*, vol. 11, no. 1, pp. 22–28, 1998.

[13] L. M. Zahn, H. Kong, J. H. Leebens-Mack et al., "The evolution of the SEPALLATA subfamily of MADS-box genes: A preangiosperm origin with multiple duplications throughout angiosperm history," *Genetics*, vol. 169, no. 4, pp. 2209–2223, 2005.

[14] L. J. Cseke, S. B. Cseke, N. Ravinder et al., "SEP-class genes in Populus tremuloides and their likely role in reproductive survival of poplar trees," *Gene*, vol. 358, no. 1-2, pp. 1–16, 2005.

[15] S. Pelaz, G. S. Ditta, E. Baumann, E. Wisman, and M. F. Yanofsky, "B and C floral organ identity functions require SEPALLATTA MADS-box genes," *Nature*, vol. 405, no. 6783, pp. 200–203, 2000.

[16] O. A. Shulga, A. V. Shchennikova, G. C. Angenent, and K. G. Skryabin, "MADS-box genes controlling inflorescence morphogenesis in sunflower," *Russian Journal of Developmental Biology*, vol. 39, no. 1, pp. 2–5, 2008.

[17] G. Theißen, "Development of floral organ identity: Stories from the MADS house," *Current Opinion in Plant Biology*, vol. 4, no. 1, pp. 75–85, 2001.

[18] Y. Xu, L. L. Teo, J. Zhou, P. P. Kumar, and H. Yu, "Floral organ identity genes in the orchid Dendrobium crumenatum," *Plant Journal*, vol. 46, no. 1, pp. 54–68, 2006.

[19] Z.-X. Lu, M. Wu, C.-S. Loh, C.-Y. Yeong, and C.-J. Goh, "Nucleotide sequence of a flower-specific MADS box cDNA clone from orchid," *Plant Molecular Biology*, vol. 23, no. 4, pp. 901–904, 1993.