

ISOLASI DAN KARAKTERISASI GEN PEMBENTUK PIGMENT ANTOSIANIN PADA TANAMAN ANGGREK *Dendrobium* ‘Enobi’ DAN *Phalaenopsis* HIBRIDA BERBUNGA VARIGATA

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INTI SARI

Anggrek *Dendrobium* dan *Phalaenopsis* merupakan tanaman yang banyak dibudidayakan karena keindahannya. Bunga varigata dicirikan dengan adanya perbedaan warna pada satu bunga. Fenotip varigata yang menarik, dapat meningkatkan nilai komersial tanaman hias. Penelitian terkait gen yang berperan dalam pembentukan warna bunga menarik untuk diteliti dengan tujuan pemuliaan tanaman. Pigmen utama bunga berwarna ungu adalah antosianin. Salah satu gen kunci dalam biosintesis antosianin adalah *Chalcone Synthase* (*CHS*). Analisis gen *CHS* kemungkinan menunjukkan perubahan yang terjadi dalam proses pembentukan warna pada bunga, untuk itu sebagai pendukung dalam mengetahui cara kerjanya maka dilakukan analisis kadar pigmen antosianin. Dengan begitu, tujuan dari penelitian ini adalah mengetahui struktur gen *CHS* pada proses pembentukan warna bunga dengan menggunakan bunga *Dendrobium* ‘Enobi’ dan *Phalaenopsis* hibrida berbunga varigata. Metode yang digunakan pada penelitian ini: 1) pengamatan morfologi bunga, 2) isolasi genom DNA bunga, 3) amplifikasi gen *CHS* menggunakan *polymerase chain reaction* (*PCR*) dengan spesifik primer *CHS* F1 dan *CHS* R1, serta 4) analisis kadar pigmen antosianin. Hasil pengamatan morfologi bunga menunjukkan fenotip varigata telah muncul sejak kuncup baik pada *Dendrobium* ‘Enobi’ dan *Phalaenopsis* hibrida berbunga varigata. *Dendrobium* ‘Enobi’ berbunga varigata memiliki luas area putih yang lebih luas daripada ungu, sedangkan *Phalaenopsis* hibrida berbunga varigata memiliki luas area ungu yang lebih luas daripada putih. Perbedaan struktur gen *CHS* pada zona ungu dan putih *Dendrobium* ‘Enobi’ meliputi perbedaan panjang sekuens yaitu zona ungu 1213 bp; zona putih 1217 bp, mutasi di beberapa titik pada zona putih, letak motif protein yaitu PLN03170 *super family* zona ungu pada interval 118-825 bp; zona putih 156-923 bp, perbedaan ukuran asam amino zona ungu 507 asam amino; zona putih 557 asam amino, dan perbedaan letak motif asam amino PS00441 CHALCONE_SYNTH (*Chalcone and stilbene synthases active site*) zona ungu pada interval 295-331 bp; zona putih 93-109 bp. Sedangkan Perbedaan struktur gen *CHS* pada *Phalaenopsis* hibrida berbunga varigata meliputi perbedaan panjang sekuens yaitu zona ungu 1170 bp; zona putih 1200 bp, mutasi di beberapa titik pada zona putih, macam motif protein yaitu pada zona ungu PLN03173 *super family*; zona putih PHA 03247 *super family*, PLN03173 *super family*, PLN 03168 *super family*, PLN03170 *super family*, perbedaan ukuran asam amino zona ungu 454 asam amino 478 asam amino, dan letak motif asam amino PS00441 CHALCONE_SYNTH zona ungu pada interval 366-382 bp; zona putih 308-324 bp. Ketidakstabilan ukuran motif asam amino dan adanya mutasi di beberapa titik pada zona putih kemungkinan dapat menyebabkan tidak munculnya pigmen antosianin pada zona putih. Hal ini didukung dengan hasil analisis kadar pigmen antosianin yaitu terdapat perbedaan kadar antosianin yang berbeda signifikan antara zona ungu dan putih yang mempengaruhi perbedaan warna bunga, semakin tinggi kadar antosianin maka warna bunga semakin gelap.

Kata kunci: Anggrek, Antosianin, Bunga, *CHS*, *Dendrobium*, *Phalaenopsis*, Varigata

ISOLATION AND CHARACTERIZATION OF ANTHOCYANIN PIGMENT FORMING GENE OF VARIEGATED FLOWER IN *Dendrobium* ‘Enobi’ AND *Phalaenopsis* HYBRID ORCHID

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ABSTRACT

Dendrobium and *Phalaenopsis* orchids are plants which are widely cultivated due to their attractiveness. Variegated flowers are characterised by the presence of different colours in one flower. Attractive variegated phenotype can increase the commercial value of ornamental plants. Research related to genes that play a role in the formation of flower colour is interesting to examine for plant breeding. The main pigments of purple flowers are anthocyanins. One of the essential genes in anthocyanin biosynthesis is Chalcone Synthase (*CHS*). Analysis of the *CHS* gene may reveal alterations that occur in the process of colour formation in flowers; for this reason, an anthocyanin pigment content analysis was carried out as support in knowing how it operates. The objective of this study is to determine the *CHS* gene's structure in the flower colour formation process using variegated-flowered of *Dendrobium* ‘Enobi’ and *Phalaenopsis* hybrid. The methods applied in this study are: 1) floral morphology observation, 2) Genome DNA isolation, 3) *CHS* gene amplification using polymerase chain reaction (PCR) with specific primers *CHS* F1 and *CHS* R1, and 4) analysis of anthocyanin pigment content. The results of flower morphology observations indicated that the variegated phenotype had appeared since the buds in both *Dendrobium* ‘Enobi’ and *Phalaenopsis* hybrid variegated flower. *Dendrobium* ‘Enobi’ variegated flower has a wider white area than purple, while *Phalaenopsis* hybrid has a wider purple area than white. Differences in *CHS* gene structure in the purple and white zones of *Dendrobium* ‘Enobi’ variegated flower are different in sequence length, the purple zone has 1213 bp and white zone 1217 bp; mutations at several points in the white zone, different location of the protein motif PLN03170 superfamily in purple zone at intervals 118-825 bp and white zone 156-923 bp; different size of amino acids in purple zone has 507 amino acids and white zone 557 amino acids; and different location of the amino acid motif PS00441 CHALCONE_SYNTH (Chalcone and stilbene synthases active site) in purple zone at intervals of 295-331 bp and white zone 93-109 bp. Meanwhile, the different in the structure of *CHS* gene in *Phalaenopsis* hybrid variegated flower are different in sequence length, the purple zone has 1170 bp and white zone 1200 bp; mutations at several points in white zone; different types of protein motifs, in purple zone is PLN03173 super family and white zone are PHA 03247 superfamily, PLN03173 superfamily, PLN 03168 superfamily, PLN03170 superfamily; different size of amino acids in purple zone has 454 amino acids and white zone 478 amino acids; and different location of the amino acid motif PS00441 CHALCONE_SYNTH in purple zone at intervals 366-382 bp and white zone 308-324 bp. The instability of amino acids size, the amino acids motif, and the mutations at several points may cause the absence of anthocyanin pigments in white zone. This was supported by the analysis of anthocyanin pigment levels, that there with significant different in anthocyanin levels between the purple and white zones, which affect flower colour differences; the higher the anthocyanin content, the darker the flower colour.

Keywords: Orchid, Anthocyanin, Flower, *CHS*, *Dendrobium*, *Phalaenopsis*, Variegated