

Conference Paper

Isolation of Dihydroflavonol-4-reductase (DFR) Gene in *Dendrobium helix* cv. Pomeo Brown

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Abstract

Dendrobium helix cv. Pomeo Brown is one of the most attractive species in Spatulata section. This orchid is an epiphyte which has clustered stem, elliptic leaves, and erected flowers with brown petals. The brown color of *D. helix* cv. Pomeo Brown is due to the anthocyanin. Biosynthetic pathway of anthocyanin is regulated by several enzymes, including key enzyme dihydroflavonol-4-reductase (DFR) which is encoded by DFR gene. This study aimed to analyze the DFR gene from *D. helix* cv. Pomeo Brown by PCR using primer which were designed based on *Dendrobium moniliforme*. The sequences amplified using these primers could not be analyzed. Based on the sequence chromatograph, it can be suggested that the amplified DNA consists of several DFR fragments.

Keywords: *Dendrobium helix* cv. Pomeo Brown; anthocyanin; DFR gene.

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1. Introduction

Here *Dendrobium* is one of the important commercial orchids in Indonesia. *Dendrobium* is the subject of conventional breeding program for orchid cultivation. This genus comprises multiple colors and forms and is a major source of cut flowers and potted plants in the orchid market. One of the *Dendrobium* species which often used as a parental in pollination is *Dendrobium helix*. This species is the most attractive species of Spatulata section. It varies particularly in its flower color, attitude and the degree of its petal twisting. Several forms of *D. helix* have been introduced into cultivation in recent years. Pomeo Brown is a cultivar of *D. helix* spread widely in New Britain and has an epiphytic herb with clustered stem, elliptic leaves, and erected flowers with brown petals [1-4].

Color formation in a flower is due to the accumulation of pigment compounds, such as anthocyanin, which is the primary flower pigment in higher plants. Investigation of color formation in a flower is required for orchids breeding programs to improve the quality of the orchids [5-7]. It is quite difficult breeding orchid to obtain a new flower color conventionally because of the low probability of getting the desirable

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colors without changing the flower's other characteristics. Development in the plant molecular genetic could give an advantage to the breeding of a novel flower color using recombinant DNA technology [8]. One of the advantages is that the desirable flower color can be obtained without interfering its other characteristics using metabolic engineering of the anthocyanin biosynthetic pathway.

The improvement of the flower color using metabolic engineering requires molecular basis of genes, especially genes that involved in anthocyanin biosynthesis [9]. Several genes which encode the enzymes of anthocyanin biosynthetic pathway have been characterized including dihydroflavonol 4-reductase (DFR) gene. DFR gene encodes DFR enzyme that catalyses a key step in the late biosynthesis of anthocyanin [7, 10]. DFR enzyme activity has been described for a number of plant species. One of the most interesting characteristics concerning the known DFR enzymes is their substrate specificity. DFR proteins of many plants can accept dihydroflavonols with different hydroxylation patterns like dihydrokaempferol (DHK), dihydroquercetin (DHQ), and dihydromyricetin (DHM) as substrates [11–13]. On the other hand, DFR activities to convert one or more substrate of dihydroflavonols were said to be low in some postulated species. Although the DFR gene has been cloned and characterized in several orchids such as *B. finlaysonianana* [12] and *C. hybrida* [14], but there is still no report about DFR gene in *D. helix*. This paper aimed to describe DFR gene in *D. helix*.

2. Material and Method

2.1. Plant material

Dendrobium helix cv Pomeo Brown samples were collected from DD'Orchid Nursery, Dadaprejo, Batu, East Java, 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. DFR gene amplification

The primer was designed based on the conserve region in *Dendrobium moniliforme*. The pair of oligonucleotides was forward F1: (5'-ATG GAG AAT GAG AAG AAG GG-3') and reverse R1: (5'-TGC AGT GAT CAT GCT TGG TG-3'). PCR reactions were carried out in a total volume of 50 μ L. DFR gene was amplified using Qiagen Rotor-Gene Q. DFR gene amplification was done by 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.

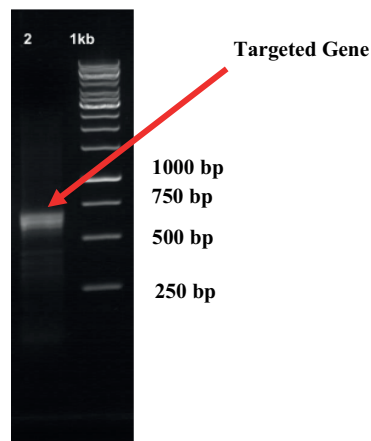


Figure 1: Result of gel electrophoresis.

2.4. DNA sequences and analysis

DFR gene sequencing was carried out in First BASE Laboratories, Malaysia. DNA sequence were analyzed using 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 with query from Gene Bank, ClustalX to make multiple alignments between DFR gene in *D. helix* cv. Pomeo Brown and another plant species.

3. Results and Discussions

The 630 bp of *DFR* gene sequence was amplified as a result (see Figure 1).

The chromatogram showed that the fragment was not specific due to the overlapping peak and abnormal structure of bases (poly G; poly C; poli A and poly T). This result indicated that there weresome amplified multiple fragments. Presumably there was multiallele possibility in *DFR* gene from *Dendrobium helix*. Some previous studies reported that *DFR* gene in some species was found as a gene cluster. In *Lotus japonicus*, *DFR* geneconsists of five locuses but it is spliced in *DFR4*. Therefore, it consists of six *DFR* genes [15]. On the other hand, *DFR* gene was found in a single gene like in *Ascocenda* spp. [9], *Brohmedia finlaysonianana* [12] and *Cymbidium hybrida* [14]. We assumed that the repetition of *DFR* gene amplification *D. helix* should be carried out using the same primer to make sure of multiallele possibility.

Sequence product in the repetitive amplification was 570 bp and the chromatogram showed that forward sequence was not specific (see Figure 2a) while reverse sequence was good (see Figure 2b).

The differences between the sequences products were presumably due to multi allele. This assumption was made based on the different substrates and metabolite products. The differences of anthocyanin component caused the variation of flower color [8, 16, 17]. This showed that DFR enzymes from varied flower color might have

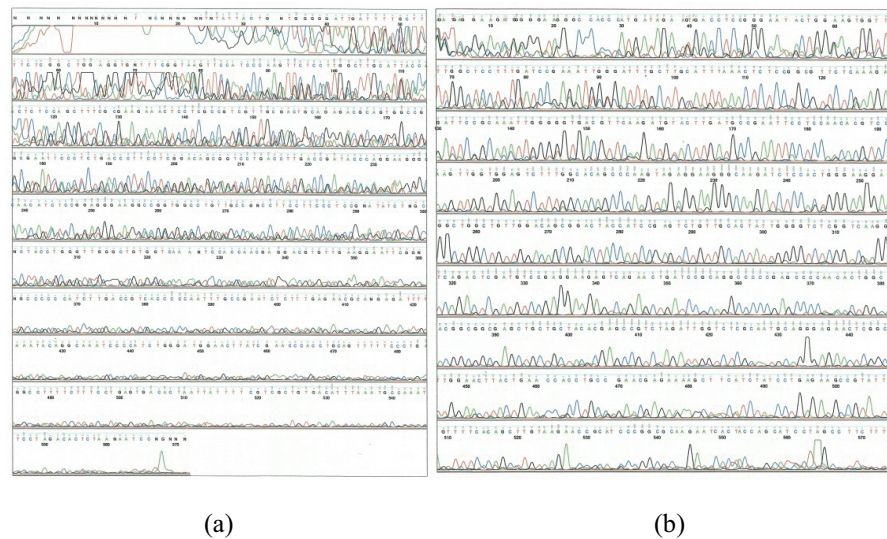


Figure 2: (a) The chromatogram of forward sequence, (b) The chromatogram of reverse sequence of DFR gene amplified from DNA genome of *D. helix*.

different structure. The difference of DFR enzymes catalyzed product is due to substrate differences [11]. The specific catalytic activity character of DFR enzyme is caused by amino acid residue in the specific active sites [18]. It was reported that most amino acid residue in plant species was Asparagine (Asn), while in another plant species, especially dicotyledone, most amino acid residue was Aspartate (Asp) [15]. The different amino acid residue in the specific active site is presumably due to the bases structure in specific *DFR* gene. There was a possibility that the bases structure in *DFR* gene was different since it did not only happen in the specific active site but also in other *DFR* gene part. The unspecific forward fragment of *DFR* gene in *D. helix* is presumably due to the different *DFR* gene domain with different amplified bases structure.

4. Conclusions

This study could not obtain agentic profile of *Dendrobium helix* cv "Pomeo Brown" because there was no consensus sequence analyzed. Presumably, there was multi allele in *DFR* gene isolated from *Dendrobium helix* cv. Pomeo Brown. Further study is needed to make sure of the presumptions.

Acknowledgements

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