Bioinformatics approach of predicted polyprenol reductase in Durian (Durio zibethinus Murr.)

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Abstract. Durio zibethinus Murr. (Malvaceae) is well known to produce secondary metabolites and has been described to contain several biological activities. The present study reports the bioinformatics approach to determine ten probably polyprenol reductase from durian (Durio zibethinus Murr.). The length of the gene was varied from 1313 to 1702 bp with protein length from 317 to 412 amino acid. Several physicochemical parameters of polyprenol reductase in D. zibethinus were varied among the genes observed. Based on stability coefficients, ten D. zibethinus genes were unstable proteins except for X5 variant. The genes were mostly stored in the plasma membrane, chloroplast thylakoid membrane, Golgi body, and endoplasmic reticulum (membrane). In contrast to this observation, a few genes existed to the microbody (peroxisome), mitochondrial inner membrane, and endoplasmic reticulum (lumen). To clarify the homology in the group of the polyprenol reductase gene in D. zibethinus, a dendrogram tree was constructed. A clustering showed that polyprenol reductase in D. zibethinus consisted of three branches. The present results indicated the prominence of understanding the disparity and function of physical and chemical features of the different amino acids in plant polyprenol reductase genes in D. zibethinus.

1. Introduction

Durian (Durio zibethinus Murr.) is a famous of the native Indonesian fruit plants. D. zibethinus widely distributed throughout the Malay Peninsula, Thailand, Malaysia, Indonesia, and New Guinea, and has been initiated to India, Sri Lanka, Myanmar, Vietnam and Jamaica [1]. Durian fruit is also called the king of fruit which is very popular with various groups of people in Indonesia because of its distinctive taste, especially the people in North Sumatra [2]. This fruit has a skin that resembles thorns and hard but also has a strong aroma of fruit flesh. Durian tree can grow both in the lowlands and highlands. Therefore this plant can be found in almost all districts/cities in North Sumatra [3].

D. zibethinus is well known to produce secondary metabolites and has been reported to contain several biological activities, for example, D. zibethinus peels extracts had the antidiabetic and antihypercholesterolemic activities [4]. Secondary metabolites such as polyisoprenoid alcohols are
recognized as polyprenol and dolichol concerning the isoprenoid structure [5-6] that are existing in all living organisms including in D. zibethinus. Furthermore, in the NCBI database, ten predicted polyprenol reductase from D. zibethinus had been deposited. This enzyme altered polyprenol compound to be dolichols in the polyisoprenoid biosynthesis [7]. Newly, expected polyprenol reductase and its bioinformatics information had been described from mangrove plant Kandelia obovata [8-9]. A work on bioinformatics of the plant polyprenol reductase from D. zibethinus is rarely studied. The present report thus purposed to analyze ten predictable polyprenols reductase genes in Durian using the bioinformatics method.

2. Materials and method

2.1. Materials

Ten probable polyprenol reductase genes from D. zibethinus placed in NCBI were obtained. The NCBI accession of the DNA and amino acid sequence was employed in this research as following. D. Zibethinus polyprenol reductase-2 like (LOC111293122) transcript variant X2 (XP_0227521604, XM_022885869), D. zibethinus polyprenol reductase (LOC111293122) transcript variant X1 (XP_022752159, XM_022896424), D. zibethinus polyprenol reductase 2-like (LOC111293122), transcript variant X6 (XP_022741608, XM_022885873), D. zibethinus polyprenol reductase 2-like (LOC111293122), transcript variant X5 (XP_022741607, XM_022885872), D. zibethinus polyprenol reductase 2-like (LOC111293122), transcript variant X4 (XP_022741606, XM_022885871), D. zibethinus polyprenol reductase 2-like (LOC111293122), transcript variant X3 (XM_022885870, XP_022741605), D. zibethinus polyprenol reductase 2-like (LOC111293122), transcript variant X1 (XP_022741603, XM_022885868), D. zibethinus polyprenol reductase 2-like (LOC111282724) (XP_022726695, XM_022870960), D. zibethinus polyprenol reductase 2-like (LOC111300810), transcript variant X3 (XP_022752162, XM_022896427), and D. zibethinus polyprenol reductase 2-like (LOC111300810), transcript variant X2 (XP_022752160, XM_022896425).

2.2. Physic and chemical features of the polyprenol reductase gene

To determine the composition, physical and chemical features in ten polyprenol reductase genes, ProtParam website was employed as previously termed [8]. The calculated elements illustrate the molecular weight, theoretical isoelectric point rates, amino acid structure, atomic configuration, extinction coefficient, projected half-life, instability index, fat coefficient, as well as typical hydrophilicity as earlier demonstrated [8].

2.3. Prospective allocation of peptide and subcellular localization of polyprenol reductase gene

To calculate transport peptide, the target P1.1 website accessible was applied. The site is based on the projected presence of any of the N-terminal pre-sequences chloroplast transport peptide (cTP), mitochondrial targeting peptide (mTP) as well as secretory pathway signal peptide (SP). In addition, PSORT Prediction was employed to define the subcellular localization of polyprenol reductase genes as precedingly reported [8].

2.4. Phylogenetic analysis of ten polyprenol reductase

The NCBI accessing numbering of the amino acid sequence of D. zibethinus was applied to this observation as previously mentioned in Materials subsection. Phylogenetic examination of deduced amino acid alignment from D. zibethinus polyprenol reductase genes was performed using CLUSTAL W ver. 1.83 [10] of the DDBJ tracked by depiction with TreeView ver. 1.6.6 [11] according to a neighbor-joining method. Bootstrap investigation with 1000 repetition was employed to measure the strong point of the nodules in the dendrogram [12].
3. Results and Discussions

3.1. Physicochemical features of the polyprenol reductase gene

Numerous line considerations of physicochemical polyprenol reductase in *D. Zibethinus* is illustrated in Table 1. The polyprenol reductase in Durian comprises of ten variations: X1, X1.XM, X2, X2.XM, X3, X3.XM, X4, X5, X6, and XM. The length of the genes was varied with the genes observed: 1313-1702 bp. Molecular weights were 111416.22 to 144862.56. It is remarkable the variety of corresponding theoretical isoelectric point values, the total atomic numbering, scattering coefficient, instability coefficient, and common mean hydropathicity together with the studied genes (Table 1).

The calculated half-life time interval was varied along the genes, X1.XM (1.2 h), X1-X6 (4.4 h), and X5 and XM (30 h). This variation result was similar those reported from oxidosqualene cyclase genes (4.4 h) [12], mangrove actin genes (1.3-30 h) [13]. *Kandelia obovata* polyprenol reductase gene (1.2-30 h) [8-9]. The probable half-life of polyprenol reductase in this work, therefore, was comparable to polyprenols reductase from other preponderances of plants.

According to stability coefficients, entirely genes were non-stable proteins, except for X5 variant. A small number of plant polyprenol reductase genes have been reported as stable genes in Euphorbiaceae tribe, for instance, *Glycine max*, *G. arboretum*, and *G. raimondii* [15]. Stable genes also have been depicted in oxidosqualene cyclase genes [12], actin genes from mangrove plants [13]. These findings indicated that the significant facts for difference and character of physicochemical features of the divergent amino acids in *D. zibethinus* polyprenol reductase genes.

Searching to *D. zibethinus* enzyme through KEGG (Kyoto Encyclopedia of Genes and Genomes) databases (https://www.genome.jp/kegg-bin/get_htext#A1) found seven enzymes, namely oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, and translocases. Polyprenol reductase belongs to oxidoreductases. In the database, polyprenol reductase included in performing on the CH-CH group of donors with NAD+ or NADP+ as an acceptor in the form of polyprenol reductase 2-like or isoform X1. The function of this enzyme is still an open question.

| *D. zibethinus* gene | X1 | X1.XM | X2 | X2.XM | X3 |
|----------------------|----|-------|----|-------|----|
| Length of genes/bp   | 1702 | 1697 | 1639 | 1697 | 1609 |
| Molecular weight     | 144862.56 | 144349.58 | 139621.90 | 144423.66 | 136602.29 |
| Theoretical isoelectric point values | 4.99 | 4.98 | 5.00 | 4.98 | 5.01 |
| Total number of atoms | 18764 | 18592 | 18070 | 18603 | 17702 |
| Extinction coefficient | 20250 | 21875 | 19875 | 21875 | 18875 |
| Half-life period     | 4.4h | 1.2h | 4.4h | 4.4h | 4.4h |
| Instability coefficient | 42.43 | 48.19 | 43.11 | 48.24 | 41.52 |
| Aliphatic index      | 25.97 | 24.69 | 25.81 | 24.63 | 26.29 |
| Grand average of hydropathicity | 0.619 | 0.639 | 0.624 | 0.637 | 0.619 |

| *D. zibethinus* gene | X3.XM | X4 | X5 | X6 | XM |
|----------------------|-------|----|----|----|----|
| Length of genes/bp   | 1690 | 1546 | 1472 | 1481 | 1313 |
| Molecular weight     | 143815.96 | 131361.62 | 125100.41 | 125984.21 | 111416.22 |
| Theoretical isoelectric point values | 4.98 | 5.01 | 5.03 | 5.03 | 5.04 |
| Total number of atoms | 18526 | 17008 | 16239 | 16358 | 14396 |
| Extinction coefficient | 21750 | 18500 | 16875 | 16875 | 15750 |
| Half-life period     | 4.4h | 4.4h | 30h | 4.4h | 30h |
| Instability coefficient | 48.19 | 42.20 | 40.61 | 41.19 | 45.06 |
| Aliphatic index      | 24.62 | 26.13 | 25.56 | 25.86 | 23.84 |
| Grand average of hydropathicity | 0.636 | 0.625 | 0.611 | 0.589 | 0.575 |

Table Continued
3.2. Prospective transport of peptide and subcellular localization of polyprenol reductase gene

Table 2 describes the viewpoint of the possible allocation peptide in the possibility of the expected transform peptide in *D. zibethinus* polyprenol reductase genes. The dependability were defined: chloroplast transit peptide, mitochondrial target peptide, the indicator peptide of the secretory pathway, and the likelihood view. The regulators of chloroplast peptide nevertheless no sign peptide were comparatively intermediate rate, possessed that occasional chloroplast transit peptide or somewhat in advancement show peptide of excretion pathway in Durian. It is conspicuous that mitochondria target peptide assessment distinguished from 0.017 to 0.164, signifying that is estimated to be present.

| Nucleotide ID | Reliability | Chloroplast transit peptide | Mitochondrial target peptide | Signal peptide of secretory pathway | Reliability prediction |
|---------------|-------------|-----------------------------|------------------------------|------------------------------------|------------------------|
| X1            | 0.161       | 0.017                       | 0.880                        | 2                                  |
| X1.XM         | 0.363       | 0.116                       | 0.022                        | 5                                  |
| X2            | 0.161       | 0.017                       | 0.880                        | 2                                  |
| X2.XM         | 0.401       | 0.105                       | 0.020                        | 5                                  |
| X3            | 0.161       | 0.017                       | 0.880                        | 2                                  |
| X3.XM         | 0.401       | 0.105                       | 0.020                        | 5                                  |
| X4            | 0.161       | 0.017                       | 0.880                        | 2                                  |
| X5            | 0.468       | 0.164                       | 0.042                        | 4                                  |
| X6            | 0.267       | 0.037                       | 0.215                        | 5                                  |
| XM            | 0.335       | 0.138                       | 0.058                        | 5                                  |

The maximum mitochondrial objective peptide was X5 variant, a *D. zibethinus* polyprenol reductase gene. The little assessment of mitochondrial mark peptide was opposing to preceding results on the centered mitochondrial target peptide midst the cycloartenol genes from mangrove plants [12], actin genes from mangroves [13], and mangrove species *Kandelia obovata* polyprenol reductase [7-8]. Nevertheless, the small worth was comparable points found in reported plant polyprenol reductase [15]. It is remarkable that consistency expectation (2-5) was slightly developed rate associating to triterpene synthase genes from mangrove trees (2-4) [12], actin genes from mangroves (2-3) [13], and probably polyprenol reductase genes from *K. obovata* (4-5) [7-8].

Table 3 displays the subcellular localization of polyprenol reductase genes in *D. zibethinus*. The subcellular localization of ten genes has stored in the plasma membrane, chloroplast thylakoid membrane, Golgi body, endoplasmic reticulum (ER-membrane and lumen), microbody (peroxisome), and mitochondrial inner membrane. It is surprising to note that only one gene (X1.XM) had no place in the plasma membrane (Table 3). Similarly, all the genes resided in chloroplast membrane except for XM. Other localizations such as Golgi body or endoplasmic reticulum (ER-membrane) shows variation among the genes observed (Table 3). The polyprenol reductase genes from *D. zibethinus* localized in Golgi body or endoplasmic reticulum agreed on previous results [16], where the zibethinins, are deposited in swellings of the ER and Golgi body which develop to protein storage vacuoles [16]. Furthermore, the polyprenol reductase gene, XM was distinguishable to store only in the ER lumen. On the other hand, two genes, X1.XM and X2.XM resided in the mitochondrial inner membrane (Table 3).
### Table 3. Subcellular localization of predicted polyrenol reductase in *D. zibethinus*

| Nucleotide ID | Plasma Membrane | Chloroplast Membrane | Golgi Body | Endoplasmic Reticulum (membrane) | Microbody (Peroxisome) | Mitochondrial Inner Membrane | Endoplasmic Reticulum (Lumen) |
|---------------|-----------------|----------------------|------------|---------------------------------|------------------------|------------------------------|-------------------------------|
| X1            | 0.600           | 0.451                | 0.400      | 0.300                           | nd                     | nd                           | nd                            |
| X1.XM         | nd              | 0.311                | nd         | 0.748                           | 0.622                  | nd                           | nd                            |
| X2            | 0.600           | 0.451                | 0.400      | 0.300                           | nd                     | nd                           | nd                            |
| X2.XM         | 0.440           | 0.311                | nd         | 0.748                           | 0.622                  | nd                           | nd                            |
| X3            | 0.600           | 0.451                | 0.400      | 0.300                           | nd                     | nd                           | nd                            |
| X3.XM         | 0.440           | 0.311                | nd         | 0.850                           | 0.748                  | nd                           | nd                            |
| X4            | 0.600           | 0.451                | 0.400      | 0.300                           | nd                     | nd                           | nd                            |
| X5            | 0.600           | 0.451                | 0.400      | 0.300                           | nd                     | nd                           | nd                            |
| X6            | 0.600           | 0.720                | 0.400      | 0.300                           | nd                     | nd                           | nd                            |
| XM            | 0.640           | nd                   | 0.460      | 0.685                           | nd                     | nd                           | 0.100                         |

#### 3.3. Phylogenetic tree of polyrenol reductase gene and potency of Durian fruit

To verify the homology amongst the polyrenol reductase gene in Durian, a clustering was formed (Figure 1). The similarity among polyrenol reductase of *D. zibethinus*, varies from 51-100%, correspondingly. These DNA sequences coded 317-412 amino acids excesses for ten circumstances, respectively. The phylogenetic tree characterizes three branches; where *D. zibethinus_XP 022741607* stood alone with another branch *D. zibethinus_XP 022741606*. The largest branch consists of eight polyrenol reductase genes (Figure 1).

Among the Malvaceae family in the NCBI database, there are forty-one polyrenol reductase genes, among them, genus *Gossypium* has the largest (24 entries), followed by *Durio* with ten genes, *Herrania* (4 polyrenol reductase genes), and three genes belong to *Theobroma*. The occurrence of abundance dolichols in *D. zibethinus* was reinforced by the earlier reports on true and associate mangroves [6-9]. The enzyme shifted the polyrenol to dolichols in the polysoprenoid biosynthesis. The occurrence of polyrenol reductase may correlate with the presence of dehydodolichyl diphosphate synthase from *D. zibethinus* in the database.

Very newly it has been termed that salt stress alters the polysoprenoid concentrations in mangrove plants [6]. The change of polysoprenoids supported the accepted observations on the increase of polysoprenoid upon abiotic and biotic tolerance [6]. It has been reported that Durian seeds have been shown to contain a complex of secondary metabolites [17], in addition to a potential worth supplementary food constituents and bioactive compounds in preference to removal as excess [17].

As exotic seasonal fruit especially in North Sumatra, Durian has been reported to have a source of food and high nutritional value, as well as a prospective therapeutic agent such as antioxidant [18]. Durian also had the local-wisdom uses as a drug and pharmaceutical characteristics. The *D. zibethinus* tree, for example, fruits, hulls, leaves, and roots has long time been used to delight numerous kinds of illnesses and collective diseases in human [18]. Pharmacologically Durian fruit has been shown to affect innumerable components of metabolic syndromes, such as against anovulation and menstrual disturbances [19]. Furthermore, Durian fruit intake has also been described to reduce hyperlipidemia, hyperglycemia, cardiovascular syndromes, and inflammation together with oxidative stress [20].
Figure 1. Clustering of ten polyprenols reductase in D. zibethinus. Dendrogram of deduced amino acid sequences was done with the neighbor-joining approach of the CLUSTAL W [10]. The specified measure signifies 0.1 amino acid switch each place. Numbers show bootstrap assessment from 1000 measurements [12]. The NCBI locus numbers of the amino acid sequence applied this examination are mentioned in the Materials subsection.

4. Conclusions
The current work clarified the prominence of thoughtful the disparity and character of physicochemical features of the divergent amino acids in plant polyprenol reductase genes in D. zibethinus. The subcellular localization in ten polyprenol reductase genes has stored in the plasma membrane, chloroplast thylakoid membrane, Golgi body, endoplasmic reticulum (ER-membrane and lumen), microbody (peroxisome), and mitochondrial inner membrane.

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