Bioinformatics approach of polyprenol reductase in *Hevea brasiliensis*

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**Abstract.** *Hevea brasiliensis* Mull. Arg. (rubber plant) is now used utterly for the commercial production of natural rubber, mainly of cis-1,4-polyisoprenoid. The current study assesses the bioinformatics approaches to assay four probably polyprenol reductase genes from *Hevea brasiliensis* on NCBI database as well as expected the physicochemical, subcellular localisation, and phylogenetic of polyprenol reductase. Several parameters of physicochemical polyprenol reductase in *H. brasiliensis* were varied among the genes observed. The predictable half-life of polyprenol reductase in this study was similar to polyprenols reductase from other majorities of plant species. Based on stability coefficients, there were no stable proteins; all polyprenol reductase genes were non-stable proteins. It is notable that mitochondria target peptide value diverse from 0.053 to 0.101, signifying that is expected to be a presence. To clarify the homology in the midst of the polyprenol reductase gene in Euphorbiaceae family, a dendrogram tree was constructed. The close relationship among polyprenol reductase genes: *environs may interpret* Kandelia obovata, Ricinus communis, Manihot esculenta with rubber plant in the polyprenol reductase environs in the tropical rain forests. The present results indicated the prominence of understanding the variation and role of physical and chemical characteristics of the distinct amino acids in plant polyprenol reductase genes in *H. brasiliensis*.

1. **Introduction**

Over 2500 plant species are recognised to yield natural rubber consists mainly of cis-1,4-polyisoprenoid; however, only rubber plant (*Hevea brasiliensis* Mull. Arg.) is now used exclusively for the commercial production of natural rubber [1]. *H. brasiliensis* is widely studied for their biosynthesis, structural characterisation, breeding, and biological activity [2-4]. Polysiprenoids have been reported in various tissues of *H. brasiliensis* [5-6]. Polysiprenoids have two kinds of polysiprenoids concerning the α-isoprene construction: polyprenol (α-unsaturated isoprenoid alcohols) and dolichol (α-saturated isoprenoid alcohols). Polyprenols were found to be dominated over dolichols in nascent leaves of young *H. brasiliensis* shoots [5], containing shorter (C₅₀-C₆₅) and longer...
polyprrenols (up to C$_6$) [5-6]. By contrast, dolichols were predominated in _H. brasiliensis_ seeds, young shoots, young roots, and young leaves [6].

Despite abundance polyisoprenoids in _H. brasiliensis_, limited studies have been focused on polyprrenol reductase occurred in the rubber plant. Polyprrenol reductase has been shown to change polyprrenol to dolichols in the dolichol biosynthetic [7]. Recently, predicted polyprrenol reductase and its bioinformatics data had been reported from _Kandelia obovata_ [7-8]. Thus the current study purposed to evaluate the bioinformatics analysis on projected polyprrenol reductase in _H. brasiliensis_.

2. Materials and method

2.1. Materials

Four projected polyprrenol reductase genes from _H. brasiliensis_ stored in NCBI were gathered. The NCBI locus numbers of the DNA and amino acid sequences utilised in this study are in this way: XM\_021812510 and XP\_021668202 (_H. brasiliensis_ polyprrenol reductase 2-like, transcript variant X1), XM\_021812518 and XP\_021668201 (_H. brasiliensis_ polyprrenol reductase 2-like, transcript variant X2), XM\_021812527 and XP\_021668219 (_H. brasiliensis_ polyprrenol reductase 2-like, transcript variant X3), XM\_002495052 (_H. brasiliensis_ polyprrenol reductase 2-like, transcript variant X4).

2.2. Physical and chemical characteristics of the polyprrenol reductase gene

Protparam online (web.expasy.org/protparam/) was used to determine the structure, physicochemical characteristics of polyprrenol reductase genes from _H. brasiliensis_. The calculated factors designate the molecular mass, theoretical isoelectric point values, total number of atoms, extinction coefficient, predictable half-life, instability index, aliphatic index, and grand average hydropathicity as earlier reported [9].

2.3. Possible transfer of peptide and subcellular localisation of polyprrenol reductase gene

To predict transit peptide, the target P1.1 server online (www.ebs.dtu.dk/services/targetp/) was applied. The position is according to the expected existence of any of the N-terminal pre-sequences chloroplast transit peptide (cTP), mitochondrial targeting peptide (mTP), and secretory pathway signal peptide (SP). Additionally, PSORT (protein subcellular localisation prediction tool) prediction online (psort.hgc.jp/form.html) was employed to control the subcellular determination of polyprrenol reductase genes as earlier termed [10].

2.4. Phylogenetic analysis of polyprrenol reductase from Euphorbiaceae family

The NCBI locus numbers of the sequence of Euphorbiaceae family used this investigation in this manner: _Hevea brasiliensis_ X2 (XM\_021812518), _H. brasiliensis_ X1 (XM\_021812510), _H. brasiliensis_ X3 (XM\_021812527), _Manihot esculenta_ X3 (XM\_021755036), _M. esculenta_ X2 (XM\_021755035), _M. esculenta_ X1 (XM\_021755034), _Ricinus communis_ X3 (XM\_021755036), _R. communis_ X2 (XM\_015715302), _R. communis_ X1 (XM\_002512961), _Jatropha curcas_ X8 (XM\_012210100), _J. curcas_ X7 (XM\_020677198), _J. curcas_ X6 (XM\_020677193), _J. curcas_ X5(XM\_020677189), _J. curcas_ 4 (XM\_012210077), _J. curcas_ 3 (XM\_012210077), _J. curcas_ 2 (M\_012210083), and _J. curcas_ X1(XM\_012210091).

3. Results and Discussions

3.1. Physicochemical characteristics of the polyprrenol reductase gene in _H. brasiliensis_

Table 1 depicts the several parameters of physicochemical polyprrenol reductase in _H. brasiliensis_. The length of the genes was alternated with the genes ascertained. Several lines of coded amino acids were 320 to 339. It is important to note the heterogeneity of relative molecular weight, theoretical
isoelectric point value, the total number of atoms, extinction coefficient, instability coefficient, and general average hydropathicity along with the analysed genes.

The approximated half-life interval was no significant difference amidst the genes and was shown much longer that triterpene and cycloartenol synthase genes [9], mangrove actin genes [10], K. obovata polypropenol genes [8]. However, the predictable half-life of polypropenol reductase in this study was similar to polypropenol reductase from other majorities of plant species [11]. Based on stability coefficients in the present results, there were no stable proteins, and all genes were non-stable proteins (Table 1). A few genes have been reported as stable genes from plant polypropenol reductase genes, such as Glycine max, G. arboreum, and G. raimondii [11]. Established genes also have been described in β-amyrin synthase (BgAS) from Bruguiera gymnorrhiza and cycloartenol synthase (ReCAS) from Rhizophora stylosa [9], mangrove actin genes: B. gymnorrhiza BgAct1, KcAct1 from K. candel, and RsAct1 from R. stylosa [10]. The present results indicated the significance of insight the variation and role of physical and chemical characteristics of the altered amino acids in plant polypropenol reductase genes [11].

Table 1. Physicochemical characteristics of the H. brasiliensis polypropenol reductase

| H. brasiliensis variant | X1 | X2 | X3 | X4 |
|------------------------|----|----|----|----|
| Length of genes/bp     | 1475 | 1396 | 1495 | 1280 |
| Number of encoded amino acids | 339 | 339 | 320 | - |
| Molecular weight       | 125460.57 | 118224.85 | 127236.51 | 109039.56 |
| Theoretical isoelectric point values | 4.94 | 5.03 | 5.01 | 5.03 |
| Total number of atoms  | 16178 | 15290 | 16412 | 14028 |
| Extinction coefficient  | 18750 | 16625 | 19000 | 16875 |
| Half-life period       | 7.2h | 30h | 4.4h | 4.4h |
| Instability coefficient| 46.67 | 41.17 | 46.26 | 50.29 |
| Aliphatic index        | 25.42 | 25.21 | 25.35 | 24.92 |
| Grand average of hydropathicity | 0.649 | 0.603 | 0.644 | 0.661 |

3.2. Possible transfer of peptide and subcellular location

Table 2 displays the likelihood of the potential transfer peptide in the probability of the promising transfer peptide in H. brasiliensis polypropenol reductase genes. Four reliabilities were determined: chloroplast transit peptide, mitochondrial target peptide, the signal peptide of the secretory pathway, and the prediction prospect. The charges of chloroplast peptide however no indication peptides were short, shown that different chloroplast transit peptide or comparatively great indicator peptide of secretion pathway in rubber tree polypropenol reductase genes. It is notable that mitochondria target peptide value diversified from 0.053 to 0.101, signifying that it is expected to be a presence. The uppermost mitochondrial target peptide was X2 variant, an H. brasiliensis polypropenol reductase gene. This consequence well agreed with the prior report on the maximum mitochondrial target peptide amongst the phytosterol genes from mangrove trees was detected in KcCAS, K. candel cycloartenol synthase gene [9].

Table 2. The expectation of the potency transit peptide in H. brasiliensis

| H. brasiliensis variant | Reliability |
|------------------------|-------------|
| Chloroplast transit peptide | Mitochondrial target peptide | Signal peptide of secretory pathway | Reliability prediction |
| X1                     | 0.076       | 0.053       | 0.102       | 5       |
| X2                     | 0.174       | 0.101       | 0.115       | 5       |
| X3                     | 0.079       | 0.054       | 0.115       | 5       |
| X4                     | 0.079       | 0.054       | 0.115       | 5       |
Table 3 shows the subcellular location of polyprenl reductase genes in *H. brasiliensis*. The subcellular localisation of these genes frequently existed in the plasma membrane and the Golgi body. Only two variants (X1 and X2) were located in the endoplasmic reticulum (membrane and lumen). The X3 was a presence in chloroplast thylakoid membrane and mitochondrial inner membrane, the X3 was found in the mitochondrial inner membrane and mitochondrial intermembrane space (Table 3). Very newly, it has been reported that the expressed gene of two triterpene synthases, *BgbAS* and *RsM1* increased the triterpene concentration of plasma membrane portions along with traced in the plasma membrane [9, 13]. Likewise, it has been accepted interpretations that the plasma membrane is the first defence counter to conversions in the physical chemical adaptable to the changing environs [14]. A number of salt tolerance genes from *R. stylosa* localised in the plasma membrane sustained the earlier documents on the prominence of the lipid bilayer for abiotic stress tolerance along with salinity stress [10].

The variation of subcellular was worthy among the genes detected, expressing that the significant role of a secondary composition of plant polyprenl reductase in the plant family. Polyprenl may involve an essential function in the early stages of protein N-associated glycosylation, and the enzyme was needed for the changing of polyprenl to dolichol [8].

| *H. brasiliensis* variant | Plasma Membrane | Golgi Body | endoplasmic reticulum (membrane) | endoplasmic reticulum (lumen) |
|--------------------------|------------------|------------|----------------------------------|-------------------------------|
| X1                       | 0.640            | 0.460      | 0.685                            | 0.100                         |
| X2                       | 0.640            | 0.460      | 0.685                            | 0.100                         |
| X3                       | 0.600            | 0.400      | nd                               | nd                            |
| X4                       | 0.600            | 0.400      | nd                               | nd                            |

**Table 3.** Subcellular localisation of *H. brasiliensis* polyprenl reductase gene

| *H. brasiliensis* variant | Chloroplast thylakoid membrane | Mitochondrial inner membrane | Mitochondrial intermembrane space |
|--------------------------|--------------------------------|------------------------------|----------------------------------|
| X1                       | nd                             | nd                           | nd                               |
| X2                       | nd                             | nd                           | nd                               |
| X3                       | 0.441                          | 0.365                        | nd                               |
| X4                       | nd                             | 0.681                        | 0.331                            |

*nd* = not detected

3.3 Phylogenetic examination of polyprenl reductase gene

To clarify the homology between the polyprenl reductase genes in Euphorbiaceae family, a dendrogram was constructed. The polyprenl reductase 2-like (LOC110656001) of *H. brasiliensis*, variants X1, X2, X3, and X4 sequences were 1475, 1396, 1495 and 1280 bp, correspondingly. These DNA sequences encoded 339, 339, and 320 amino acids residues for three circumstances, respectively. Variant X1 and X2 shared 95% identities in their amino acid sequences and 100% between X1 and X3 in their amino acid sequences. X1 and X4, X3 and X4 displayed high homologies in amino acid (96 %). Variant X2 had 95% similarity in amino acid with X3; variant X2 and X4 depicted the lowest homology.

The clustering tree depicts that there are three branches of the tree. The rubber plant was situated in the main branch. It is interesting to note that polyprenl reductase of *H. brasiliensis* joined with four *Ricinus communis* and three *Manihot esculenta* polyprenl reductases. This similar pattern was found in *K. obovata* polyprenl reductase which also close to *R. communis* [7]. The distribution of polyisoprenoid in *R. communis* contained shorter polyprenl (C60-C65), no found dolichol in leaves. In contrast to this observation, dolichol with chain length (C80-C90) and no polyprenels detected in the roots [12].
The close relationship among polyprenol reductase genes: *their environments can show K. obovata, R. communis, M. esculenta* with rubber plant as mentioned previously in the polyprenol reductase in the tropical rain forests [7-8, 12]. The obvious homology and connection these genes in the phylogenetic tree may be the consequence of tropical atmospheric [12, 15]. The perennial plant such as *H. brasiliensis* is widespread in the tropical zone along with *R. communis* in the coastal plant, *K. obovata* of mangrove species [12, 15].

**Figure 1.** Clustering tree of plant polyprenol reductase from Euphorbiaceae together with three predicted polyprenols reductase from *H. brasiliensis*. The directed scale epitomises 0.1 amino acid replacements each location. Numerals are showing bootstrap assessment from 1000 duplicates. The NCBI locus numbers of the amino acid sequence of analyses are described in the Materials and method section.
4. Conclusions
This study clarified the variation and role of physical and chemical characteristics of the *H. brasiliensis* polyprenol reductase genes. Our report promoted preceding studies on the subcellular location of salt tolerance genes positioned in the plasma membrane. The detectable similarity and joining polyprenol reductase genes of *H. brasiliensis* in the dendrogram may be the outcome of tropical environmental.

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