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Isolation, Structural Studies and Homology Modelling of 6-Phosphogluconate Dehydrogenase in Coconut

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ABSTRACT: 6-phosphogluconate dehydrogenase (6PGD) is an important protein present in both eukaryotes and prokaryotes. The key role of this protein is that its acts as an enzyme in the conversion of 6-phosphogluconate (6PGD) to ribulose 5-phosphate (R5P) in oxidative pentose phosphate pathway (OPPP). All higher plants possess different isoforms of 6PGD in cytosol and plastid. Since the structure of this protein is not known in coconut, a study was carried out to investigate the primary, secondary and tertiary structure of 6PGD. NAD binding 2 domain and 6PGD domains were identified as conserved domains. *pI* (isoelectric point) value was found as 6.4 in acidic character, while the instability index (II) was found as 25.04 with accepted stable protein. Secondary structure analysis revealed high percentage of α-helices. The 3D structure of protein was deduced using homology modelling with SWISS-MODEL. The accuracy of the predicted 3D structure checked using Ramachandran plot analysis showed that 96.2% of the residues were in favoured region. The result of this study contributes to understanding of 6PGD protein structure in coconut and will be useful for the further study of protein-biomolecular interactions.

KEYWORDS*:* Coconut, 6-PGD (6-phosphogluconate dehydrogenase), Homology modeling, 3D structure

I. INTRODUCTION

6-phosphogluconate dehydrogenase (6PGD) is an enzyme in the oxidative pentose phosphate pathway (PPP). In higher plants, the oxidative pentose phosphate pathway operates in both the cytoplasm and plastids. 6PGD (EC1.1.1.44) catalyses the conversion of 6-phosphogluconate (6PGD) to ribulose 5-phosphate (R5P), a reaction that occurs in the pentose phosphate pathway that generates NADPH and pentose sugars [1].This reaction is a key step in the oxidative pentose phosphate pathway because it is unidirectional in most organisms [2]. The deleterious effects generated by compromising 6PGD activity have been ascribed to the disruption of glycolysis by 6PGD inhibition of phosphoglucose isomerase [3].

II. RELATED WORK

In perennial plants, such as poplar (*Populus gelrica*), 6PGD levels in the stem are influenzed by the seasonal variation. During winter, the 6PGD levels were found to be higher as the growth proceeded and in spring, the levels decreased as the growth retards [4]. Many researchers have reported the increased activity of 6PGD in various parts of virus-infected plants [5]. The maize genome encoded three copies of 6PGDH. Double mutants of the maize cytosolic isozymes, PGD1 and PGD2, have no obvious phenotype [6]. The third 6PGDH isozyme, PGD3, was predicted to be plastid localized, based on the available data in the literature and protein targeting prediction algorithms [7]. Chloroplast localized 6PGD plays a crucial role in the development of endosperm and starch accumulation in maize [8]. In *Pseudomonas chlororaphis*, O6 mutation in the *edd* gene encoding the 6-phosphogluconate dehydratase, leads to root colonization and was correlated with reduced induction of systemic resistance [9]. Developing embryos of *Brassica napus* L. (oilseed rape) showed high levels of fatty acid synthesis as a result of increase in 6PGD in oxidative pentose phosphate pathway in both cytosol and plastids [10]. A progressive increase in the expression of 6PGD proteins during the

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somatic embryogenesis of Prince Rupprecht's larch (*Larix principis-rupprechtii Mayr*) has been reported [11]. The activities of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) were found to increase in suspension cultured *Arabidopsis thaliana* cells in the exponential growth stage. Prokaryotic and eukaryotic 6PGDs are proteins of about 470 amino acids whose sequences are highly conserved. 6PGDH protein is a homodimer in which the monomers act independently. Each contains a large, mainly alpha-helical, domain and a smaller beta-alpha-beta domain, containing a mixed parallel and antiparallel 6-stranded beta sheet. NADP is bound in a cleft in the small domain, the substrate binding in an adjacent pocket [12] [13].

All higher plants so far examined have distinct cytosolic and plastidic 6PGDH isoforms [14][15][16]. Increased levels of 6PGD during embryo development have earlier been reported in coconut [17] and date palm [18]. In oil palm (*Elaeis guineensis* Jacq), specific activities of G-6-PDH and 6-PGDH and the concentration of nitrogen in the cytosol were identified as molecular markers for oil content in ripening fruits [19]. Since this protein has major role in somatic and zygotic embryogenesis of higher plants, the present study mainly focuses on the homology modelling and structural identification of coconut 6PGDH. The study of this protein has not yet reported in coconut so that the structural identification may useful for the study of protein-biomolecular interactions.

III. METHODOLOGY

Protein extraction and SDS PAGE: Proteins were extracted from the matured zygotic embryos excised from 11-12 month old coconut [20] and run on sodium dodecyl sulfate polyacrylamide gel electrophoresis with a 12% acrylamide separating gel and 5% acrylamide stacking gel [21].

Mass spectrometric analysis of protein band: Selected protein band (approx.55 kDa) was excised from the protein profile and they were characterized by MALDI-TOF/TOF MS followed by Peptide Mass Fingerprinting (PMF).

Bioinformatics analysis: The mass spectral data were queried against the NCBI database using the MASCOT search engine (http://www.matrixscience.com/). The amino acid sequences were used to confirm the full length of protein sequence using Uniprot and molecular weight of the protein using the ExPASy Prot Param tool (http://web.expasy.org/protparam/). Conserved domains were identified using SMART databases [22]. Helical wheel projection was carried out to illustrate the alpha helical properties of 6PGDH domains. The self optimized prediction method with alignment (SOPMA) was used to predict the secondary structure and the secondary structural properties including α helix, Extended strand, Beta turns, Random coil and other states [23]. After the sequence comparison, three-dimensional model of 6PGD was predicted by homology modelling method using the SWISS MODEL (http://swissmodel.expasy.org/) [24]. The predicted 6PGD model was validated using RAMPAGE server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

IV. RESULTS AND DISCUSSION

Conserved domain prediction and helical wheel projection

The amino acid sequence of coconut 6PGD retrieved from the MASCOT search engine (http://www.matrixscience.com/) were used for the conserved domain prediction and helical wheel projection. Two conserved domains were identified in coconut 6PGD by using SMART database. One is NAD binding 2 domain and the other is 6PGD domain. NAD binding 2 domain covers around 4-178 amino acids and 6PGD domain around 182 to 476 aminoacids (Fig.1). Same types of domains have observed in Tb6PGDH and Ll6PDH [25][26].

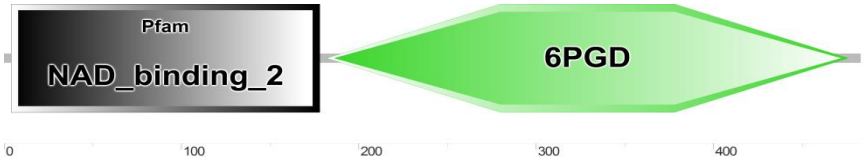

Fig.1. Conserved domains of coconut 6PGDH (NAD binding 2 domain and 6PGD domain).

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Alpha helical properties of coconut 6PGDH were identified using the helical wheel projection software. In NAD binding 2 domain, 84 hydrophilic residues (circles) and 43 hydrophobic residues (diamonds) were present, Whereas in 6PGD domain, hydrophilic residues were 94 and hydrophobic were 60. The hydrophobicity and hydrophilicity of the domains were indicated in color codes. The most hydrophobic residue is green, and the amount of green is decreasing proportionally to the hydrophobicity, with zero hydrophobicity coded as yellow. NAD binding 2 domain contained more green residues than the 6PGD so the hydrophobicity of this domain is considered as high. Hydrophilic residues are coded red with pure red being the most hydrophilic (uncharged) residue, and the amount of red decreasing proportionally to the hydrophilicity. The 6PGD domain contained more red residues than NAD binding 2 domain (Fig.2 A and Fig.2B).

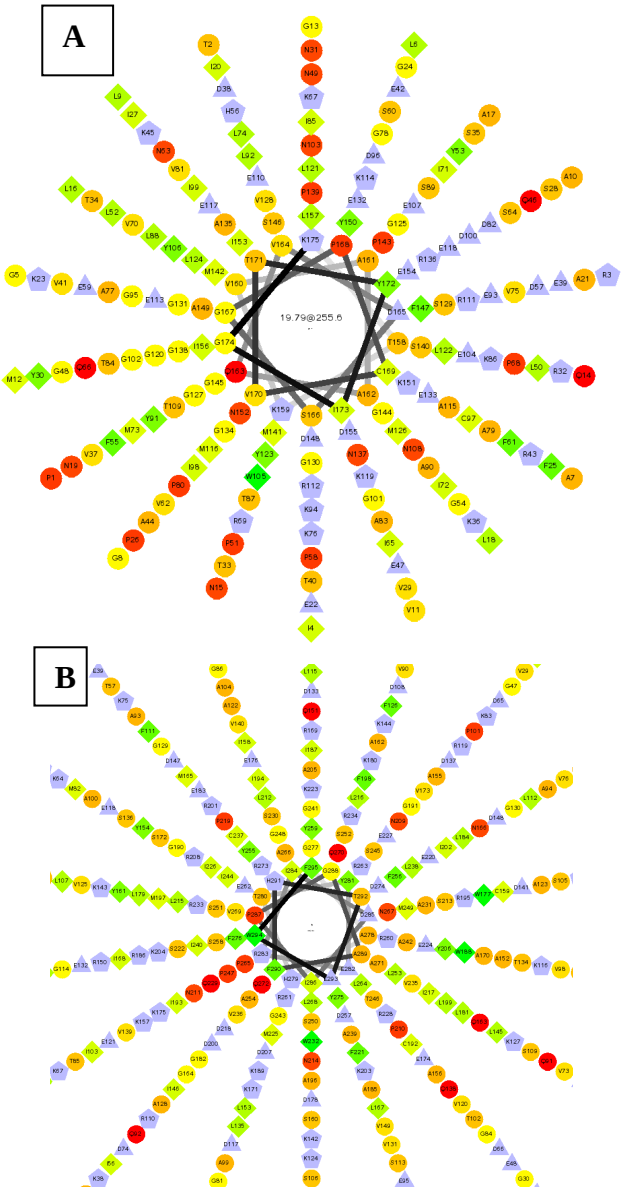

Fig.2. Helical wheel projection of NAD binding 2 domain (Fig 2A) and 6PGD domain (Fig.2B).

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Primary structure prediction

Primary structure prediction was carried out by using the ExPASy Prot Param tool (http://web.expasy.org/protparam/). Results showed that 6PGD has 483 amino acid residues and the estimated molecular weight is 53.24 kDa. The amino acid sequences of almost 40 different 6PGDs have been reported including human [27] mouse [28], rat [29] and pig [30]. Crystal structures of recombinant *Lactococcus lactis* 6-phosphogluconate dehydrogenase (LlPDH) shares significant sequence identity with the enzymes from sheep liver and the protozoan parasite *Trypanosoma brucei* for which structures have been reported.

The computed isoelectric point (pI) value of 6PGD protein was 6.04. pI is the pH at which the surface of protein is covered with charge but net charge of protein is zero. At pI, proteins are stable and compact. Computed pI value of 6PGD was less than 7 (pI<7) indicates that these proteins are considered as acidic. The computed isoelctric point (pI) will be useful for developing buffer system for purification by isoelectric focusing method. The total number of negatively charged residues (Asp + Glu) was 66 and Total number of positively charged residues (Arg + Lys) was 63.

The aliphatic index (AI), which is defined as the relative volume of a protein occupied by aliphatic side chains, is regarded as a positive factor for the increase of thermal stability of globular proteins [31]. Aliphatic index for the 6PGD protein sequences was 88.49. The very high aliphatic index of 6PGD protein sequences indicates that these proteins may be stable for a wide temperature range. The instability index provides an estimate of the stability of protein in a test tube. A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable [31].The instability index value for the coconut 6PGD proteins were found to be 25.04, indicates 6PGD protein as stable protein. The Grand Average hydropathicity (GRAVY) value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence. GRAVY indices of 6PGD are ranging -0.248. This low range of value indicates the possibility of better interaction with water.

Secondary structure prediction

The secondary structure of 6PGD was predicted using the SOPMA program. The results revealed that alpha helices (56.11 %) dominated among secondary structure elements. Random coil (22.7 %), extended strand (11.59 %) and beta turn (9.52%) are presented at varying percentage (Fig.3. These results were similar with the Oa6PGDH and Tb6PGDH [13][25].

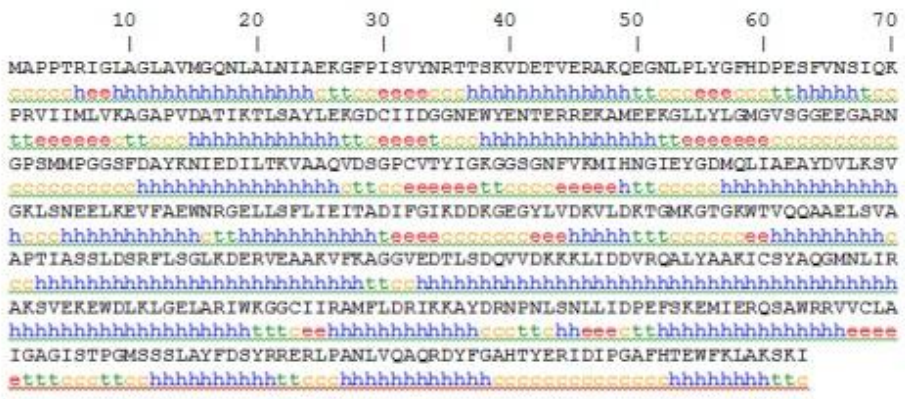

Fig.3. Secondary structures of the 6-phosphogluconate dehydrogenase protein predicted by SOPMA.

Tertiary structure prediction

The tertiary sturucture of 6PGD was predicted from SWISS-MODEL. The obtained structure was further validated through Ramachandran plot by using RAMPAGE server. The number of residues in favoured region was 96.2%, allowed region were 3.2% and disallowed region were 0.6% (Fig.4a). The tertiary structure was viewed by the

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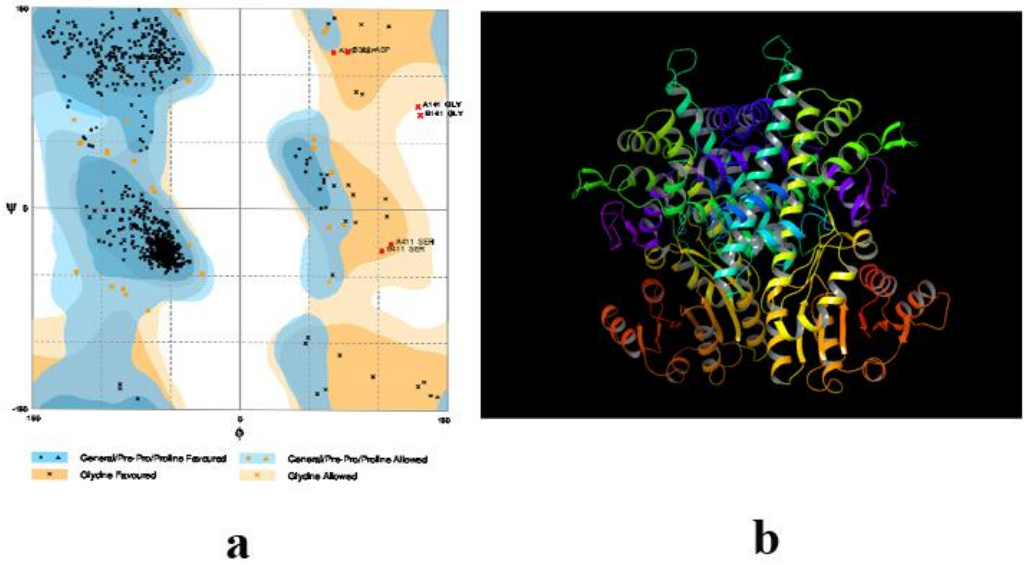
RasMol visualization program. The total numbers counted were 14662. In that 666 H-bonds, 47 helices, 34 strands and 78 turns were present (Fig.4b). These results were comparable with the result obtained in maize [32].



a **b**

Fig.4. a) Ramachandran plot of 6PGD obtained through RAMPAGE server .b) 3D structure of coconut 6PGD

V. CONCLUSION

This study provides the primary, secondary and tertiary structure of coconut 6PGD.The primary and secondary structure analysis gives the detailed idea of the charge, mass, conserved domains, and stability, hydrophobic, hydrophilic and other related properties of coconut 6PGD.The homology modeling predicted a 3D structure of the protein which has not reported yet in coconut. This 3D structure will be useful for the further study of protein-bimolecular interactions for improvement of embryogenesis in coconut.

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