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Research Article

In silico sequence analysis of predicted beta-amylase 7-like protein in *Juglans regia* L.

Emre Sevindik

Faculty of Agriculture, Department of Agricultural Biotechnology, Adnan Menderes University, South Campus, Cakmar, Aydin, Turkey; E-mail: ph.d-emre@hotmail.com

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ABSTRACT

Walnut (*Juglans regia* L.) is a deciduous tree of the Juglandaceae family. Beta-amylase (β-amylase, EC 3.2.1.2) is an enzyme that catalyses hydrolysis of glycosidic bonds in polysaccharides. In this study; sequence, physicochemical, and three-dimensional analyses of predicted β-amylase 7-like protein in *Juglans regia* using various bioinformatic tools were conducted. The physicochemical properties of the predict β-amylase 7-like protein were analyzed by using ExPASy ProtParam tool that revealed the molecular weight (MW), Isoelectric Points (*pI*), total number of negatively charged residues (Asp + Glu), total number of positively charged residues (Arg + Lys), instability index, aliphatic index, and GRAVY (Grand Average of Hydropathy) values. Subcellular localization using CELLO v.2.5, putative phosphorylation sites using NetPhos 3.1 server, domain analysis using Pfam, and secondary structure prediction using SOPMA were accomplished. To predict the 3D structure of the predict β-amylase 7-like protein, homology models were applied using PSIPRED, RAMPAGE, and PyMOL programs. The results of our study provide insight into fundamental characteristics of the predicted β-amylase 7-like protein in *Juglans regia.*

Keywords: *Juglans regia*; β-amylase 7-like; In silico.

1. INTRODUCTION

The genus *Juglans* (family Juglandaceae) comprises 7 to 45 species depending on the taxonomic study. The genus is distributed mostly across the temperate and subtropical regions of the Northern Hemisphere, with several species also found in Central America and along the Andes Mountains in Western South America [1]. Walnut (*Juglans regia* L.) is a species of deciduous tree of the family Juglandaceae. Its leaves, husks, bark, and fruits are used as a raw herb. In the literature, antibacterial and antifungal properties of the fruit extracts of walnut have been described [2]. There are also reports of antioxidant [3, 4], and insecticidal [5] properties of extracts of walnut green husk. Walnuts are mostly consumed in the form of dried fruits.The tree bark of walnut, fruit bark, green fruit bark and leaf parts are widely used in the pharmaceutical and cosmetic industries, and as a stain in the carpet and textile industry [6].

Amylase is a kind of enzyme that catalyses the breakdown of starch into sugars. This enzyme is found in plants and in some bacteria. All types of amylases belong to glycoside hydrolases with α-1,4-glycosidic bonds in polysaccharides, including amylose, amylopectin, glycogen, or their degradation products [7]. Beta-amylase is an exoamylase that attacks the nonreducing ends of starch molecules, producing, β-maltose and β-limit

dextrin as products. β-amylases are strictly plant enzymes that have been reported in ungerminated wheat and soybean seeds; germinating barley, rice, sorghum, and wheat seeds; sweet potato roots; broad bean leaves; and pea seedling roots [8]. Additionally, β-amylase has widespread applications in many industries such as foods, brewing, textiles, adhesives, detergents, pharmaceuticals, and sewage treatments [9].

The aim of this study was to generate predicted 3D structure of β-amylase 7-like protein by using comparative homology modeling. Also, primary and secondary structure analyses were performed utilizing various bioinformatics tools.

2. MATERIALS AND METHODS

The protein sequence of β-amylase 7-like (acession no: XP_018859154.1) in *Juglans regia* was retrieved from NCBI (https://www.ncbi.nlm.nih.gov/protein). The physicochemical analysis and amino acid contents of the pro-teins were analyzed by ExPASy'sProtParam (http://web.expasy.org/protparam/) that is also used to determine isoelectric point (*pI*), mole-cular weight (MW), total number of positive (+R) and negative (-R) residues, extinction coefficient (EC), instability index (II), aliphatic index (Al), and GRAVY values. The putative phosphorylation sites of the β-amylase 7-like protein were determined by NetPhos 3.1 (http://www.cbs.dtu.dk/services/NetPhos/). Secondary structure prediction was performed using SOPMA server (http://npsa-pbil.ibcp.fr/). For domain analysis, Pfam (http://pfam.xfam. org/) was used. Subcellular localization was predicted using CELLO v.2.5 (http://cello.life.nctu.edu.tw/). Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan) was used to identify known motifs in the sequence. The average amino acid rates were determined by MEGA 6.0 [10]. To predict the 3D structure of the β-amylase 7-like protein, homology model was performed using PSIPRED v3.3 (http://bioinf.cs.ucl.ac.uk/psipred/). The results were checked and verified by a Ramachandran plot analysis in RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php), which determined the best predicted models. Finally, 3D comparative analysis was performed using PyMOL (https://www.pymol.org/) (TM) (Schrodinger, LLC).

3. RESULTS AND DISCUSSION

The physicochemical analysis of the predicted β-amylase 7-like was performed using ExPASy ProtParam (the results were shown in Table 1). This protein had 693 amino acids with a molecular weight of 78124.14 Daltons and a *pI* of 5.73. The total number of negati-vely charged residues (Asp + Glu, 94) was found higher than the total number of positively charged residues (Arg + Lys, 76). Instability index (43.93), aliphatic index (78.48) and GRAVY value (-0.389) were also determined. The subcellular localization prediction of unknown proteins contributes to understanding of their functions [11].

Table 1. The physiochemical properties of the predicted β-amylase 7-like protein.

Parameters	Value
Molecular weight	78124.14
Theoretical *pI*	5.73
Total number of negatively charged residues (Asp + Glu)	94
Total number of positively charged residues (Arg + Lys)	76
Instability index	43.93
Aliphatic index	78.48
GRAVY	-0.389

Table 2. Secondary structure of the predicted β-amylase 7-like protein.

Parameters	Number of amino acids	Amino acids (%)
Alpha helix (Hh)	243	34.81
3_{10} helix (Gg)	0	0.00
Pi helix (Ii)	0	0.00
Beta bridge (Bb)	0	0.00
Extended strand (Ee)	122	17.48
Beta turn (Tt)	69	9.89
Bend region (Ss)	0	0.00
Random coil (Cc)	264	37.82
Ambiguous states	0	0.00
Other states	0	0.00

The subcellular localization was performed using CELLO v.2.5, and the protein was found to be localized in cytoplasmic, chloroplast and nuclear. The secondary structure of the protein was predicted using SOPMA (Table 2). It was observed that random coil was predominant (37.82%) followed by an alpha helix (34.81%) and an extended strand (17.48%). Also, a beta turn was predicted (9.89%). Random coils have important functions in proteins for flexibility and conformational changes such as enzymatic turnover [7].

Our findings could be related with the enzymatic function of the protein.The domain analysis was conducted using Pfam database and glycosyl hydrolase family 14 was detected. Glycoside hydrolases (GHs), a widely distributed group of enzymes, cleave glycosidic bonds in glycosides, glycans and glycoconjugates, and they can play key roles in the development of biofuels and in disease research. GHs such as cellulases, xylanases, and other glycosidases are being used to produce sugars from pretreated biomass substrates, which are then fermented to produce ethanol or butanol as renewable alternatives to gasoline [12, 13]. The Motif scan tool was used to determine different motifs (Table 3).

The seven types of motifs were observed while the highest number of motifs were N-myristoylation site and casein kinase II phosphorylation site with 11 times, and the lowest number of motifs were amidation site, β-amylase active site 1, and glycosyl hydrolase family 14 as once. Myristoylation is an irreversible, post-translational protein modification found in fungi, higher eukaryotes and viruses. Myristoylation can influence the conformational stability of individual proteins as well as their ability to interact with membranes or the hydrophobic domains of other proteins. Myristoylation plays a critical role in many cellular pathways, especially in the areas of signal transduction, apoptosis, and extracellular export of proteins [14].

Casein kinase II (CKII) is a multifunctional protein kinase that has been implicated in the regulation of central cellular functions, such as cell division and growth, mitosis, signal transduction, gene expression, and DNA replication [15, 16]. The most abundant amino acid composition in the predicted β-amylase 7-like protein Gly (9.1%), while the minimum amino acid ratio in the predicted β-amylase 7-like protein were Cys and Trp (1.8%) (Fig. 1). Phosphorylation processes are important mechanisms regulating cellular functions. Phosphorylation serves to effect critical post-translational modification of proteins having profound effects on their functions, which in turn governs the metabolic processes in a cell and tissue [17]. NetPhos 3.1 server was used to detect the putative phosphorylation sites (Fig. 2). The confidence rate that these were true Phosphorylation sites was above the threshold (0.5) and the output score was given in a 0.0-1.0 range.

ERRAT is a protein structure verification algorithm that analyzes statistics of non-bonded interactions between different atom types based on characteristic atomic interaction [18]. The overall quality factor was found as 66.121 (Fig. 3). The stereochemical quality of the modeled protein was analyzed by RAMPAGE (Fig. 4).

Table 3. The motifs of the predicted β-amylase 7-like protein by Motif Scan.

Motif information	No. of sites	Amino acid residues
Amidation site	1	376-379
N-glycosylation site	2	317-320, 588-591
Casein kinase II phosphorylation site	11	11-14, 184-187, 202-205, 237-240, 312-315, 340-343, 366-369, 373-376, 398-401, 424-427, 692-695
N-myristoylation site	11	46-51, 69-74, 152-157, 177-182, 232-237, 306-311, 345-350, 429-434, 486-491, 537-542, 584-589
Protein kinase C phosphorylation site	8	75-77, 98-100, 174-176, 209-211, 312-314, 373-375, 409-411, 465-467
Beta-amylase active site 1	1	342-350
Glycosyl hydrolase family 14	1	264-684

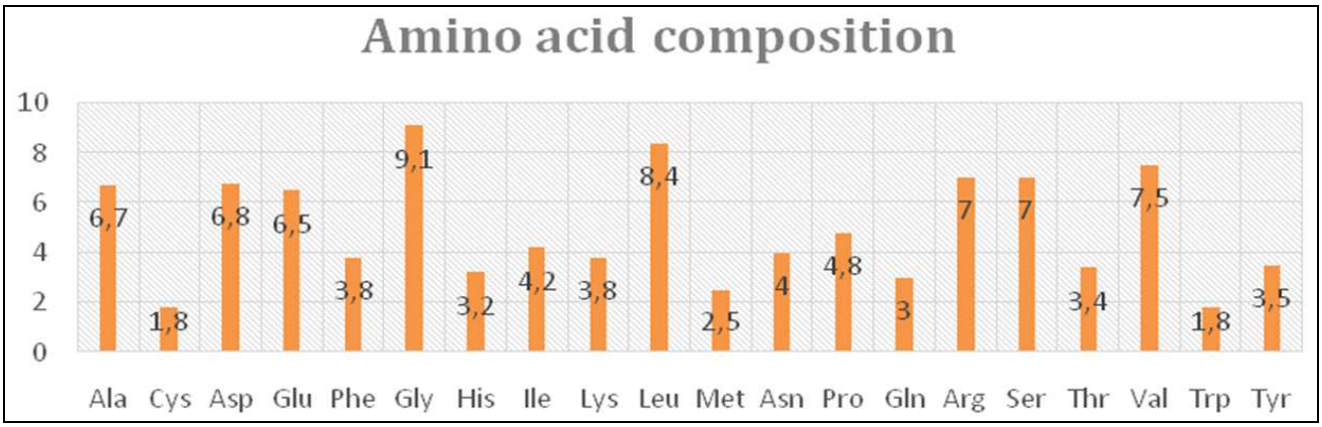


Figure 1. The average amino acid composition of predicted β-amylase 7-like protein from *Juglans regia*.

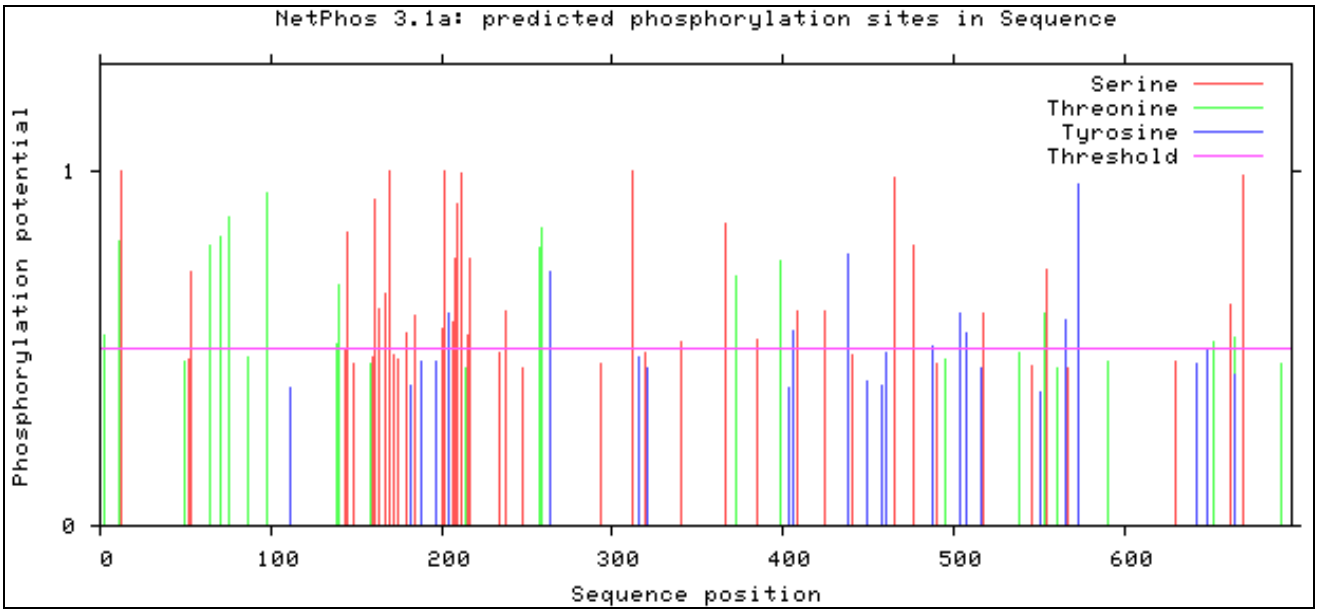


Figure 2. Putative phosphorylation sites of the predicted β-amylase 7-like protein determined with a score above a threshold of 0.5.

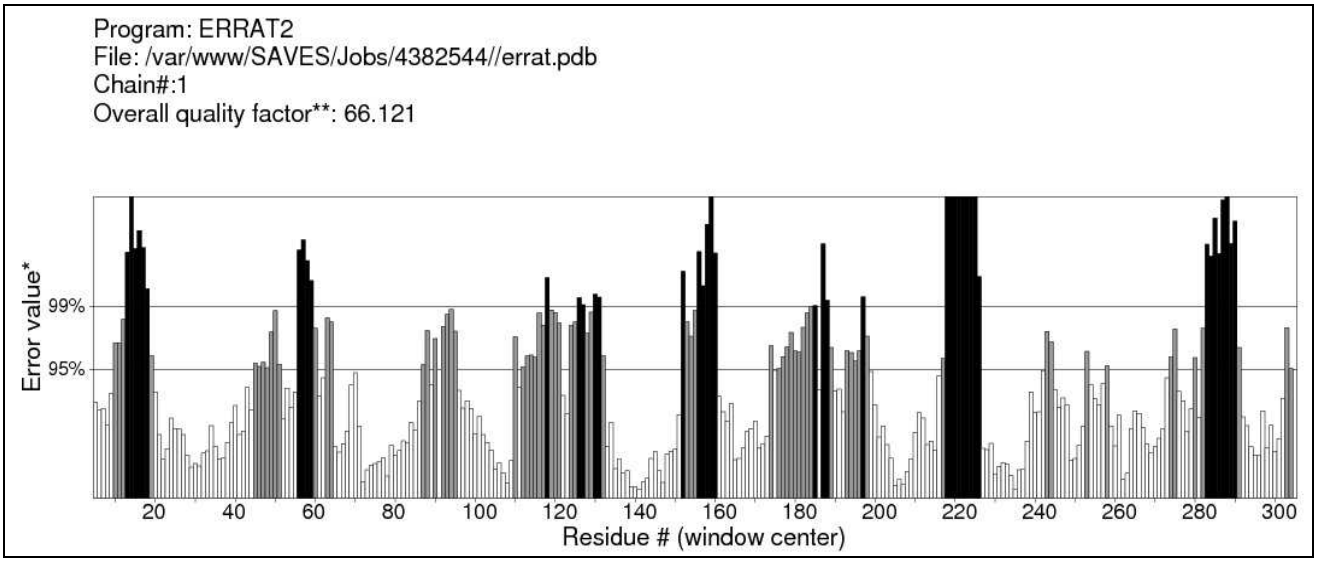
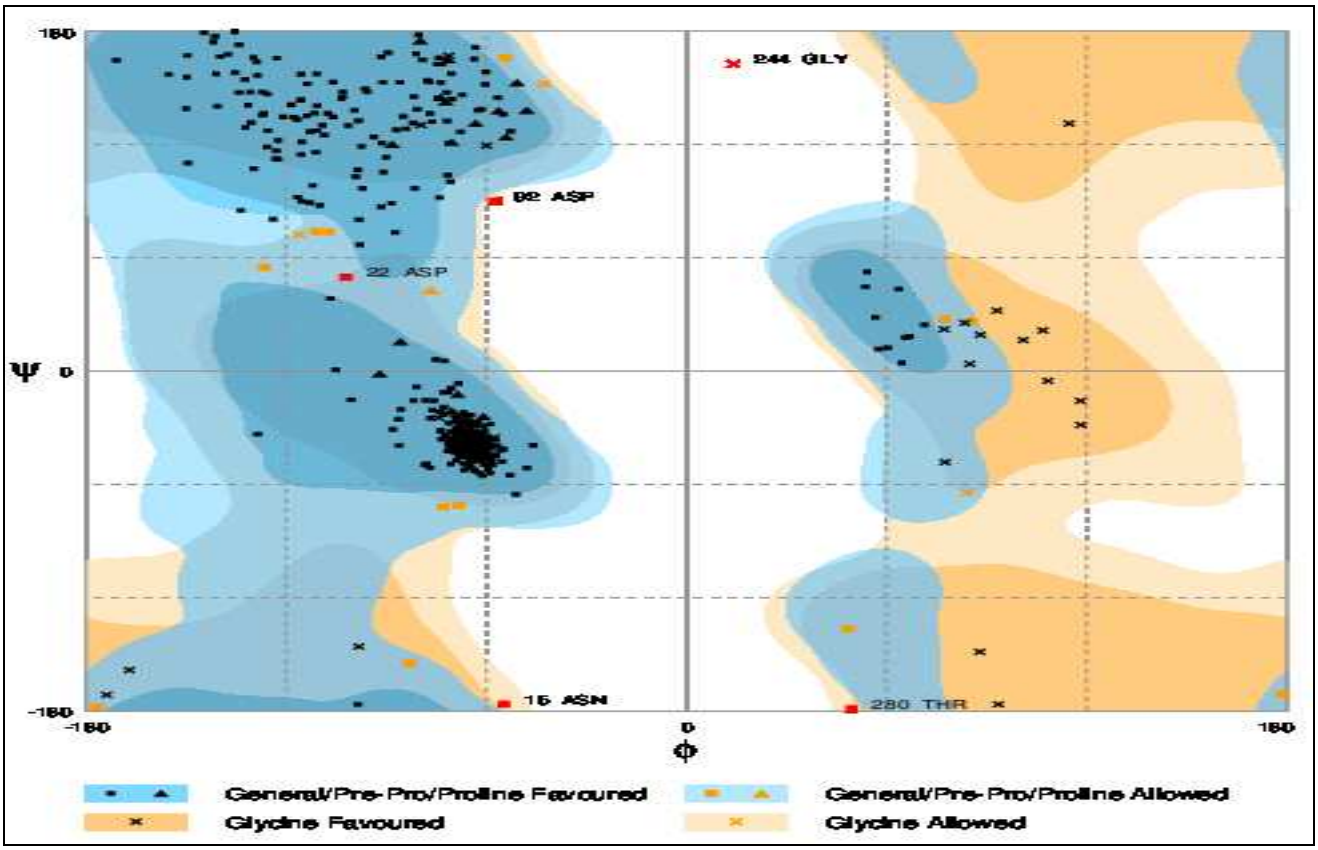


Figure 3. Overall quality factor evaluated by ERRAT.



Number of residues in favoured region (~98.0% expected) : 414 (95.2%)
Number of residues in allowed region (~2.0% expected) : 16 (3.7%)
Number of residues in outlier region : 5 (1.1%)

Figure 4. RAMPAGE values for indicating the number of residues in favored, allowed, and outlier regions.

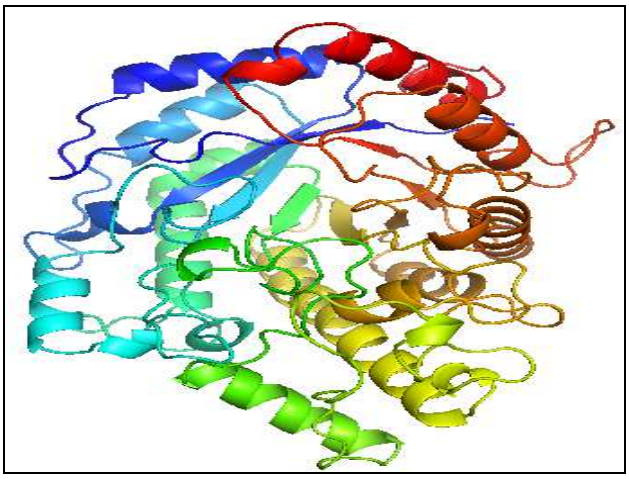


Figure 5. The 3D structure of the predicted β-amylase 7-like protein of *Juglans regia* by PyMOL.

Ramachandran plot analysis showed only 1.1% residues in outlier region, 3.7% allowed region and 95.2% in favored region, indicating that the models were of reliable and good quality. The three-dimensional structure of the predict β-amylase 7-like protein was constructed using the PyMOL program. The alpha helix and beta helix structures were demonstrated (Fig. 5). The three-dimensional structure of the proteins contributes to the understanding of protein function and active regions, and facilitates drug design [7].

4. CONCLUSION

In this study, in silico analysis was carried out using bioinformatic tools such as ExPASy ProtParam, CELLO v.2.5., MEGA 6.0, SOPMA, Pfam, NetPhos 3.1, ERRAT, PSIPRED v3.3, RAMPAGE and PyMOL for β-amylase 7-like protein in walnut. The results of this study will pave the way for further research on β-amylase 7-like protein in different plant species and will illuminate the future in silico studies.

TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

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