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In silico analysis on catalase protein from *Nicotiana tabaccum* L.

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Abstract

Catalases are antioxidant enzymes which are responsible for decomposition of hydrogen peroxide to water and oxygen. Catalase activities have been shown to be influenced by environmental factors and stress conditions. In this study, in silico analysis on the structure and possible functions of the catalase protein which was retrieved from genbank and abbreviated as NtCAT-1 (Accession no: NP_001312341.1) in Nicotiana tabacum L. was performed via bioinformatic tools. The results of this sudy suggested that the ORF of NtCAT-1 gene is 1479 bp and encodes a polypeptide of 492 amino acids. The predicted polypeptide was revealed as a 56.82 kDa protein with a pI of 6.27. The polypeptide had an aliphatic index of 71.52 and the grand average of hydropathicity (GRAVY) of -0.519. NtCAT-1 protein is hydrophilic and localised in Peroxisome. NtCAT-1 had two conserved domains at the positions of 18-399 and 421 and 486. had the catalase activity motif (CAM) at the position of 54–70 and heme-binding site (HBS) at the position of 344– 352. A highly reliable 3D structure was obtained and from Ramachandran plot analysis it was found that the portion of residues falling into the most favoured regions was 97.23%. The results of this study will provide fundamental information for further research in silico studies on catalase protein in different plant species.

Keywords: *Nicotiana tabaccum L., catalase, in silico.*

Nicotiana tabaccum L. katalaz proteininin *in siliko* analizi

Öz

Katalazlar, hidrojen peroksitin su ve oksijene ayrışmasından sorumlu olan antioksidan enzimlerdir. Katalaz aktivitelerinin çevresel faktörlerden ve stres koşullarından etkilendiği gösterilmiştir. Bu çalışmada Nicotiana tabaccum L. katalaz proteininin biyoinformatik araçlarla in siliko analizi yapılmıştır. Bu araştırmanın sonuçları,

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NtCAT-1 geninin ORF'sinin 1479 bp olduğunu ve 492 amino asitlik bir polipeptidi kodladığını göstermiştir. Öngörülen polipeptit, 6.27'lik bir pl ile 56.82 kDa'lık bir protein olarak ortaya çıkmıştır. Polipeptit, 71.52'lik bir alifatik indekse ve -0.519'luk büyük hidropatisite (GRAVY) ortalamasına sahiptir. NtCAT-1 proteini hidrofiliktir ve peroksizomda lokalizedir. NtCAT-1, 18-399 ve 421 ve 486 pozisyonlarında iki korunmuş domaine sahiptir. 54-70 pozisyonunda katalaz aktivite motifine (CAM) ve 344-352 pozisyonunda heme-bağlama bölgesine (HBS) sahiptir. Son derece güvenilir bir 3B yapı elde edilmiş ve Ramachandran çizim analizinden, en çok tercih edilen bölgelere düşen rezidülerin %97.23 olduğu bulunmuştur. Bu çalışmanın sonuçları, farklı bitki türlerinde katalaz proteini ile ilgili in siliko çalışmalarında daha ileri araştırmalar için temel bilgiler sağlayacaktır.

Anahtar kelimeler: *Nicotiana tabaccum L., katalaz, in siliko*

1. Introduction

Plant growth was affected from abiotic stresses factors such as drought, salinity, temperatures and other extreme environmental conditions. When plants exposed to these stressors; reactive oxygen species (ROS) are produced and damage the cellular components [1,2]. Plants have both non-enzymatic and enzymatic scavenging systems to eliminate these aggressive oxygen species as protective mechanisms [3,4]. Hydroxyl radicals are very reactive, and it is hard to control them directly, so aerobic organisms use superoxide and hydrogen peroxide, which are the less reactive precursor forms [4].

Catalases are antioxidant enzymes which are responsible for decomposition of hydrogen peroxide to water and oxygen and consist of two groups such as monofunctional catalases and catalase-peroxidases. Non-heme manganeses (Mn-catalases) are initially referred to as pseudocatalases. Non-heme manganeses form a minör group that can be the third group of catalytically active enzymes and present only in bacteria [5]. Among the other two groups, the monofunctional catalases are the best characterized. Although both types are heme enzymes with high catalase activities, they show significant differences such as no sequence similarity, different protein structures and active sites. They both found in all aerobic living organisms [6]. Catalase activities have been shown to be influenced by environmental factors and stress conditions such as; drought, salinity, plant pathogens and insects, light, temperature, 0_2 and CO_2 concentration [7-10]. *Nicotiana tabaccum* L. is an annually grown herbaceous plant; which is the major source of nicotine. Besides mostly being used as cigars, *Nicotiana tabaccum* is also used as an insecticide too [11].

Hence, thorough study of *in silico* analysis of the catalase gene and enzyme present in *Nicotiana tabaccum* can clarify its role in defence mechanism against infection. In the era of genomics, bioinformatics has become highly significant, assisting in the genome-wide discovery and characterization of potential genomic regions of different enzymes for a variety of industrial applications. Therefore, present communication deals with the bioinformatics analysis on the characterization of the protein sequence of catalase from *Nicotiana tabaccum* and subjected to structural and phylogenetic analysis.

The results of the present study provide additional evidence into basic bioinformatic characteristics of the *Nicotiana tabacum* catalase protein.

2. Materials and methods

The amino acid sequence of catalase protein (Accession no: NP_001312341.1) from *Nicotiana tabacum* was retrieved from NCBI (https://www.ncbi.nlm.nih.gov/protein) [12] and abbreviated as NtCAT-1. Online biotechnology tools were used to predict the structural properties and phylogenetic analysis of the NtCAT-1. Blast analysis was performed by using the amino acid sequence of NtCAT-1 and aligned according to their structure similarities from NCBI BLAST tool (https://www.ncbi.nlm.nih.gov/protein) [12]. The ProtParam software (http://web.expasy.org/protparam/) [13] was used to identify the theoretical pI, molecular weight (MW) and the grand average of hydropathicity (GRAVY) of the NtCAT-1. The positions of the conserved domains were calculated by using PFAM server (http://pfam.xfam. org/) [14]. The heme-binding site and catalytic active site of NtCAT-1 protein were detected by using InterPro [15]. Subcellular localization was predicted by using PlantmPLoc [16]. Conserved domains of the protein were predicted by NCBI conserved domain search (https://www.ncbi.nlm.nih.gov/protein) [12] and the three-dimensional (3D) structure of *NtCAT-1* was predicted by using I_TASSER (https://zhanggroup.org/I-TASSER/) [17] and a Ramachandran plot analysis was performed by MolProbity [18]. NetPhos 3.1 (http://www.cbs.dtu.dk/services/NetPhos/) [19] was used to determine the putative phosphorylation sites of the NtCAT-1 protein. Different motifs of the NtCAT-1 protein were determined by using Motif scan tool (http://myhits.isb-sib.ch/cgi-bin/motif_scan) [20].

BioEdit 7.2 [21] was used to analyse the open reading frame (ORF) of the *NtCAT-1* gene (Accession no: NM_001325412.1) and conduct multiple alignment of the deduced amino acid sequence of the NtCAT-1 with the other catalases, including *Nicotiana tomentosiformis* catalase isozyme 1 (XP_009590727.1), *Capsicum annuum* catalase (PHT66671.1) *Solanum lycopersicum* catalase isozyme 1 (NP_001234827.1), *Prunus persica* catalase (CAD42909.1), *Ziziphus jujuba* catalase (NP_001310800.1), *Olea europaea* var. *sylvestris* catalase (XP_022880282.1), *Theobroma cacao* catalase 2 (EOY15259.1), and *Eriobotrya japonica* catalase 1 (AGE15298.2). MEGA 6 software [22] was used to construct a phylogenetic tree using the neighbour-joining (NJ) method with 1000 bootstrap replicates.

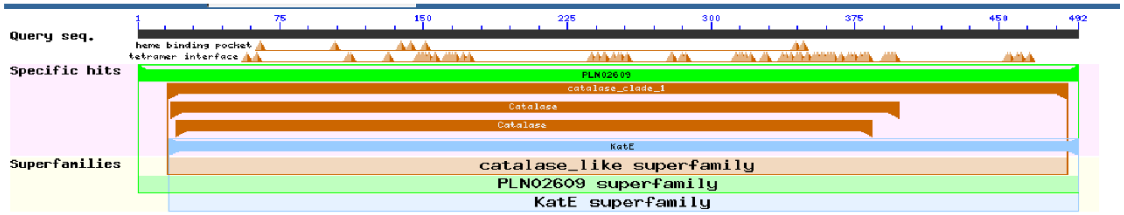
3. Results and discussion

The ORF of *NtCAT-1* gene is 1479 bp long and encodes a polypeptide of 493 amino acids. The predicted polypeptide was revealed as a 56.82 kDa protein with a pI of 6.27. The polypeptide had an aliphatic index of 71.52 and the grand average of hydropathicity (GRAVY) of -0.519, indicating that the NtCAT-1 protein is hydrophilic. According to the ProtParam (http://web.expasy.org/protparam/) [13] results; NCAT-1 is localised in Peroxisome. A search of the NCBI Conserved Domain Database (https://www.ncbi.nlm. nih.gov/protein) [12] indicated that the predicted protein belongs to catalase_clade_1 (Figure 1A) and PFAM server (http://pfam.xfam. org/) [14] results clearly indicated that NtCAT-1 had two conserved domains at the positions of 18-399 and 421 and 486.

According to the InterPro software [15] NtCAT-1 had the catalase activity motif (CAM) at the position of 54–70 and heme-binding site (HBS) at the position of 344– 352 (Figure 1B). As NtCAT-1 contains the heme-binding site and conserved catalytic active site, which are very critical in catalytic function and the heme ligand, NtCAT-1 is accepted as

a typical catalase. The presence of the deduced amino acid sequence of NtCAT-1 showed the evidence of a putative peroxisomal targeting signal (PTS1) motif QKVASRLTLKPTM that contains conserved QVV/I/L amino acid residues at the position of 480–482 (Figure 1B). The heme binding site residues were found to be at the positions of 62, 63, 64, 65, 102, 121, 122, 123, 136, 137, 138, 143, 148, 151, 207, 208, 324, 340, 343, 344, 347, 348, 351, 352, 355. These results clearly demonstrated that NtCAT-1 is a peroxisomal catalase, which is consistent with the results of previous studies [23-25].

A



B

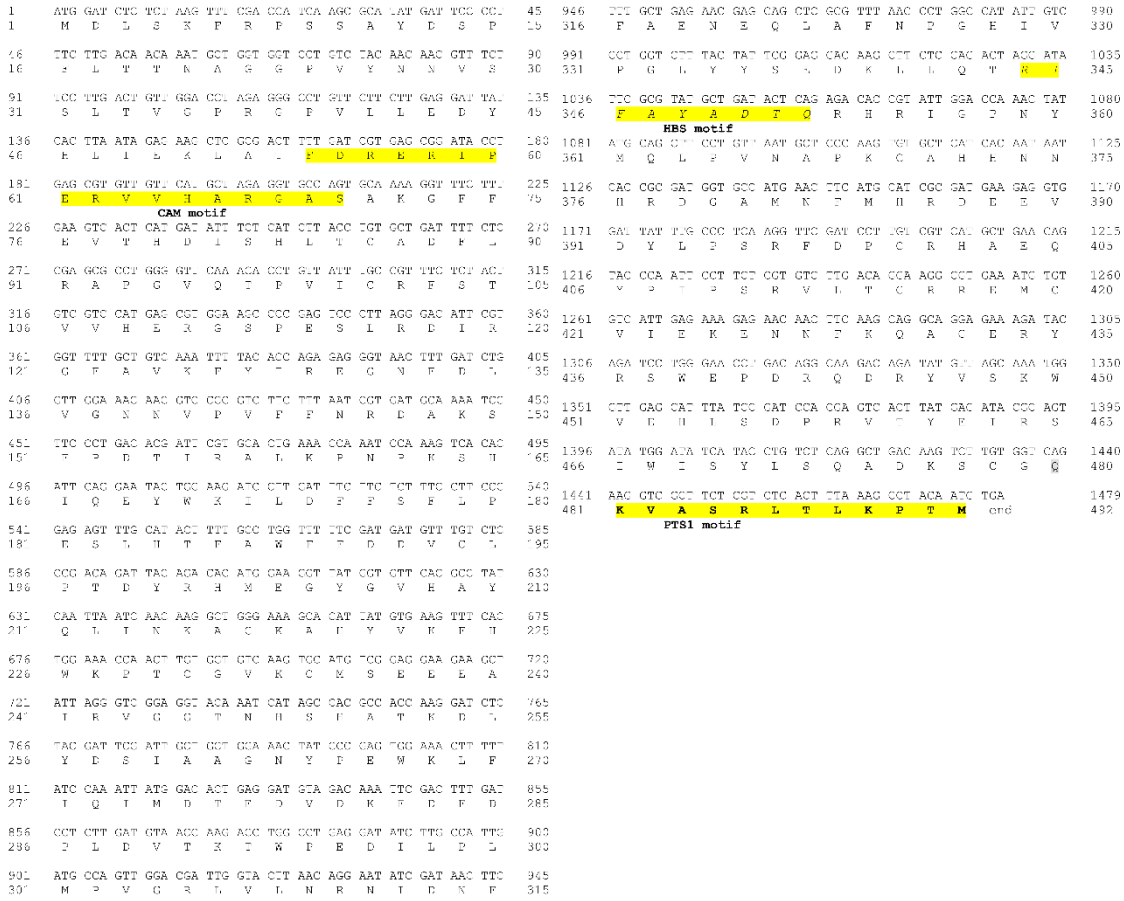


Figure 1. Open reading frame and amino acid residues of NtCAT-1 A) Conserved protein domains of NtCAT-1 B) cDNA and amino acid sequences of NtCAT-1. CAM residues were shown as highlighted, HBS motif was shown as highlighted and italic, PTS1 motif was shown as highlighted and bold.

The 3D structure of the proteins has very important role in understanding protein functions and determining the active sites. This kind of analysis provide important data for drug design studies [26]. The putative 3D structure of NtCAT-1 was predicted by I-TASSER (https://zhanggroup.org/I-TASSER/) [17] in order to reveal relationship between the structure–function with an estimated TM-score of 0.99±0.04 which suggested that the predicted structure is highly reliable. As shown in Figure 2, the predicted structure of NtCAT-1 is a homotetramer which has conceptually four subunits, which is in similar fashion with a pervious study [27].

According to the blast analysis as performed by using the amino acid sequence of NtCAT-1 according to their structure similarities; NtCAT-1 shared the highest similarity with the template protein 4QOL-A with a Query Cover of 95%, Percent Identity of 50.42% with an E-value of 5e-166. Ramachandran plot analysis helps to identify the protein structure on the premise of phi (φ) and Psi ψ angles to determine the quality of protein 3D structures. According to the results based on the Ramachandran plot analysis by MolProbity [18]; 99.9% (1947 out of 1948 residues) were in allowed (>99.8%) regions. There were 1 outliers (phi, psi): D 484 Ser (109.6, -32.6)) so > 99% residues have allowed conformations. From Ramachandran plot analysis it was found that the portion of residues falling into the most favoured regions was 97.23%, (Figure 3). Ramachandran plot for general, isoleucine and valine, Pre-proline, glycine, *trans* proline and *cis* proline and are also done and found that they all fall under allowed regions so this result provided the reliability of the model.

Figure 2. Predicted 3D-structure model of NtCAT-1 protein using I-Tasser (https://zhanggroup.org/I-TASSER/) [17]

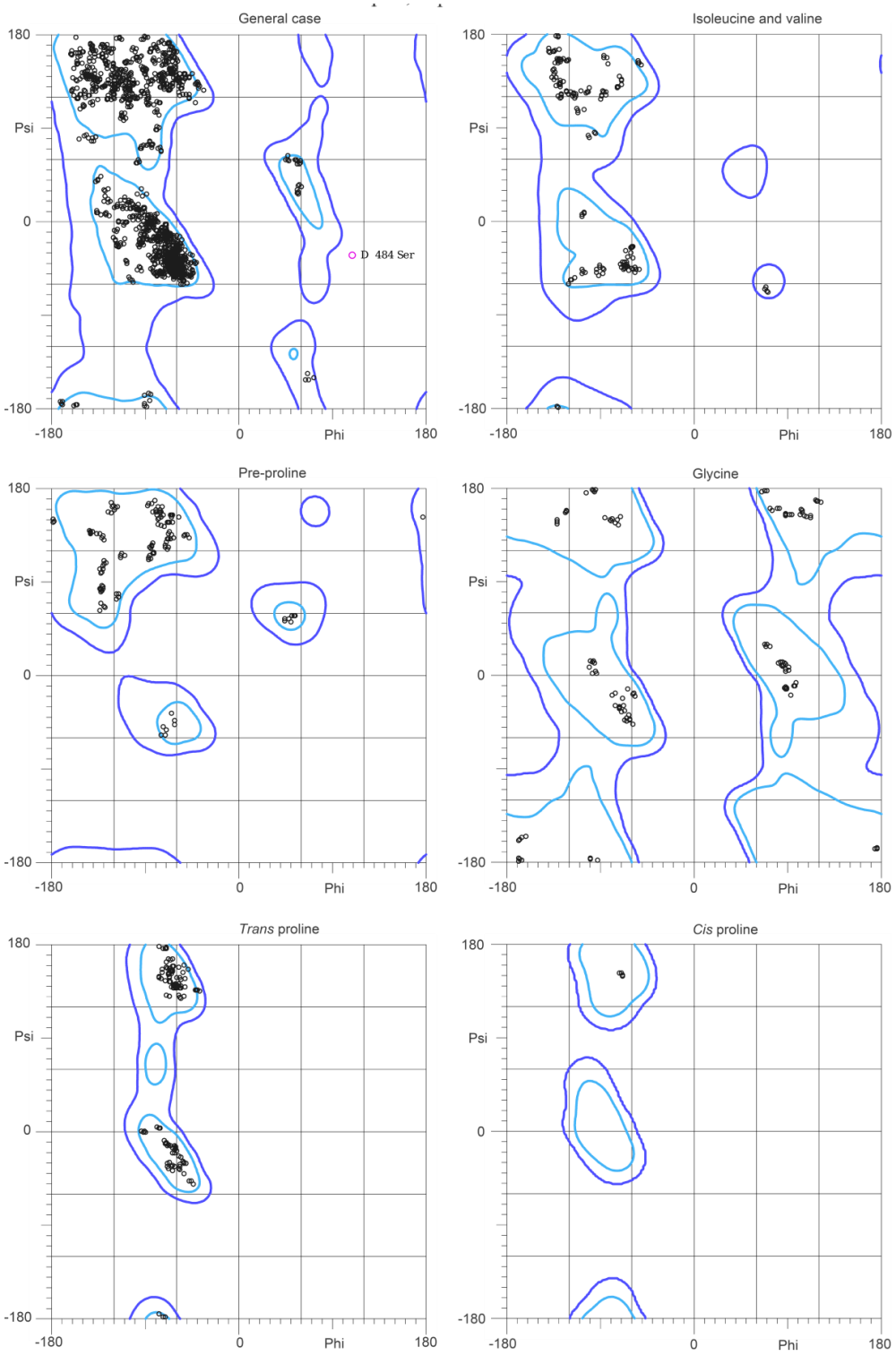


Figure3. Ramachandran plot analysis using MolProbity [18]. Distribution of (ϕ,ψ) angles of NtCAT-1 protein structures shown on Ramachandran plot map.

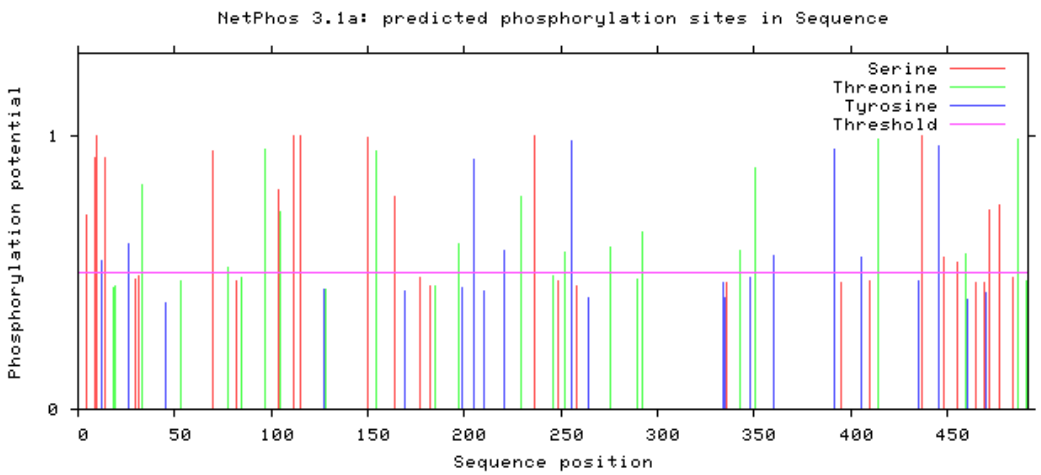


Figure 4. Putative phosphorylation sites of the NtCAT-1 protein by NetPhos (http://www.cbs.dtu.dk/services/NetPhos/) [19] with a score above a threshold of 0.5.

Different motifs (Table 1) were determined by Motif scan tool (http://myhits.isb-sib.ch/cgi-bin/motif_scan) [20]. Through the seven types of motifs, casein kinase II phosphorylation site and N-myristoylation site have the highest numbers of motifs as 8 and 6 respectively (Table 1). N-myristoylation is a modification in which the covalent attachment of a 14-carbon saturated fatty acid called myristate to the N-terminal glycine residue of proteins. Myristoylation has an effect on the conformational stability of proteins and their interaction with membranes or the hydrophobic domains of other proteins [31]. Casein kinase 2 (CK2) is an active kinase which is expressed constitutively, has key roles in a broad range of cellular events such as transcription and translation, ribosome biogenesis, cell cycle progression, and apoptosis [32].

Table 1. The motifs of the NtCAT-1 protein by Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan) [20]

Motif information	**Number of sites**	**Amino acid residues**
Amidation site	1	414-417
N-glycosylation site	2	28-31, 247-50
Casein kinase II phosphorylation site	8	10-13, 85-88, 115-118, 150-153, 236-239, 292-295, 395-398, 472-475
N-myristoylation site	4	111-116, 245-250, 332-337, 479-484
Protein kinase C phosphorylation site	6	70-72, 115-117, 154-156, 351-353, 414-416, 487-489
Catalase proximal active site signature	1	54-70
Catalase family profile	1	14-492
Catalase proximal heme-ligand signature	1	344-352

BLASTp analysis of NCBI database (https://www.ncbi.nlm.nih.gov/protein) revealed that NtCAT-1 deduced amino acid sequences had the closest match to *Nicotiana tomentosiformis* catalase isozyme 1 (99.39%) among other plants examined. A putative calmodulin binding domain is located from the 413th to 453rd residues in NtCAT-1. In a similar fashion, a putative calmodulin binding domain is located between the 415th and 451st residues in IbCAT2 of *Ipomea batatas* [23]. Previous studies revealed that calmodulin and calcium have an exclusive part in posttranslational regulation of catalase in Arabidopsis and sweet potato [33]. The PTS1 motif (QK-L), which has been shown to commonly exist in many CATs and directed catalase import into peroxisome was identified at the C-terminus of NtCAT-1. According to the results, NtCAT-1 could be considered as potentially regulated and activated by calmodulin and calcium.

CAM and HBS motifs from the selected species were also identified (Figure 5A). The results showed that there was the amino-acid composition of the HBS motif is more polymorphic than the CAM and PTS1 motifs. and the QKL/I/V amino acid residues in the PTS1 motif were highly conserved. Amino acid smilarities and conserved residues were shown in (Figure 5A).

The NJ tree of NtCAT-1 also proved that NtCAT-1 had the highest similarity with *Nicotiana tomentosiformis* catalase isozyme 1 (Figure 5B). NJ tree also revealed that NtCAT-1 shared the lowest similarity with *Olea europaea* var. *sylvestris* catalase among the plant catalases examined. NJ tree construction is used in many protein studies to cluster the protein in interest with other related proteins. These results suggest that; the NtCAT-1 protein encodes a putative peroxisomal catalase, which is likely regulated and activated by calmodulin and calcium (Figure 5A).

4. Conclusion

In this study, *in silico* analysis of *Nicotiana tabacum* catalase protein was carried out using bioinformatic tools. The results of this study will provide fundamental information for further research *in silico* studies on catalase protein in different plant species. Additional detailed experimental studies are needed such as protein structure and crystallography are needed which will help to understand the role of the protein in plant defence.

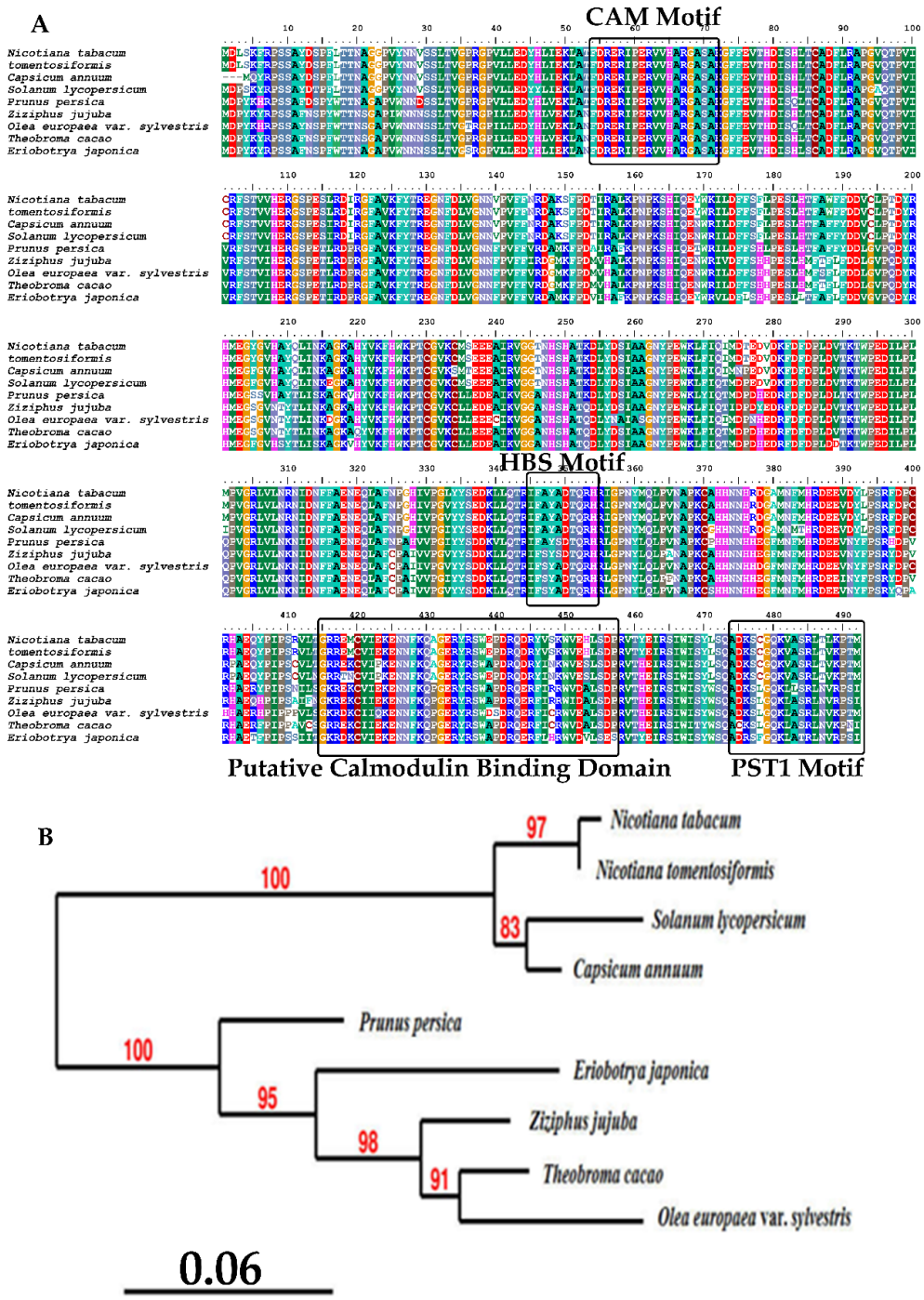


Figure 5. The relationship of NtCAT-1 with other related plant catalases A) Graphic view of the alignment of NtCAT-1 by using BioEdit 7.2 [21] with other catalases from different plant species. CAM motif, HBS motif, calmodulin binding domain and conserved QVV/I/L motif for PTS1 were marked with rectangular. B) Phylogenetic analysis of NtCAT-1. Bootstrap values over 50% are shown.

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