

Comparative Insilico Analysis of Ascorbate Peroxidase Protein Sequences from Different Plant Species

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Abstract

The existence of isoform diversity among antioxidant enzymes contributes to the spatial and temporal fine tuning of cellular responses. In plants heme binding ascorbate peroxidase (APX) (EC, 1.11.1.11) presents a crucial line of defense against reactive oxygen species. The present study aims to provide a comparative view of the functional attributes of major isoforms of APX in plants species. A total of 64 protein sequences of APX were subjected to homology search, multiple sequence alignment, phylogenetic tree construction, and motif analysis. The phylogenetic tree constructed revealed different clusters based on heme binding APX in respect of dicot and monocot plants such as different source of plant species represented by *Oryza sativa*, *Arabidopsis thaliana*, *Sorghum bicolor*, *Zea mays*, *Ricinus communis*, *Populus trichocarpa*, *Vitis vinifera*, and *Selaginella moellendorffii*. The multiple sequence alignment of these APX protein sequences from different plants showed conserved regions at different stretches with maximum homology in amino acid residues. The motif analysis revealed a conserved peroxidase domain uniformly observed in all APX irrespective of variable plant species suggesting its possible role in structural and enzymatic functions. The signature amino acids sequence of VFYQMGLSDKDIVALSGGHTLGRCH, NNGLHIAIRLCQPIKEQFPIITYADFYQLAGVVAVEVTGGPTIPMHPGRV and LFEDPSFRPYVEKYAKDQDAFFKDYAEAHMKLSELGF, related with the plant heme binding peroxidase as well as chloroplastic and cytosolic peroxidase signature was frequently observed and seemed to be related with the structure and enzymatic function in all APX protein sequences. The findings of the present study may be useful for designing degenerate primers or probes specific for APX and possibly presents the first line of defense amongst all the APX isoforms involved in the cellular antioxidant defense pathway, during exposure to abiotic stresses.

Keywords: Antioxidant enzyme; Ascorbate peroxidase (APX); Protein sequence

Introduction

Aerobic life has developed by exploiting the abundance of environmental oxygen (O₂) in the atmosphere to oxidize organic compounds, thus obtaining chemical energy in a highly efficient manner. Paradoxically, the univalent reduction of molecular oxygen in metabolic reactions produces a plethora of partially reduced intermediates, commonly known as reactive oxygen species (ROS). If their levels are not tightly controlled, these chemical species can react with the majority of biological molecules and cause serious cellular damages [1-2]. ROS are byproducts of aerobic metabolism and are produced in excess within plant cells under abiotic and biotic stresses [3-4]. However, ROS are also important in many physiological processes and their balance is of the utmost importance. As a result, a complex system, comprising enzymatic and nonenzymatic mechanisms, maintains the delicate balance between oxidant and antioxidant compounds in the cell [5]. Ascorbate peroxidase (APX) is known play the most essential role in scavenging ROS and protecting cells against these toxic effects in higher plants, algae, euglena and other organisms [6,9]. In plants, ascorbate peroxidases (EC, 1.11.1.11) catalyze the conversion of H₂O₂ to H₂O using ascorbate as the specific electron donor in this enzymatic reaction [9]. APX is the largest class of the nonanimal peroxidase superfamily, and its members are found in all living organisms except Diplomonads, Parabasalids, Apicomplexa, Amoebozoa, and animals [10]. Increased activity of different APX isoforms in response to environmental stresses such as salinity and drought has been reported in different plant species, indicating possible functional specialization of the respective isoenzymes in eliminating H₂O₂ in cells [11-12]. APX in higher plants are encoded by small multigene families and different isoforms are classified according to their subcellular localization. Soluble isoforms

are found in cytosol and chloroplast stroma, while membrane-bound isoforms are found in peroxisomes and chloroplast thylakoids. The final subcellular localization of the isozyme is determined by the presence of organelle specific targeting peptides and transmembrane domains that are found in the protein N-terminal and C-terminal [13], APXs purified from different plant species and tissues, such as tea leaves, maize (*Zea mays*) seedlings and leaves, and potato (*Solanum tuberosum*) tubers, have been isolated in both monomeric and dimeric forms [14]. Expression of this gene has been reported to be enhanced in plants by drought and salt [15-16].

This paper reports in silico characterization of amino acid sequence of heme binding peroxidase from different plants for homology search, multiple sequence alignment, phylogenetic tree construction, and motif analysis using various bioinformatics tools proposing new strategies for plant and crop improvement to combat stressful condition.

Materials and Methods

Retrieval of ascorbate peroxidase protein sequences

For the identification of APX in various plants, the homology search

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Received July 17, 2011; Accepted October 15, 2011; Published October 19, 2011

Citation: Pandey S, Negi YK, Marla SS, Arora S (2011) Comparative Insilico Analysis of Ascorbate Peroxidase Protein Sequences from Different Plant Species. J Bioengineer & Biomedical Sci 1:103. doi:10.4172/2155-9538.1000103

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of the APX proteins was done through Blast search tool of NCBI (<http://www.ncbi.nlm.nih.gov/BLAST/>) using Blastp and tblastn algorithm and their amino acid sequence of different source organism available in GenBank were downloaded from NCBI (<http://www.ncbi.nlm.nih.gov/>). Only reference sequences were retrieved while non reference sequences were removed.

Multiple sequence alignment

All the sequences of APX were aligned using ClustalW [18] to find out the similarity present among the sequences of the same family.

Phylogenetic analysis

Phylogenetic analysis of the sequences was done by Molecular Evolutionary Genetic Analysis (MEGA) software (version 4.0.02) [19], using UPGMA method. Each node was tested using the bootstrap approach by taking 1,000 replications and a random seeding of 64,238 to ascertain the reliability of nodes. The number is indicated in percentages against each node. The branch lengths were drawn to scale indicated.

Motif analysis

Analysis of conserved motifs was performed by means of the online MEME (Multiple Expectation Maximization for Motif Elicitation) tool version 3.5.7 [20] using minimum and maximum motif width of 20 and 50 residues respectively and maximum number of 10 motifs, keeping rest of the parameters at default.

Results and Discussion

Multiple sequence alignment

A total of 64 full-length amino acid sequences of Ascorbate peroxidase (APX) enzyme from different plants were considered for comparative In Silico analysis (Table 1).

To investigate the APX sequence features among various plants we performed multiple sequence alignments of the 64 amino acid sequences of APX. Conserved region of all proteins are shown in Figure 1 (shown as supplementary). Multiple sequence alignment highlighted the sequence conservation of amino acid residues among different members of APX families in the species. This conservation however, is concomitant with differences sufficient enough to support variations which are subsequently reflected at the structural and functional levels.

Phylogenetic analysis

To examine the phylogenetic relationship among APX from different plants a rooted tree was constructed from alignments of their amino acid sequences (Figure 2). The phylogenetic analysis of APX across all plant species clearly reveals four clusters: cluster A, cluster B, cluster

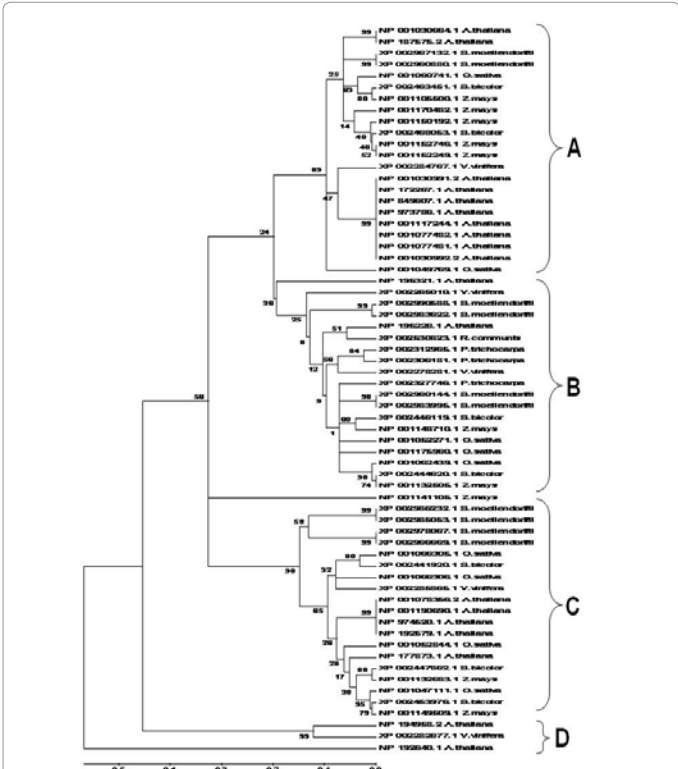


Figure 2: Phylogenetic tree of APX protein constructed by UPGMA method of MEGA version 4.0.02. Each node was tested using the bootstrap approach by taking 1,000 replications.

C and cluster D. Phylogenetic tree results outline the development of APX in Arabidopsis thaliana, Oryza sativa, Zea mays, Sorghum bicolor, Populus, Vitis vinifera, Ricinus communis and Selaginella bryopteris many of them exhibited orthologous and paralogous relations with each other. This indicates that this protein gene family is strictly conserved and has evolved from ancestral plants.

Motif analysis

An extensive search of the motifs and their positions was done by MEME software which identified several conserved motifs in the protein sequences of APX (Table 2 and Figure 3). Motif analysis also communicated the same fundamental necessity for the development of this gene family. Motifs which contain the signature sequences are either well conserved or are having substitutions which do not change their activity, while the ones which do not have a direct impact on the active site contain altered residues and are clearly the outcome of accumulation of mutations or have been subjected to rearrangements. A total of ten motifs labelled as 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were observed in all 64 sequences when subjected to MEME [21-23]. In all plant heme peroxidase, motif-1 was most commonly observed which is functionally related to its detoxication of H₂O₂ or reactive oxygen species both cytosolic and chloroplast cell compartment as well as having heme binding peroxidase properties. While Motif-4, which also have similar function as Motif-1 was present in all APX isoforms. Motif 2 contains Casein kinase II phosphorylation site and signature of chloroplastic and cytosolic ascorbate peroxidase [24]. Beside this, Motif-3 & 5, 7, and 9 also most frequently present in APX isoforms which are functionally related with chloroplastic and cytosolic and non animal peroxidase [25]. Motif 6 is present in all APX isoforms

S.No.	Plant	Total No.
1.	Arabidopsis Thaliana	19
2.	Zea mays	10
3.	Selaginella moellendorffii	10
4.	Oryza sativa	9
5.	Sorghum bicolor	7
6.	Vitis vinifera	5
7.	Populus trichocarpa	3
8.	Ricinus communis	1

Table 1: List of apx protein sequences from different source organisms.

S. NO.	ACCESSION NO.	M= 1	M= 2	M= 3	M=4	M=5	M= 6	M= 7	M= 8	M= 9	M= 10
1	NP_001066306.1	+	+	+	+	+	+	+		+	
2	NP_001062439.1	+	+	+	+	+	+	+	+		
3	NP_001052844.1	+	+	+	+	+	+	+	+	+	
4	NP_001052271.1	+	+	+	+	+	+	+	+		
5	NP_001047111.1	+	+	+	+	+	+	+	+	+	+
6	NP_001066305.1	+	+	+	+	+	+	+		+	
7	NP_001060741.1	+	+	+	+	+	+	+			
8	NP_001049769.1	+	+	+	+	+	+	+			
9	NP_001078356.2	+	+	+	+	+	+	+	+	+	
10	NP_001117244.1	+	+	+	+	+	+	+			
11	NP_001077482.1	+	+	+	+	+	+	+			
12	NP_001077481.1	+	+	+	+	+	+	+			
13	NP_001030992.2	+	+	+	+	+	+	+			
14	NP_001030991.2	+	+	+	+	+	+	+			
15	NP_001030664.1	+	+	+	+	+	+	+			
16	NP_974520.1	+	+	+	+	+	+	+	+	+	
17	NP_973786.1	+	+	+	+	+	+	+			
18	NP_187575.2	+	+	+	+	+	+	+			
19	NP_849607.1	+	+	+	+	+	+	+			
20	NP_192579.1	+	+	+	+	+	+	+	+	+	
21	NP_195226.1	+	+	+	+	+	+	+			
22	NP_177873.1	+	+	+	+	+	+	+		+	+
23	NP_172267.1	+	+	+	+	+	+	+			
24	NP_001190690.1	+	+	+	+		+	+	+	+	
25	XP_002463451.1	+	+	+	+	+	+	+			
26	XP_002441920.1	+	+	+	+	+	+	+		+	
27	XP_002447862.1	+	+	+	+	+	+	+			
28	XP_002468053.1	+	+	+	+	+	+	+			
29	XP_002444620.1	+	+	+	+	+	+	+			
30	XP_002446119.1	+	+	+	+	+	+	+			
31	XP_002453976.1	+	+	+	+	+	+	+		+	+
32	NP_001170482.1	+	+	+	+	+	+	+			
33	NP_001152746.1	+	+	+	+	+	+	+			
34	NP_001152249.1	+	+	+	+	+	+	+			
35	NP_001150192.1	+	+	+	+	+	+	+			
36	NP_001148710.1	+	+	+	+	+	+	+	+		
37	NP_001132505.1	+	+	+	+	+	+	+	+		
38	NP_001149509.1	+	+	+	+	+	+	+	+	+	+
39	NP_001105500.1	+	+	+	+	+	+	+			
40	NP_001132683.1	+	+	+	+	+	+	+	+	+	
41	XP_002530823.1	+	+	+	+	+	+	+	+		
42	XP_002312965.1	+	+	+	+	+	+	+	+		
43	XP_002306181.1	+	+	+	+	+	+	+	+		
44	XP_002284767.1	+	+	+	+	+	+	+			
45	XP_002278281.1	+	+	+	+	+	+	+	+		
46	XP_002285865.1	+	+	+	+	+	+	+	+	+	+
47	XP_002960144.1	+	+	+	+	+	+	+	+		
48	XP_002983995.1	+	+	+	+	+	+	+	+		
49	XP_002986232.1	+	+	+	+	+	+	+	+	+	
50	XP_002978067.1	+	+	+	+	+	+	+	+	+	+
51	XP_002967132.1	+	+	+	+	+	+	+			
52	XP_002985053.1	+	+	+	+	+	+	+	+	+	
53	XP_002960880.1	+	+	+	+	+	+	+			
54	XP_002966669.1	+	+	+	+	+	+	+		+	+
55	XP_002327746.1	+	+	+	+	+	+	+	+		
56	XP_002983622.1	+	+	+	+	+	+	+	+		
57	XP_002990588.1	+	+	+	+	+	+	+	+		
58	NP_195321.1	+	+	+	+	+	+	+	+		
59	XP_002265010.1	+	+	+	+	+	+	+	+		+
60	NP_001141105.1	+	+	+	+	+	+	+			
61	NP_194958.2		+	+	+	+	+	+	+		
62	XP_002282677.1		+	+	+	+	+	+			
63	NP_001175960.1		+					+			
64	NP_192640.1		+	+	+		+	+			+

Table 2: Distribution of motifs among 64 APX proteins sequences from different source organisms.

except *Vitis vinifera* [XP_002282677.1] which possess myristoylation site. Motif 8 is functionally related with glycosylation site. Motif-10 contains Protein kinase C phosphorylation site. Multilevel consensus sequences for the MEME defined motifs are shown in [Table 3](#).

Conclusion

In silico analysis of ascorbate peroxidase protein sequences and its comparison with other APX has revealed the sequence-based similarity existed among different APX isoforms and clustering in distinct groups based on its source among different plants and nature of the mechanism of enzymatic activity against the antioxidant defense

mechanism in plants. In silico domain analysis confirms the existence of the different groups of ascorbate peroxidase based on the presence of unique domains, a heme binding domain found in all isoforms of APX. The presence or absence of specific domains was directly in relation with the structural and functional organization of different isoforms of ascorbate peroxidase.

Amino acid sequence similarity specific for different groups could be utilized for designing strategy for cloning the putative genes based on PCR amplification using degenerate primers and potentially useful for the development of transgenic crop plants tolerant to abiotic stresses.



Figure 3: Block Diagram of Multilevel consensus sequences for the MEME defined motifs of APX proteins: Ten motifs were obtained by MEME software. Different motifs are indicated by different filled boxes with numbers 1 to 10.

Motif	Width	E value	Multilevel consensus sequences
1	36	5.8e-1798	VFYQMGLSDKDIVALSGGHTLGRCHPERSGWEGPWT
2	50	4.4e-2164	NNGLHIAIRLCQPIKEQFPITTYADFYQLAGVVAVEVTGGPTIPMHPGRV
3	37	3.2e1587	LFEDPSFRPYVEKYAKDQDAFFKDYAEAHMKLSELGF
4	39	5.7e-1546	YMKQVEKCRDLRGLIAEKNCHPIMVRLAWHDAGTYDCN
5	26	1.0e-1086	WLKFDNSYFKEILEGEKEGLQLPTD
6	19	3.9e-642	TKTGGPNGSMRFDQEQSHG
7	19	5.0e-612	KPQCPPEGRLPDATKGCPH
8	36	1.5e-185	KSCNKTSTVLAQSAFGVAVAAAVVIWSYFYEVNKKM
9	14	4.0e-177	YTKNGPGAPGGQSW
10	50	3.1e-175	EKFVAAKYSTGKKELSDSMKQKIRAEYEGFGGSPDKPMQSNYFLNIMIII

Table 3: Multilevel consensus sequences for the MEME defined motifs.

All motif regions of the Ascorbate peroxidase shade future structure-based studies and also the evolution of enzymatic activities of APX isoforms for improve abiotic stress tolerance in transgenic.

Acknowledgement

We acknowledge valuable suggestion of Dr. Anil Kumar, Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar. We thank, Anshita Goel, Dept. of molecular biology & genetic engineering, G.B.P.U.A.&T., Pantnagar for her suggestions during manuscript preparation.

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