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RESEARCH ARTICLE

In silico analysis and homology modelling of antioxidant proteins of barley

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SUMMARY

Barley (*Hordeum vulgare* L.) is an important major cereal grain. It is used as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods and often associated with beneficial health effects. In this paper, a bioinformatics and molecular modeling approach was adopted to explore properties and structure of barley antioxidant proteins by studying the antioxidative proteins *viz*., ascorbate peroxidase (APX), phospholipid hydroperoxide glutathione peroxidase-like protein (PHGPX) and 2-Cys peroxiredoxin BAS1 (2-CPs). The properties of these proteins have been interpreted by physico-chemical characterization including pI, EC, AI, GRAVY and instability index. Functional characterization was done by predicting motifs, patterns, disulfide bridges and secondary structure. Three dimensional structures for these proteins were not available as yet at PDB. Therefore, homology models for these antioxidant proteins were developed by using SWISS MODEL server. The model was analyzed for its fold reliability by using server ProSA, ERRAT server was used for analyzes the statistics of non-bonded interactions between different atom types. The model was validated using protein structure checking tool WHAT IF. These structures will provide a good foundation for functional analysis of experimentally derived crystal structures.

Key Words : Homology modeling, Antioxidant

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Barley is a member of Poaceae family. It is a self-pollinating, diploid species with a widely adaptable crop as barley is more tolerant of soil salinity than wheat; Barley has a short growing season and is also relatively drought tolerant (Antonio, 1996). *Hordeum vulgare* has antioxidative property and with other health benefits. Barley's dietary fibre also provides food for the "friendly" bacteria in the large intestine and so protects intenstine and reduces the risk of colon cancer. It has DPPH$^{\bullet}$ scavenging activity and ABTS$^{\bullet+}$ scavenging activity or reducing power could be used to assess barley antioxidant activity. It has phenolic compounds and flevonoids compounds due to which it gains an antioxidative properties. A detailed analysis of the antioxidant protein sequences, their probable structures and mode of action has yet to be accomplished (Sharp and Raven, 2003).

Oxidation is a process where electrons are transferred from one atom to another, with the molecule losing an electron being oxidized. Free radicals are generated when oxidation occurs during aerobic respiration (Pietta, 2000). Reactive oxygen species (ROS) are the oxygen-centered free radicals. These exist in different forms such as super-oxides (O_2•-), peroxyls (ROO•), alkoxyls (RO•), hydroxyls (HO•), and nitric oxides (NO•) (Pietta, 2000).Reactive oxygen species (ROS) are generated by exogenous sources, including prooxidant allelochemicals. Stress/starvation is an important endogenous source that generates ROS (Ahmad and Pardini, 1990). Even under optimal conditions, many metabolic processes, including

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the chloroplastic, mitochondrial, and plasma membrane-linked electron transport systems of higher plants, produce reactive oxygen species (ROS). Fortunately, there are natural defense mechanisms to reduce the damage done by free radicals. Antioxidants and antioxidant enzymes interrupt the cascades of uncontrolled oxidation in some organelles (Shigeoka *et al.*, 2002). The antioxidant defense is primarily constituted by the actions of glutathione peroxidase (GPX), superoxide dismutase, catalase and ascorbate peroxidase (Barbehenn, 2002). In this study the antioxidant proteins of spinach have been selected for which three dimensional structures were not available at the protein data bank (PDB). These proteins are ascorbate peroxidase (APX), phopholipid hydroperoxide glutathione peroxidase-like protein (PHGPX) and 2-Cys peroxiredoxin BAS1 (2-CPs).

Ascorbate peroxidase is a member of the heme peroxidase family of enzymes which are found in most life forms from yeasts to humans. They have many biological functions including antibacterial action, hormone synthesis and hydrogen peroxide removal (Sharp and Raven, 2003). APX exist as isoenzyme and plays an important role in the metabolism of H_2O_2 in higher plants. APX isoenzymes are distributed in four distinct cellular compartments: stromal APX (sAPX), thylakoid membrane-bound APX (tAPX) in chloroplast, microbody (including glyoxisome and peroxisome) membrane bound APX (mAPX), and cytosolic APX (cAPX) (Ishikawa *et al.*, 1998). Interestingly, APX isoenzymes of chloroplasts in higher plants are encoded by only one gene, and their mRNAs are generated by alternative splicing of the gene's two 3'-terminal exons. Manipulation of the expression of the enzymes involved in the AOS-scavenging systems by gene-transfer technology has provided a powerful tool for increasing the present understanding of the potential of the defence network against oxidative damage caused by environmental stresses.2.

2-Cysteine peroxiredoxins (2-CPs) constitute a ubiquitous group of peroxidases that reduce cell-toxic alkyl hydroperoxides to their corresponding alcohols (Baier and Dietz, 1999). Despite the presence of elaborate enzymatic and nonenzymatic antioxidative defense mechanisms, ROS escape from detoxification and oxidize organic compounds such as proteins, nucleic acids, terpenoids and fatty acids to the respective peroxides (Baier and Dietz, 1998). These protein displayed reduced glutathione-dependent phospholipid hydroperoxide peroxidase activity, but differs from counterpart mammalian enzymes that instead contain an active seleno-Cys. LePHGPx functioned as a cytoprotector in yeast (Saccharomyces cerevisiae), preventing Bax, hydrogen peroxide, and heat stress induced cell death, while also delaying yeast senescence (Chen S., *et al.*, 2004).

In silico approaches provide a viable solution to major drawbacks of experimental analysis of protein in regards to physicochemical and structural properties. Time requirement, high cost and the methods without amenable to high throughput techniques are the major drawbacks of experimental analysis of protein whereas computationally based characterization of the features of the proteins found or predicted in completely sequenced proteomes is an important task in the search for knowledge of protein function.

MATERIAL AND METHODS

Sequences of antioxidant proteins of spinach were retrieved from the NCBI (http://www.ncbi.nlm.nih.gov/), a public domain protein database (Bairoch and Apweiler, 2000) as shown in table 1. The antioxidant proteins sequences were retrieved in FASTA format and used for further analysis.

For physico-chemical characterization, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction co-efficient (Gill and Von Hippel, 1989), instability index (Guruprasad *et al.*, 1990), aliphatic index (Ikai, 1980) and grand average hydropathy (GRAVY) (Kyte and Doolottle, 1982) were computed using the Expasy's ProtParam server (Gasteiger, 2005) (http://us.expasy.org/tools/protparam.html). The results are shown in Table 2.

The SOSUI server (http://bp.nuap.nagoya-u.ac.jp/sosui/) performed the identification of transmembrane regions. Table 3 represents the transmembrane region identified for these antioxidant proteins. Prosite and MOTIFSCAN had been used for computing profile and pattern. Prosite is a database of protein families and domains (Falquet *et al*., 2002). Table 5 represents the output of Prosite and MOTIFSCAN that was recorded in terms of the length of amino residues of protein with specific profiles and patterns.

SOPMA (Geourjon and Deleage, 1995) was employed for calculating the secondary structural features of the antioxidant protein sequences considered for this study. The results were presented in Table 3.

The modeling of the three dimensional structure of the protein was performed by homology modeling server, Swissmodel (http://swissmodel.expasy.org/) (Arnold *et al.*, 2006). The overall stereochemical property of the protein was assessed by Ramchandran plot analysis (Ramachandran *et al.*, 1963). The validation for structure models obtained from the three software tools was performed by using WHAT IF (Vriend, 1990), ProSA (Wiederstein and Sippl, 2007) and ERRAT. The results of WHAT IF, ProSA (https:// prosa.services.came.sbg.ac.at/prosa.php) and ERRAT (http:/ /nihserver.mbi.ucla.edu/ERRATv2/) analysis are shown in Table 4. Structural analysis was performed and figures representations were generated with Swiss PDB Viewer (Guex and Manuel, 1997).

RESULTS AND DISCUSSION

Table 1 shows antioxidant proteins of spinach considered in this study with their accession numbers and retrived in

FASTA format for further analysis. The parameter pI, EC, AI, GRAVY and instability index, which was computed using Expasy's ProtParam tool had been shown in table 1. isoelectric point (pI) value was calculated because at that value protein is stable and compact. pI value is the pH at which protein has no net charge. The computed pI value of APX (AAL08496.1, CAA06996.1, AAL08495.1, CAA03952.1, CAC69935.1), PHGPX (BAC55016.1) and 2-Cysteine peroxiredoxins (Q96468.1, CAA84396.1) were less than 7 (pI<7) indicates that these antioxidant proteins were considered as acidic. The computed isoelctric point (pI) will be useful for developing buffer system for purification by isoelectric focusing method. Extinction co-efficient computed for the wavelength 276, 278, 279, 280 and 282 nm of which 280 nm is favored because proteins (Cys, Trp and Tyr amino acids) absorb light strongly. The high EC of ascorbate peroxidase (AAL08496.1, CAA06996.1) and 2-Cys peroxiredoxin BAS1 indicates presence of high concentration of Cys, Trp and Tyr. The computed extinction co-efficients help in the quantitative study of protein–protein and protein–ligand interactions in solution. 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 (Guruprasad *et al.*, 1990). The instability index value for the spinach antioxidant proteins were found to be ranging from 25.17 to 47.98. The result classified APX (CAA06996.1, CAC69935.1), and PHGPX (BAC55016.1) as stable protein (Table 1).

The aliphatic index (AI) is defined as the relative volume of a protein occupied by aliphatic side chains. It is a positive factor for the increase of thermal stability of globular proteins. Aliphatic index for the antioxidant protein sequences ranged from 71.37 to 91.00. The very high aliphatic index of all antioxidant protein sequences indicates that these antioxidant proteins may be stable for a wide temperature range whereas the lower thermal stability of APX was indicative of a more flexible structure when compared to other antioxidant protein.

SOSUI distinguishes between membrane and soluble proteins from amino acid sequences, and predicts the transmembrane helices for functional analysis. All of the antioxidant proteins were soluble as shown in table 1.

The functions of antioxidant proteins of spinach were analyzed by submitting the amino acid sequence to Prosite server and MOTIFSCAN servers. The motifs found, typically around 10 to 20 amino acids in length, because specific residues site or regions conserved in both structure and sequence during evolution o biologically important proteins (Sigrist *et al.*, 2002). Prosite analysis and MOTIFSCAN (http://myhits.isb-sib.ch/cgi-bin/motif_scan) suggested that the functionality of these proteins with profiles and patterns identified for characteristic functionality were represented in Table 2.

The secondary structure of spinach antioxidant proteins were predicted by SOPMA (Self Optimized Prediction Method with Alignment) with default parameter which correctly predicts 69.5% of amino acids for a state description of the secondary structure prediction (Geourjon and Deléage, 1995). Results are represented in Table 3 with parameters Alpha helix

Table 1 : Protein sequences considered for the study and Parameters computed using Expasy's ProtParam tool And Transmembrane regions identifi ed by SOSUI server

Antioxidant Proteins	Accession No.	Sequence length	Description	M.wt	pI	-R	+R	EC	II	AI	GRAVY	Type of protein
Ascorbate peroxidase	AAL08496.1	256	Ascorbate peroxidase	27639.3	5.10	35	24	21805	37016	80.16	-0.189	Soluble protein
	CAA06996.1	250	Ascorbate peroxidase	27435.2	5.85	35	30	23045	47.98	79.28	-0.406	Soluble protein
	AAL08495.1	153	Ascorbate peroxidase	16859.9	5.97	23	20	11640	40.62	71.37	-0.626	Soluble protein
	CAA03952.1	158	Ascorbate peroxidase	17003.5	6.42	19	18	11640	42.11	83.42	-0.264	Soluble protein
	CAC69935.1	355	Monodehydroascorbate reductase	38946.1	7.61	41	42	51590	36.88	79.35	-0.157	Soluble protein
(PHGPX)	BAC55016.1	169	Phospholipid hydroperoxide glutathione peroxidase-like protein	18531.9	5.44	21	19	16055	25.17	78.46	-0.268	Soluble protein
2-Cys peroxiredoxin BAS1	Q96468.1	210	RecName: Full=2-Cys peroxiredoxin BAS1, chloroplastic; AltName: Full=Thiol-specific antioxidant protein; Flags: Precursor	23298.6	5.48	29	25	21555	29.91	91.00	-0.117	Soluble protein
	CAA84396.1	210	bas1 protein	23298.6	5.48	29	25	21555	29.91	91.00	-0.117	

Pi helix Beta bridge Extended strand Beta turn Bend region Random coil Ambigous states.

The modeling of the three dimensional structure of the protein was performed by three homology modeling server, Swiss Model. SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the ExPASy web server, or from the program DeepView (Swiss Pdb-Viewer). The purpose of this server is to make Protein Modelling accessible to all biochemists and molecular biologists worldwide.

The model was analyzed for its Fold reliability by using server ProSA server. The recognition of errors in experimental and theoretical models of protein structures is a major problem in structural biology. ProSA calculates an overall quality score for a specific input structure. If this score is outside a range characteristic for native proteins the structure probably contains errors. A plot of local quality scores points to problematic parts of the model which are also highlighted in

Table 2 : Functional characterization of proteins of spinach at Prosite and MOTIFSCAN

Antioxidant Proteins	Accession No.	Motif Found	Profile	Position in the protein
Ascorbate Peroxidase	AAL08496.1	PEROXIDASE_2 (APLMLRLAWHSA)	PEROXIDASE_4	34-45
		PEROXIDASE_1 (DIVALSGGHTL)		156-166
				75-256
	CAA06996.1	PEROXIDASE_2 (SPLMLRLAWHSA)	PEROXIDASE_4	33-44
		PEROXIDASE_1 (DIVALSGGHTL)		155-165
				74-250
	AAL08495.1	PEROXIDASE_1 (DIVALSGGHTL)	PEROXIDASE_4	58-68
				1-153
	CAA03952.1	PEROXIDASE_2 (SPLMLRLAWHSA)		33-44
(PHGPX)	BAC55016.1	GLUTATHIONE_PEROXID_1(GKVLLIVNVASQCGLT)	GLUTATHIONE_PEROXID_3	31-46
		GLUTATHIONE_PEROXID_2(LAFPCNQF)		68-75
				1-169
2-Cys peroxiredoxin BAS1	Q96468.1		THIOREDOXIN_2	18-177

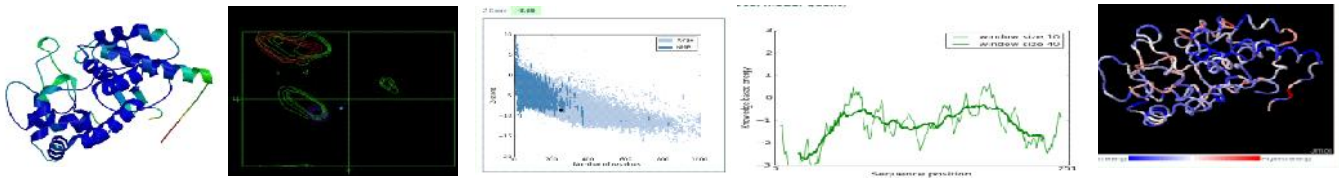
Table 3 : Calculated secondary structure elements by SOPMA

	AAL08496.1	CAA06996.1	AAL08495.1	CAA03952.1	BAC55016.1	Q96468.1
Alpha helix	35.55%	36.80%	32.03%	34.18%	30.18%	33.33%
3_{10} helix	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Pi helix	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Beta bridge	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Extended strand	12.50%	11.20%	11.76%	14.56%	21.30%	20.95%
Beta turn	6.25%	8.00%	12.42%	7.59%	10.65%	9.05%
Bend region	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Random coil	45.70%	44.00%	43.79%	43.67%	37.87%	36.67%
Ambigous states	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table 4 : What if analysis

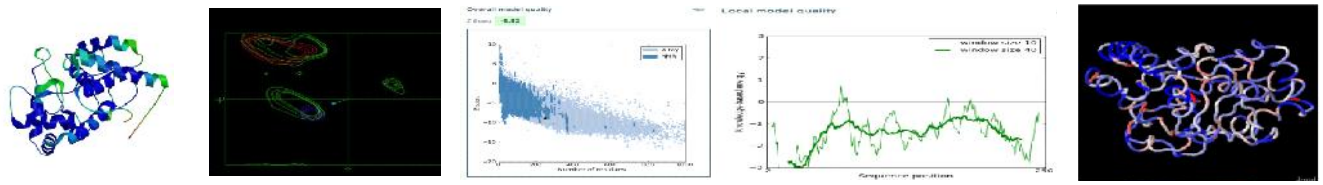
Structure Z-scores,	1st generation packing quality	-0.64	-0.711	-2.144	-1.045	-1.986	0.551
positive is better than	2nd generation packing quality	-0.265	0.146	-1.092	0.54	-0.7	0.102
average	Ramachandran plot appearance	1.092	1.28	-0.357	1.734	1.539	-0.239
	chi-1/chi-2 rotamer normality	2.381	2.713	2.004	2.894	1.537	2.986
	Backbone conformation	0.726	0.744	0.339	1.247	1.303	-0.243
RMS Z-scores, should be	Bond lengths	0.616 (tight)	0.626(tight)	0.628(tight)	0.611(tight)	0.604(tight)	0.652(tight)
close to 1.0	Bond angles	0.867	0.851	0.886	0.859	0.887	0.853
	Omega angle restraints	0.8	0.799	0.647	0.738	0.85	0.764
	Side chain planarity	1.523	1.433	1.412	1.368	1.527	1.549
	Improper dihedral distribution	1.617(loose	1.486	1.464	1.452	1.577(loose)	1.364
	Inside/Outside distribution	0.994	0.979	0.992	1.027	0.952	0.976

AAL08496.1



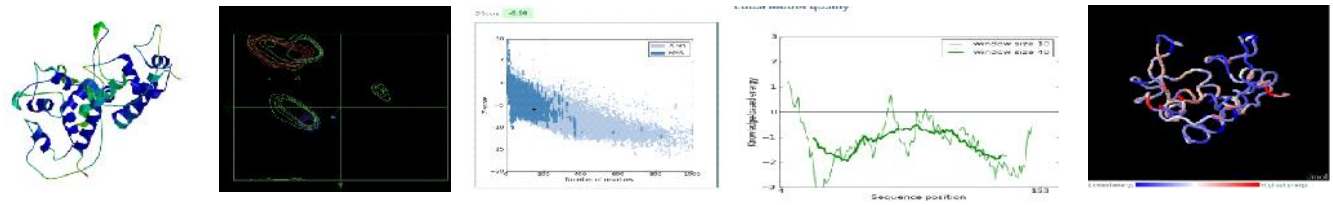
(A) (B) (C) (D) (E)

CAA06996.1



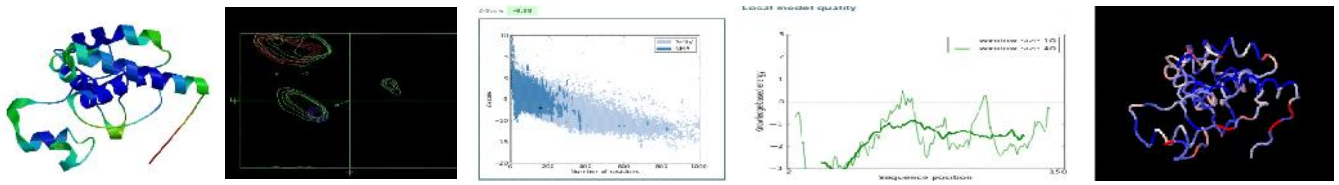
(A) (B) (C) (D) (E)

AAL08495.1



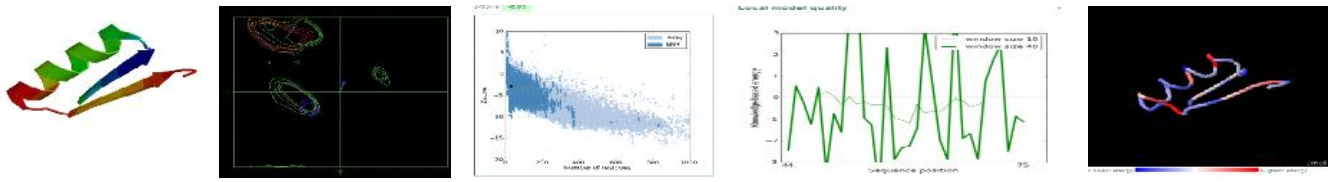
(A) (B) (C) (D) (E)

CAA03952.1



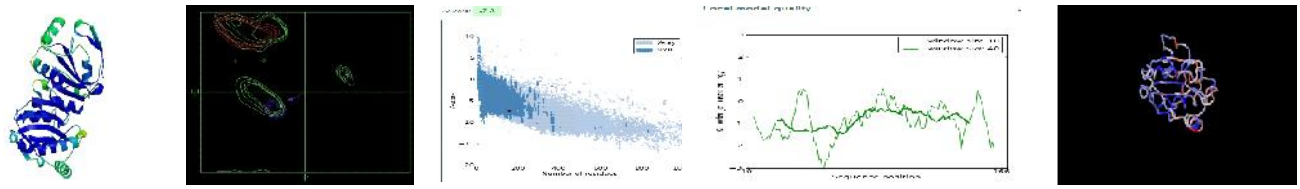
(A) (B) (C) (D) (E)

BAC55016.1



(A) (B) (C) (D) (E)

Q96468.1



(A) (B) (C) (D) (E)

(A) Model Symmentry (B) Ramachandran plot (C) Overall model quality and Z score (D) Local model quality (E) Energy level in Jmol Format

Fig. 1 : Modeled structure (SWISS MODEL), Ramachandran's Map and ProSA analysis of Antioxidant proteins

a 3D molecule viewer to facilitate their detection. The z-score indicates overall model quality (As shown in analysis). Its value is displayed in a plot that contains the z-scores of all experimentally determined protein chains in current PDB. Plot of residue scores groups of structures from different sources (X-ray, NMR) are distinguished by different color. It can be used to check whether the z-score of the input structure is within the range of scores typically found for native proteins of similar size. Results are shown in Fig. 1.

ERRAT server was used for analyzes the statistics of non-bonded interactions between different atom types. ERRAT is a protein structure verification algorithm that is especially well-suited for evaluating the progress of crystallographic model building and refinement. The program works by analyzing the statistics of non-bonded interactions between different atom types.

The modeled structures of barley antioxidant proteins were also validated by other structure verification servers WHAT IF (Vriend, 1990). Standard bond angles of the four models are determined using WHAT IF. The results were shown in Table 5. The analysis revealed RMS Z-scores were almost equal to 1 suggesting high model quality. The predicted structures conformed well to the stereochemistry indicating reasonably good quality.

Conclusion:

The modeling of the three dimensional structure of the protein was performed by three homology modeling server, Swiss Model. Model of all antioxidant proteins show reliable folding, validated by ERRAT and WHAT IF servers. These structures will provide a good foundation for functional analysis of experimentally derived crystal structures.

REFERENCES

Ahmad, S and Pardini, R.S. (1990). Mechanisms for regulation of oxygen toxicity in phytophagus insects. *Free Radical Biol. Med.,* **8** : 401-413.

Antonio, S. (1996). I semi della civiltà. Grano, riso e mais nella storia delle società umane" prefazione di Luigi Bernabò Brea Avenue Media, Bologna.

Arnold, K., Bordoli, L., Kopp, J. and Schwede, T. (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modeling. *Bioinformatics,* **22**: 195-201

Baier, M. and Dietz, K.J. (1998). The costs and benefits of oxygen for photosynthesizing plant cells. *Prog Bot.,* **60** : 282–314.

Baier, M. and Dietz, K.J. (1999). Protective Function of Chloroplast 2-Cysteine Peroxiredoxin in Photosynthesis. Evidence from Transgenic Arabidopsis. *Plant Physiol.,* **119** : 1407-1414.

Bairoch, A. and Apweiler, R. (2000). The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000" *Nucleic Acids Res.,* **28**: 45-48.

Barbehenn, R.V. (2002). Gut-based antioxidant enzymes in a polyphagous and graminivorous grosshopper. *J. Chem. Ecol.,* **28** : 1329-1347.

Chen, S., Vaghchhipawala, Z., Li, W., Asard, H., and Dickman, M.B. (2004). Tomato Phospholipid Hydroperoxide Glutathione Peroxidase Inhibits Cell Death Induced by Bax and Oxidative Stresses in Yeast and Plants. *Plant Physiol.*, **135**.

Falquet, L., Pagni, M., Bucher, P., Hulo, N., Sigrist, C.J., Hofmann, K. and Bairoch, A. (2002). The PROSITE database, its status in 2002" *Nucleic Acids Res.,* **30** (1) : 235-238.

Gasteiger, E. (2005). Protein Identifi cation and Analysis Tools on the ExPASy Server. In: John M. Walker ed, The Proteomics Protocols Handbook, Humana Press 571-607.

Geourjon, C. and Deléage, G. (1995). SOPMA: signifi cant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Comput. Appl. Biosci.,* **11** : 681-684.

Gill, S.C. and Von Hippel, P.H. (1989). Extinction co-efficient. *Anal. Biochem.,* **182**: 319- 328.

Guex, N. and Manuel, C.P. (1997). Data Modeling, Analysis and Classifi cation SWISSMODEL and the Swiss-Pdb Viewer: An environment for comparative protein modeling. *Electrophoresis,* **18**: 2714-2723.

Guruprasad, K., Reddy, B.V.P. and Pandit, M.W. (1990). Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. *Prot. Eng.,* **4** : 155-164.

Ikai, A.J. (1980). Thermo stability and aliphatic index of globular proteins. *J. Biochem.,* **88**: 1895-1898.

Ishikawa, T., Yoshimura, K, Sakai, K., Tamoi, M., Takeda, T. and Shigeoka, S.(1998). Molecular characterization and physiological role of a glyoxysome-bound ascorbate peroxidase from spinach. *Plant Cell Physiol.,* **39** (1) : 23-34.

Kyte, J. and Doolottle, R.F. (1982). A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.,* **157**: 105- 132

Pietta, P.G. (2000). Flavonoids as antioxidants. *J. Natural Products,* **62** : 1035-1042.

Ramachandran, G.N., Ramakrishnan, C. and Sasisekhran, V. (1963). Stereochemistry of polypeptide chain configuarations. J. Mol. Biol., **7**: 95-99.

Sharp, K.H. and Raven, E. (2003). Enzyme – Substrate Interactions in Ascorbate Peroxidase. *Nature Struct. Biol.,* **10**, 303.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y. and Yoshimura, K. (2002). Regulation and function of ascorbate peroxidase isoenzymes. *J. Exp. Bot.,* **53** (372) : 1305-1319.

Sigrist, C.J., Cerutti, L., Hulo, N., Gattiker, A., Falquet, L., Pagni, M., Bairoch, A. and Bucher, P. (2002). Prosite : A documented database using patterns and profiles as motif descriptors. *Briefings in Bioinformatics*, **3** (3) : 265-274.

Vriend, G. (1990). What if : a molecular modelling and drug design program. *J. Mol. Graph.*, **8** : 52—56.

Wiederstein, M. and Sippl, M.J. (2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res.*, **35** : W407-410.