BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 10(2)

Isolation and *In-silico* characterization of Peroxidase isoenzymes from Wheat (*Triticum aestivum*) against Karnal Bunt (*Tilletia indica*)

Shalini Purwar*, Ankit Gupta, Geetika Vajpayee & Shanthy Sundaram

Center for Biotechnology University of Allahabad, Allahabad; Shalini Purwar - Email: purwarshalini@gmail.com; Phone: 09473850596; *Corresponding author

Received January 16, 2014; Accepted January 26, 2014; Published February 19, 2014

Abstract:
To investigate the role of Peroxidase and its physiological significance under Karnal Bunt (KB) were determined in resistant (HD-29) and susceptible genotype (WH-542) of wheat during different developmental stages. The enzymes were expressed constitutively in both the susceptible and resistant genotype. In gel assay and differential expression analysis of POD was significantly higher (p >0.05) in Sv and S2, than the S1 and S3 stages. *In silico* analysis of Peroxidase for eg. physico-chemical properties, secondary structural features and phylogenetic classification for comparative analysis. Motif and Domain analysis of Peroxidase by MEME, to be important for the biological functions, and studies of evolution. Our results clearly indicate that the enhanced expression of POD at the WS2 stage, which reinforces its role in stage dependent immunity against Karnal bunt and role of POD metabolism provides genotype and stage dependant structural barrier resistance in wheat against KB.

Keywords: Peroxidase (POD), Karnal Bunt, *Triticum*, *insilico* analysis, Fungus

Background:
Wheat is one of the important staple crops of India. Wheat occupies more than 25 million hectares area in India with a production of about 94.9 million tones. India has now emerged as second wheat producer country the after China. Although there is quantum jump in the productivity of wheat yet it's adversely affected by several fungal diseases like brown, yellow and black rusts, leaf blight, loose smut and bunt. Karnal bunt or partial bunts, caused by *Tilletia indica (Syn Neovossia indica)* although occurs sporadically but assumes epidemic proportions in certain year which cause substantial losses in both quantity and quality of wheat. Peroxidase (POD, EC 1.11.1.7) is amonomeric heme-containing enzyme with a molecular mass between 32 and 45 kDa. Plant POD is heme-proteins that use H2O2 to oxidize a large variety of hydrogen donors such as phenolic substances, amines, ascorbic acid, indole, and certain inorganic ions **[1].** These proteins are widespread in the plant kingdom and POD isoenzymes are known to occur in a variety of plant tissues. The pattern of expression of each isoform varies in the different tissues of healthy plants and is developmentally regulated and influenced by environmental factors **[2]**; however, the role of each isoform is not fully understood. Various experiments suggest the involvement of plant POD not only in biosynthetic processes related to wall development such as lignification **[3],** suberization **[2]**, and polymerization of hydroxyproline-rich glycoproteins **[4]**, but also in the regulation of cell wall elongation **[5]** and wound healing **[1]**. Also, the POD are sometimes listed as the class of pathogenesis related proteins (PR proteins), increases upon viral, bacterial, or fungal infection and resistance against infection by pathogens **[5-9]**. Such a wide spectrum of functions is consistent with the presence of several isoforms suggesting that different POD isoenzymes might be involved in distinct processes. Moreover, POD can be

considered useful markers for environmental stresses since their activity is affected by low temperature, air pollution, ozone, heavy metals, wounding, salts, UV radiation, and pathogen attack **[10].** In view of the above, present investigation has been undertaken to characterize the genotype and organ, stage dependent immunity using biochemical and *in silico* analysis during different developmental stages of resistant and susceptible wheat spikes.

Methodology:

Collection of Wheat Genotypes

In present study two genotypes (WH-542, HD-29) of bread wheat (Triticum aestivum) were used. The seeds of these genotypes- one highly susceptible and another resistance to Karnal bunt based on pathogenecity testing under field conditions were collected **[11].** Different stages of developing wheat spikes were selected on the basis of % severity of KB induced by inoculating at different growth stages the data **[11].**

In gel Assay

10% native gel was prepared and sample was loaded with loading dye, gel was run for 2 hour at 80 mv. POD analysed after electrophoresis in PAGE with the use of Guaicol as a hydrogen donor. Gel was incubated in 0.02M guaicol for 30 minute, washed and then immersed in 0.01M H2O2 for band development **[12].** A densitometry analysis was done with the help of Gene Profiler software, Alpha Innotech Corporation USA. Briefly, individual gels were scored by placing the curser over individual band and recording the relative densitometry values of gels used for expression analysis **[11].**

In Silico Analysis of POD

Protein sequences of POD from wheat, and different plants were retrieved from protein database of NCBI (National Center for Biotechnology Information, (http://www.ncbi.nlm.nih.gov/protein/) in FASTA format **Table 1 (see supplementary material).** The homology search of the POD protein was done through BLAST search tool of NCBI http://www.ncbi.nlmnih.gov). Secondary structural properties of the POD were computed by using SOPMA (Self Optimized Prediction Method with Alignment, ttp://npsapbil.ibcp.fr/cgibinnpsa_automat.pl?page=/NPSA/npsa_sopma.html) tool of NPS (Network Protein Sequence Analysis). PDB structure analysis by (http: // www.ebi.ac.uk/pdbsum/). Domain and family analysis of amino acid sequence was done using CDD tool of NCBI (http: //www.ncbi.nlm.nih.gov/cdd) and Motif analysis done by MEME (meme.nbcr.net) **[13]** and functional motif analysis of POD by PROSITE (http://prosite.expasy.org/). The ProtParam tool (http://web.expasy.org/protparam/) of ExPASy was used to compute physiochemical characterization of POD. Phylogeny analysis of wheat, and other plants POD were aligned by ClustalW tool (http:// www.ebi.ac.uk/Tools/msa/clustalw2/) and phylogenetic tree constructed by DNAman version 7.0 (www.lynnon.com).

Results & Discussion:

Karnal bunt (KB) caused by *Tilletia indica* (Syn. *Neovossia indica*) is an economically important disease of wheat. POD, a class of pathogenesis related proteins (PR proteins), increases upon fungal infection and resistance against infection by pathogens. Therefore, it is important to differentiate the resistant and susceptible genotypes based on expression of POD at, biochemical and also *in silico* analysis of different plant POD was carried out by using various computational tools.

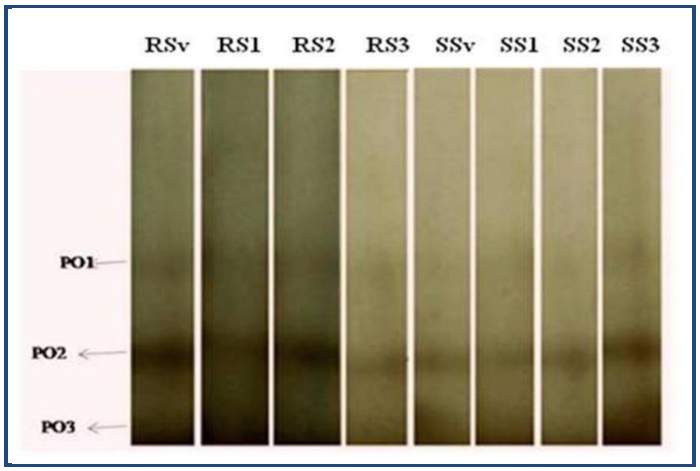

Figure 1: In Gel analysis of wheat POD at different stages (Sv, S1, S2 and S3) of developing wheat spikes in Resistant and Susceptible genotypes.

In gel analysis of POD

In order to ascertain the role of basal expression level of enzyme POD was analyzed using in gel assay at different physiological stages of wheat spikes of resistance and susceptible genotypes. POD isoenzymes were electrophoreses on native-polyacrylamide gels and stained with guaicol and hydrogen peroxide **(Figure 1).** The native gel analysis showed that there were three different bands present in both the genotypes. Differential expression analysis of POD was carried out by using Gel documentation system, POD activity was somewhat increased significantly dominant in resistant genotype than susceptible. Surprisingly, POD2 isoenzyme was significantly increased in resistant genotype, suggesting that this isoenzyme is most likely involved in defense mechanism. The expression of POD was significantly higher ($p<.05$) in S_v and S2 stage. There was decreasing trend up to S_3 stages of both genotypes indicating the enhanced lignification of cell walls in developing spikes after fertilization and grain development. During earlier stages, higher level of expression of defense enzymes and accumulation of chemical at vegetative leaf stages (Sv) certainly prevent the fungal mycelial colonization in leaves. POD activity in plants can increase in response to a variety of stresses including biotic stress, indicating that POD activities have been suggested to be involved in, cell wall biosynthesis by the polymerization of cinnamyl alcohol into lignin, in defense against attack by pathogens **[14],** and in the response to wounding **[15].** In addition, POD catalyzes polymerization of naturally occurring phenolics to produce a variety of bioactive products. POD activity is frequently increased in plant infected by pathogen, and the level of its activity is clearly correlated with disease resistance **[15].** The POD expressed constitutively in both susceptible and resistant genotype. However, the activity was higher in resistant genotype indicating that resistant genotype has significant high basal level of POD as compared to susceptible line and could be used as marker to define KB resistance. Hence, the S2 stage of wheat spike of susceptible genotype is more prone to KB infection as compared to resistant genotype.

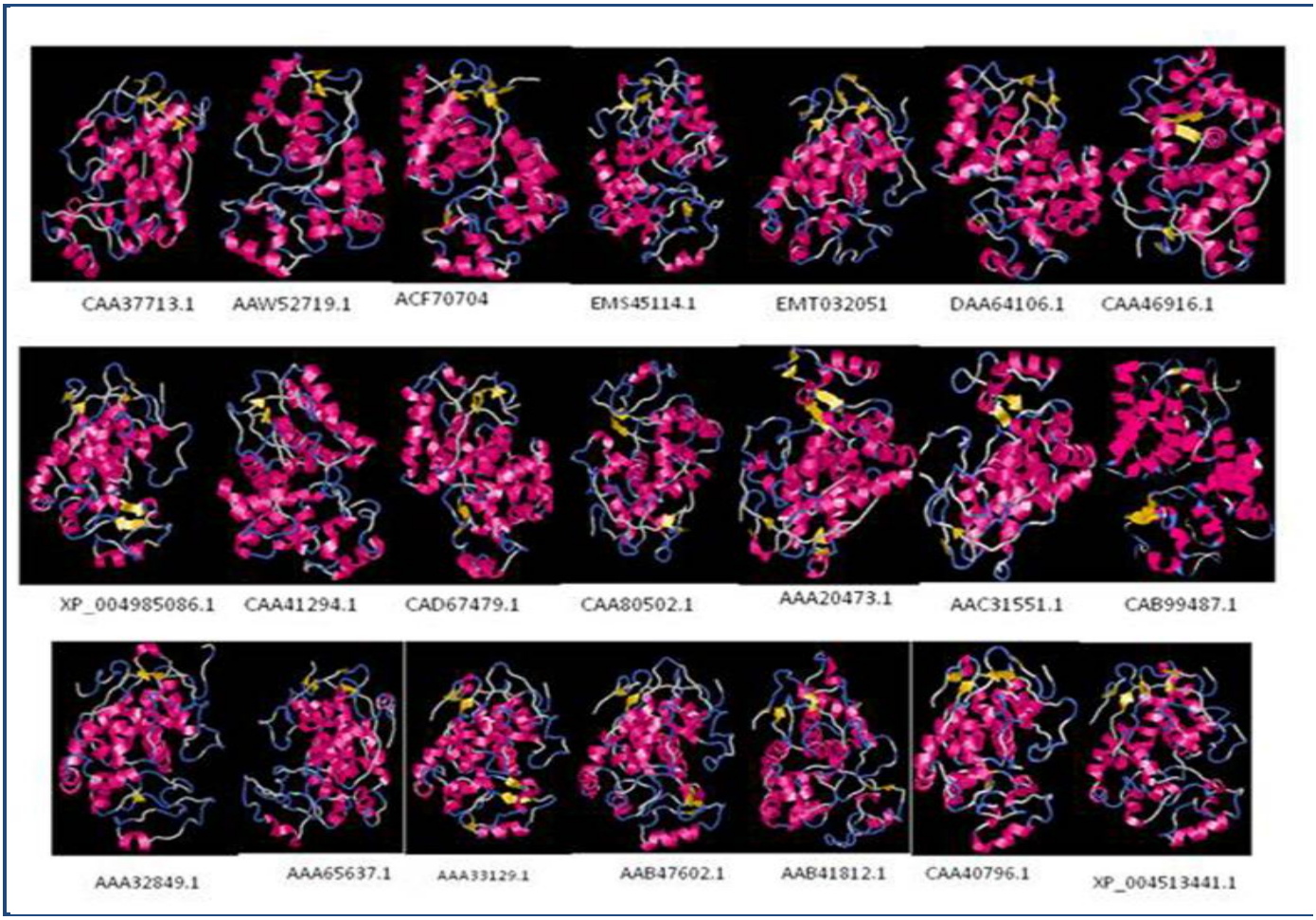

Figure 2: PDB structure of POD in selected plants.

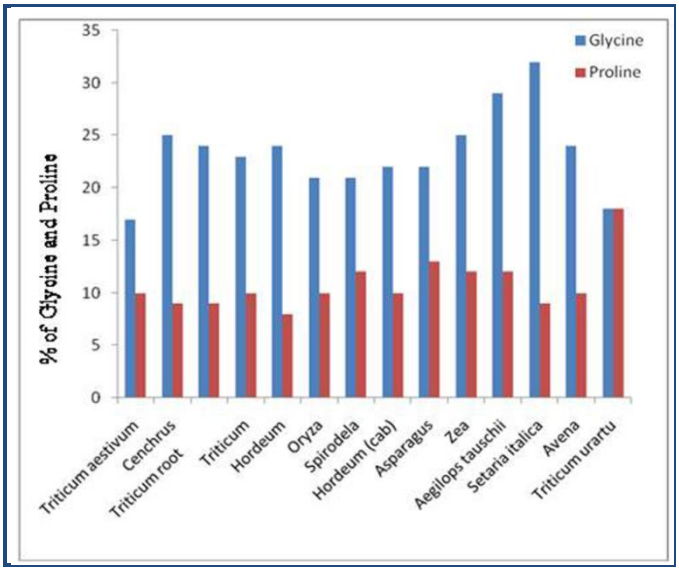

Figure 3: Glycine and Proline percentage of POD in selected plants

In silico Study of Peroxidase

Secondary structure properties, PDB Structure, Motif and Physicochemical characterization, for all the Peroxidase, were carried out by using various computational tools. SOPMA analysis was done for Peroxidase in all monocot selected plants and it showed a alpha helix occupied the largest part of the protein followed by, random coil, extended strand and beta turns, while in dicoty plant random coil occupied the largest part of the protein followed by, random coil, extended strand and beta turn **Table 2 (see supplementary material),** PDB structure also showed **(Figure 2).** High value for random coil bears important significance in the study of protein tertiary structure and related functions. Functional analysis of these proteins includes identification of important motifs **Table 3 (see supplementary material)**. There is two important motif found in both monocot and dicot plants first motif PS00436 and PS00435. PS00436 occur at starting sequence like 57-67 and PS00435 is found at middle sequence like 177 – 187. These motifs were 10 to 20 amino acids in length arise because specific residues and regions proved to be important for the biological function of a group of proteins, which are conserved in both structure and sequence during evolution. The ProtParam tool (http://web.expasy.org/protparam/) of ExPASy was used to compute glycine and proline composition (%), in the selected monocot plants. It was observed that the average percentage of glycine was higher than the proline. When compared the glycine and proline contain with in Poaceae family, *Setaria italic* have highest glycine contain while in *Triticum urartu* a diploid variety of *Triticum* have highest contain of proline. The amino acid proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental (biotic and abiotic) stresses **(Figure 3**).The

results of transgenic modifications of biosynthetic and metabolic pathways indicate that higher stress tolerance and the accumulation of compatible solutes may also protect plants against biotic and abiotic stress. The osmoprotectant role of proline has been verified in some crops by overexpressing genes involved in proline synthesis. The total number of positively (Arg + Lys) and negatively (Asp + Glu) charged residues of Peroxidase members were observed **Table 4 (see supplementary material)**. Peroxidase showed both positively and negatively charged nature, varies with their Isoelectric point. Some monocot plant Peroxidase showed higher positively charged like *Triticum aestivum*, *Cenchrus ciria, Avena*, and *Asparagus*. This possible variation might be due to their isoelectric point in acidic range. For the remaining members, the isoelectric point was within alkaline range. For the separation of the protein on a polyacrylamide gel the computed isoelectric point will be useful. Extinction coefficient for all Peroxidase was observed with in a same range except *Avena* and *Medicago*. High extinction coefficient means higher concentration of lysine, tryptophan and tyrosine. for the calculation of protein concentration in the solution the extinction coefficient can be useful. Stability of protein is described in terms of its stability index whether a protein is stable or not, can be described by its instability index. Instability index of root peroxidase of *Triticum aestivum*, and Cucumis is higher than 40 and thus describing these proteins unstable. It is noteworthy that high aliphatic index was observed for all plants Peroxidase. The higher aliphatic index indicates higher concentration of alanine, valine, isoleucine and leucine occupying the relative volume of a protein**.** Grand Average of Hydropathy (GRAVY) was computed for all the members. GRAVY index indicate the solubility of protein and its range was observed from 0.171 to -0.089 in selected plants. A positive and negative GRAVY value for POD designates it to be hydrophobic and hydrophilic in nature of Peroxidase respectively. Most of selected plant a negative GRAVY value for POD designates it to be hydrophilic in nature but diversity occurs in nature **(Table 4)**.

Domain and MEME Analysis

In silico studies revealed that peroxidase belong to the Domains (functional and/or structural units of a Protein) cl00196 (CDD accession). Heme-dependent peroxidases similar to plant peroxidases **(Figue 4)** these enzymes belong to a group of peroxidases containing a heme prosthetic group (ferriprotoporphyrin IX), which catalyzes a multistep oxidative reaction involving hydrogen peroxide as the electron acceptor. Peroxidases are found in the extracellular space or in the vacuole in plants where they have been implicated in hydrogen peroxide detoxification, auxin catabolism and lignin biosynthesis **[16],** and stress response [**17**]. Multiple Expectation-Maximization for Motif Elicitation (MEME) **[15]** is a suite of tools for motif discovery and searching. Twenty five different motifs (subdomain) between 6 and 50 residues were detected and distributed by MEME software. One to ten motifs sharing by almost all the plant groups with the few exception (**Figure 5**). Motifs one are almost conserved and found in every group and subgroup. These conserved motifs could be the essential elements determining the POD family's common molecular function among different plant species. Motif 19 absent all the monocotyledon plants. When compared a most conserved motifs in a *Triticum* group, Motifs 9 absent only in *T. aestivum*, Motif 5 absent in root part of *T. aestivum*. While Motifs 3&6 and2 & 10 absent in *T.monoccocum* and *T. ururtus*. Motif 16, 19 occurs only found in Brassicaceae plants. Unique and Absence of Motifs are either, substitution, accumulation of mutation or subjected to rearrangements. It is not necessary that changes their activity, because that do not have a direct impact on the active site contain altered residues.

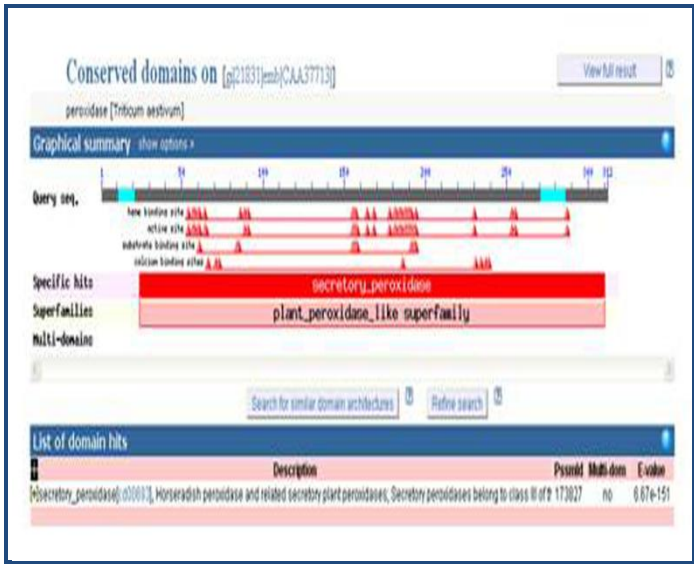

Figure 4: Functional domain analysis of POD in selected plants.

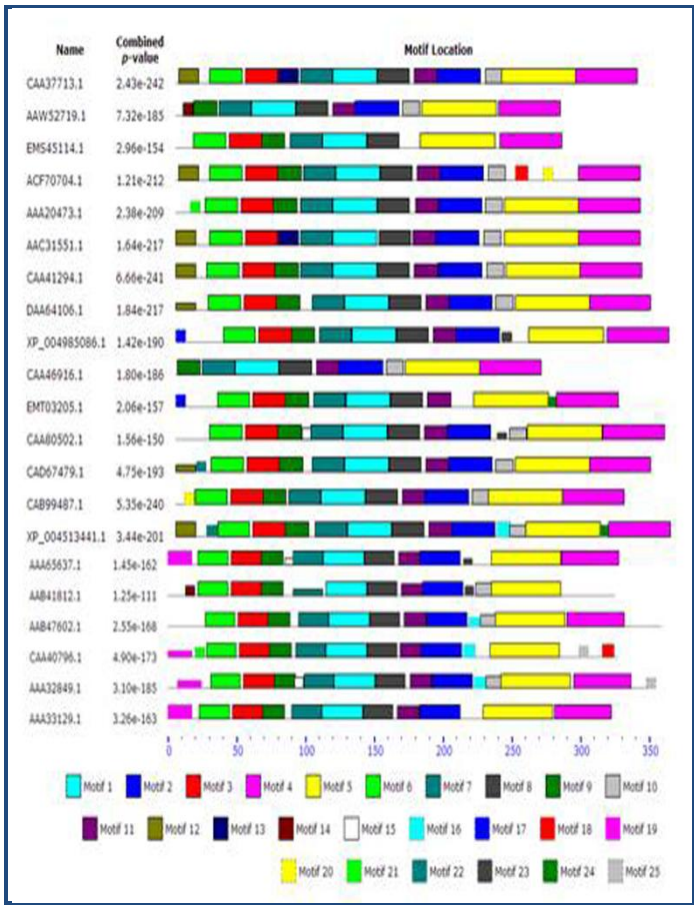

Figure 5: Schematic diagram of motif distribution of POD Proteins. MEME4.6.0 was applied to show that different sub groups were distinguished by the motif distribution.

Phylogenyn analysis of Peroxidase of Triticum aestivum with other Plants

Homology analysis depicts two main clusters A and B **(Figure 6).** On the basis of phylogenetical analysis it can conclude that overall 51.95% homology is present in monocot plants. From the thirteen species, eleven species present in cluster A and is divided in two sub-clad (A1 and A2) on the basis of homology. Cluster A, first group include *Cenchrus, Zea mays, Triticum, Oryza Avena,* consist 64 % homology and other that include, *Avena sativa, Triticum aestivum* , root peroxidase *Triticum aestivum, Hordeum and Hordeum vulgare subsp. vulgare* consist 77% homology. When homology determine in poacease family with respect to *Triticum aestivum* it observe that 84% homology present in peroxidase taken from root and leaf. Cluster B also divided in to sub-clad (B1 and B2). Monocot four species like *Triticum urartu, Setaria italica, Spirodela polyrhiza, and Asparagus* present in Clustur B along with dicot plants. This result suggests that POD genes were transfer from dicotyledonous plants to monocotyledonous or vice versa.

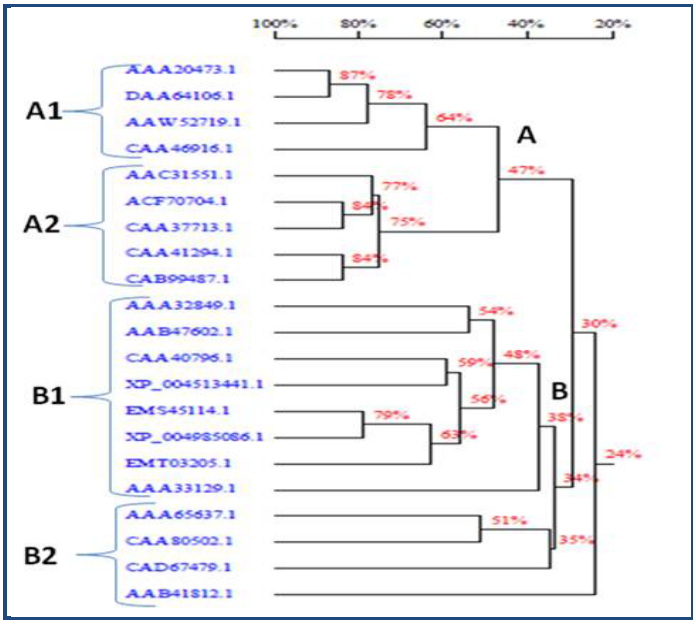

Figure 6: Homology tree of POD with selected plants.

Conclusion:

The function of POD in plant defense range from pre-formed or inducible. POD activity was significantly dominant in resistant genotype than susceptible. The expression of POD was significantly higher ($p<.05$) in S_2 stage and in subsequent stages. There was increasing trend in Sv and S_2 stages followed by decrease in S_3 stage of both genotypes indicating the enhanced lignification of cell walls in developing spikes after fertilization and grain development. In this study, physico-chemical properties, secondary structural features and phylogenetic classification will provide an insight for the biologists working with POD in order to understand the functionality of enzymes. The POD is expressed constitutively in both susceptible and resistant genotype. However, the activity was higher in resistant genotype indicating that resistant genotype has significant high basal level of these enzymes as compared to susceptible line and could be used as marker to define KB resistance.

References:

[1] Gijzen M, *Plant Physiol*. 1993 **103**: 1061 [PMID: 12232000]
[2] Sgherri CLM *et al. J Plant Physiol*. 2000 **157**: 273 DOI: 10.1016/S0176-1617(00)80048-6
[3] Escribano J *et al. J Agric Food Chem*. 2002 **50**: 6123 DOI: 10.1021/jf020356p
[4] Lagrimini LM *et al. Plant Mol Biol*. 1997 **33**: 887 [PMID: 9106512]
[5] Mojtaba Mohammadi & Homayoon Kazem, *Plant Science* 2002 **162**: 491 DOI: 10.1016/S0168-9452(01)00538-6
[6] Andrew C, *Cuming New Phytologist*. 2009 **183**: 237 [PMID: 19594699]
[7] Caruso C *et al. Phytochemistry.* 2001 **58**: 743 [PMID: 11672739]
[8] Ye XY & Ng TB, *Life Sci*. 2002 **71:** 1667 [PMID: 12137913]
[9] Ghosh M, *Ann Bot*. 2006 **98**: 1145 [PMID: 17056613]
[10] Csiszár J *et al. Plant Physiology and Biochemistry*. 2012 **52:** 119 [PMID: 22305075]
[11] Purwar S *et al. Mol Biol Rep*. 2010 **37**: 1377 [PMID: 1934760]
[12] Chandra A *et al. Acta Physiol Plant*. 2007 **29:** 361 DOI 10.1007/s11738-007-0045-2
[13] Timothy LB, *Current Protocols in Bioinformatics*. 2002 DOI: 10.1002/0471250953.bi0204s00
[14] Lange H *et al. European Polymer Journal*. 2013 **49:** 1151 DOI: 10.1016/j.eurpolymj.2013.03.002
[15] Jang IC, *Plant Physiol Biochem*. 2004 **42**: 451 [PMID: 15191750]
[16] Rosa M Pérez-Clemente *et al. Biomed Res Int*. 2013 **2013**: 654 [PMID: 23509757]
[17] Kristensen BK *et al. Plant Sci.* 1997 **122173:** 182 DOI 10.1016/S0168-9452(96)04554-2

Edited by P Kangueane

Citation: **Purwar *et al.*** Bioinformation 10(2): 087-093 (2014)

BIOINFORMATION

open access

Supplementary material:

Table 1: Selected Monocotyledonous and Dicotyledonous plant with Accession Number

Sl. No.	Plant Name	Accession No.
1	Triticum aestivum	CAA37713.1
2	Triticum monococcum	AAW52719.1
3	Triticum urartu	EMS45114.1
4	root peroxidase Triticum aestivum	ACF70704
5	Cenchrus ciliaris	AAA20473.1
6	Avena sativa	AAC31551.1
7	Hordeum vulgare	CAA41294.1
8	Zea mays	DAA64106.1
9	Setaria italica	XP_004985086.1
10	Aegilops tauschii	EMT032051
11	Hordeum vulgare subsp. vulgare	CAB99487.1
12	Oryza sativa Japonica	CAA46916.1
13	Spirodela polyrhiza	CAA80502.1
14	Asparagus officinalis	CAD67479.1
15	Solanum lycopersicum	AAA65637.1
16	Medicago sativa	AAB41812.1
17	Linum usitatissimum	AAB47602.1
18	Armoracia rusticana	CAA40796.1
19	Arabidopsis thaliana	AAA32849.1
20	Cucumis sativus	AAA33129.1
21	Cicer arietinum	XP_004513441.1

Table 2: Secondary structure prediction of Peroxidase through SOPMA

Sl. no	Protein	α Helix	310 Helix	Pi Helix	B Bridge	Extended Strand	β Turn	Bend Region	Random Coil	Ambiguous States	Other States
	Cenchrus	134	0	0	0	40	21	0	118	0	0
	Triticum	93	0	0	0	40	16	0	110	0	0
	Triticum root	133	0	0	0	42	17	0	122	0	0
	Triticum	128	0	0	0	46	17	0	121	0	0
	Hordeum	130	0	0	0	42	18	0	125	0	0
	Oryza	144	0	0	0	44	16	0	113	0	0
	Spirodela	140	0	0	0	49	13	0	127	0	0
	Hordeum	122	0	0	0	44	21	0	116	0	0
	Asparagus	124	0	0	0	40	18	0	138	0	0
	Zea	132	0	0	0	44	16	0	128	0	0
	Triticum urartu	98	0	0	0	44	11	0	107	0	0
	Aegilops tauschii	116	0	0	0	52	12	0	112	0	0
	Setaria italica	143	0	0	0	48	17	0	124	0	0
	Avena	133	0	0	0	34	13	0	134	0	0
	Arabidopsis	147	0	0	0	49	18	0	140	0	0
	Cucumis	142	0	0	0	41	15	0	124	0	0
	Solanum	138	0	0	0	50	14	0	126	0	0
	Medicago	129	0	0	0	45	15	0	136	0	0
	Linum	141	0	0	0	49	16	0	153	0	0
	Armoracia	131	0	0	0	44	19	0	133	0	0
	Cicer arietinum	137	0	0	0	44	18	0	136	0	0

Table 3: Motif prediction of Peroxidase in selected plants

S. no	Protein	Motif ID	Start	End	Active site	Motif ID	Start	End	heme-ligand signature
1	*Triticum aestivum*	PS00436	56	67	GAslLRLhFHDC	PS00435	177	187	DMVALSGAHTI
2	*Triticum urartu*	PS00436	45	56	GAslLRLhFHDC	PS00435	-	-	-
3	*Root peroxidase of Triticum aestivum*	PS00436	56	67	GAslLRLhFHD	PS00435	179	189	DMVALSGAHT
4	*Triticummonococcum*	PS00436	-	-	-	PS00435	122	132	DMVALSGAHT
5	*Avena*	PS00436	56	67	GAslLRLhFHD	PS00435	-	-	-
6	*Cenchrus*	PS00436	53	64	GAslLRLhFHDC	PS00435	178	188	EMVALSGAHT

7	*Hordeum*	PS00436	54	65	GAslLRLhFHD	PS00435	178	188	DMVALSGAHT
8	*Oryza*	PS00436	-	-	-	PS00435	111	121	DMVALSGAHT
9	*Spirodela*	PS00436	56	67	GGplLRLfFHD	PS00435	184	194	DLVLLSGGHTI
10	*Hordeum*	PS00436	46		GAslLRLhFHD	PS00435	169	179	DMVALSGAHT
11	*Asparagus*	PS00436	57	68	GAslLRLfFHD	PS00435	185	195	EMVALVGAHT
12	*Zea*	PS00436	55	66	GAslLRLhFHD	PS00435	185	195	EMATLSGAHTI
13	*Setaria italica*	PS00436	65	76	GAslLRLhFHDC	PS00435	190	200	DVVVLSGGHTI
14	*Aegilops tauschii*	PS00436	-	-	GAslLRLhFHDC	PS00435	-	-	-
15	*Arabidopsis*	PS00436	64	75	AGsiLRLhFHDC	PS00435	193	203	DLVALSGAHTF
16	*Cucumis*	PS00436	56	67	GAkiIRLhFHDC	PS00435	184	194	DLVALSGAHT
17	*Solanum*	PS00436	55	66	AAaiLRMhFHD	PS00435	184	194	DLVLLSGAHT
18	*Medicago*	PS00436	55	66	VPatLRLfFHDC	PS00435	186	196	EMIALSGAHTV
19	*Linum*	PS00436	-	-	-	PS00435	189	199	DLVALSGAHT
20	*Cicer arietinum*	PS00436	61	72	AAslLRLhFHDC	PS00435	187	197	DLVTLSGAHTI
21	*Armoracia*	PS00436	61	72	AAslIRLhFHDC	PS00435	185	195	DVVALSGAHTF

Table 4: Various parameters computed using Expasy's ProtParam tool of Peroxidase in selected plants

S. No.	Plant Protein	Accession No.	Number of amino acids	Molecular weight	pI	-R	+R	EC	II	AI	Gravy
1	Triticumaestivum	CAA37713.1	312	32381.5	8.38	19	22	11960	30.78	83.59	0.068
2	Cenchrus	AAA20473	313	32494.6	7.57	21	22	11960	35.01	81.50	0.046
3	Avena	AAC31551.1	314	32338.3	8.58	19	23	6460	36.81	80.32	0.012
4	Triticum urartu	EMS45114.1	352	38106.0	4.98	31	23	14940	31.74	88.95	0.004
5	Triticum	AAW52719.1	259	27550.9	5.75	21	18	11835	31.53	78.34	-0.089
6	Triticum root	ACF70704.	314	32559.0	9.79	15	31	9230	44.80	75.29	-0.065
7	Hordeum	CAA41294.1	315	32976.0	6.07	24	22	14940	32.74	82.19	0.008
8	Oryza	CAA46916.1	317	32875.7	5.77	23	20	11960	30.48	83.25	0.030
9	Spirodela	CAA80502.1	329	35586.4	6.65	35	34	11960	35.07	89.94	-0.017
10	Hordeum	CAB99487.1	303	32162.1	5.73	29	25	11960	35.92	79.60	-0.100
11	Asparagus	CAD67479.1	320	33946.2	9.10	23	31	18950	39.75 S	75.59	-0.184
12	Zea	DAA64106.1	320	33212.2	6.18	21	19	13575	37.55	81.25	0.046
13	Aegilops tauschii	EMT03205.1	300	32228.4	4.68	38	25	21025	29.44	87.03	-0.028
14	Setaria italica	XP_004985086.1	332	34680.1	5.54	34	29	9690	31.09	91.17	0.036
15	Arabidopsis	AAA32849.1	354	38941.2	6.42	31	30	20565	32.47	85.11	-0.066
16	Cucumis	AAA33129.1	322	34297.2	4.94	31	23	11960	42.45	80.87	-0.153
17	Solanum	AAA65637.1	328	35995.0	7.52	35	36	14940	35.66	83.23	-0.103
18	Medicago	AAB41812.1	325	35931.1	9.11	28	37	9440	29.35	84.31	-0.171
19	Linum	AAB47602.1	359	38196.9	4.69	33	22	11960	35.90	85.04	0.031
20	Armoracia	CAA40796.1	327	35126.2	7.48	29	30	13575	27.21	96.64	0.127
21	Cicer arietinum	XP_004513441.1	335	37355.1	4.67	43	27	27555	45.80	86.15	-0.150