Plant Signaling & Behavior 5:12, 1543-1546; December 2010; © 2010 Landes Bioscience

Genome-wide analysis of thioredoxin fold superfamily peroxiredoxins in Arabidopsis and rice

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Key words: antioxidant defense, chloroplast, gene family, oxidative stress, reactive oxygen species

A broad range of peroxides generated in subcellular compartments, including chloroplasts, are detoxified with peroxidases called peroxiredoxins (Prx). The Prx are ubiquitously distributed in all organisms including bacteria, fungi, animals and also in cyanobacteria and plants. Recently, the Prx have emerged as new molecules in antioxidant defense in plants. Here, the members which belong to Prx gene family in Arabidopsis and rice are been identified. Overall, the Prx members constitute a small family with 10 and 11 genes in Arabidopsis and rice respectively. The *prx* genes from rice are assigned to their functional groups based on homology search against Arabidopsis protein database. Deciphering the Prx functions in rice will add novel information to the mechanism of antioxidant defense in plants. Further, the Prx also forms the part of redox signaling cascade. Here, the Prx gene family has been described for rice.

The formation of free radicals and reactive oxygen species (ROS) occur in several enzymatic and non-enzymatic reactions during cellular metabolism. The accumulation of these reactive and deleterious intermediates is suppressed by antioxidant defense mechanism comprised of low molecular weight antioxidants and enzymes. In photosynthetic organisms, the defense against the damage from free radicals and oxidative stress is crucial. For instance, the ROS production occurs in, photosystem II with generation of singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2),[1,2] photosystem I from superoxide anion radicals (O_2^-),[3] and during photorespiration with generation of H_2O_2.[4] ROS production may exceed under environmental stress conditions like excess light, low temperature and drought.[5]

The antioxidant defense mechanism is activated by antioxidant metabolities and enzymes which detoxify ROS and lipid peroxides. The detoxification of ROS can occur in various cellular compartments such as chloroplasts, mitochondria, peroxisomes and cytosol.[6] The enzymes like ascorbate peroxidase, catalase, glutathione peroxidase and superoxide dismutase are prominent antioxidant enzymes.[6] The peroxiredoxins (Prx) emerged as new components in the antioxidant defense network of barley.[7,8] Later, Prx were studied in other plants.[9-14]

Prx can be classified into four different functional groups, PrxQ, 1-Cys Prx, 2-Cys Prx and Type-2 Prx.[15,16] They are members of the thioredoxin fold superfamily.[17,18] In this study, the *prx* genes found in Arabidopsis and rice genomes are been identified. The Arabidopsis genome encodes 10 *prx* genes classified into four functional categories, 1-Cys Prx, 2-Cys Prx, PrxQ and Type-2 Prx.[13] Of these, one each of 1-Cys Prx and PrxQ, two of 2-Cys Prx (2-Cys PrxA and 2-Cys PrxB) and six Type-2 Prx (PrxA-F) are identified[13] (**Table 1**). The members of Type-2 Prx category are more in number in Arabidopsis (**Table 1**).

In rice (rice.plantbiology.msu.edu/), there are 11 genomic loci which encode for Prx proteins (**Table 2**). These loci are distributed on chromosomes 1, 2, 4, 6 and 7 (**Table 2**). The two Prx, peroxiredoxin-2E-1 (LOC_Os01g24740) and 2-Cys peroxiredoxin BAS1 (LOC_Os04g33970) are annotated to contain a chloroplast precursor (**Table 2**). The rest of Prx genes are been annotated as "peroxiredoxin, putative, expressed" (**Table 2**). During the study, these genes are assigned to their functional groups based on BLAST searches against Arabidopsis protein database. The **Table 3** summarizes these results wherein rice LOCs and their corresponding homologs in Arabidopsis are enlisted. A nomenclature for rice *prx* genes is been proposed based on protein homology %identity/similarity with their Arabidopsis counterparts (**Tables 1 and 3**). Interestingly, a new *prx* gene (LOC_Os07g15670) annotated as "peroxiredoxin, putative, expressed" is identified making the tally of prx genes to eleven in rice as compared to ten in Arabidopsis (**Tables 1 and 2**). The BLAST search has identified its counterpart in Arabidopsis which has been annotated as "antioxidant/oxidoreductase" (AT1G21350) in the TAIR database (www.arabidopsis.org). The rice LOC_Os07g15670 and Arabidopsis AT1G21350 share protein homology %68/78 for 236 amino acids (**Table 3**).

The protein alignment study of Prx members in rice with the canonical Prx2-B and Prx2-E of Arabidopsis is shown in

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Submitted: 08/31/10; Accepted: 09/01/10

DOI: 10.4161/psb.5.12.13494

Table 1. A list of genes encoding peroxiredoxins in *Arabidopsis thaliana* L.

Locus	Annotation	Synonym	A*	B*	C*
AT1G48130	1-Cysteine peroxiredoxin 1 (ATPER1)	1-Cys Prx	216	24081.3	6.603
AT1G60740	Peroxiredoxin type 2	Type-2 PrxD	162	17471.9	5.2297
AT1G65970	Thioredoxin-dependent peroxidase 2 (TPX2)	Type-2 PrxC	162	17413.9	5.2297
AT1G65980	Thioredoxin-dependent peroxidase 1 (TPX1)	Type-2 PrxB	162	17427.8	4.9977
AT1G65990	Type 2 peroxiredoxin-related	Type-2 PrxA	553	62653.6	6.4368
AT3G06050	Peroxiredoxin IIF (PRXIIF)	Type-2 PrxF	201	21445.2	9.3905
AT3G11630	2-Cys Peroxiredoxin A (2CPA, 2-Cys PrxA)	2-Cys PrxA	266	29091.7	7.5686
AT3G26060	ATPRX Q, periredoxin Q	PrxQ	216	23677.8	10.0565
AT3G52960	Peroxiredoxin type 2	Type-2 PrxE	234	24684.0	9.572
AT5G06290	2-Cysteine Peroxiredoxin B (2CPB, 2-Cys PrxB)	2-Cys PrxB	273	29779.5	5.414

*A, amino acids; B, molecular weight; C, isoelectric point.

Table 2. Genes encoding peroxiredoxins in rice

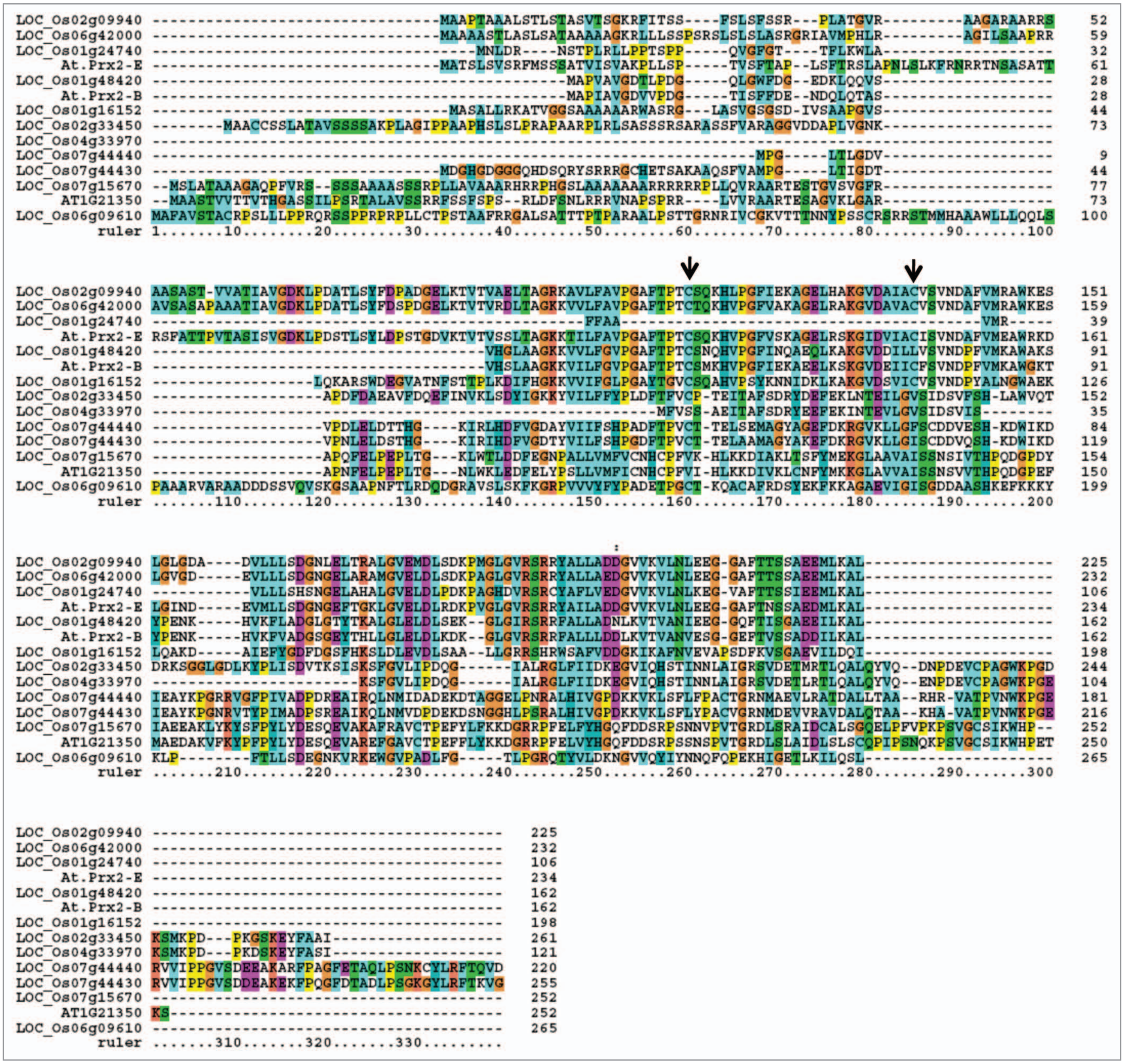
Chromosome	Locus Id	Putative function/Annotation	A*	B*	C*
1	LOC_Os01g16152	peroxiredoxin, putative, expressed	199	20873.6	8.2209
1	LOC_Os01g24740	peroxiredoxin-2E-1, chloroplast precursor, putative	107	11591.5	6.7906
1	LOC_Os01g48420	peroxiredoxin, putative, expressed	163	17290.8	5.6828
2	LOC_Os02g09940	peroxiredoxin, putative, expressed	226	23179.5	6.535
2	LOC_Os02g33450	peroxiredoxin, putative, expressed	262	28096.9	5.7709
4	LOC_Os04g33970	2-Cys peroxiredoxin BAS1, chloroplast precursor, putative, expressed	122	13410.2	4.3705
6	LOC_Os06g09610	peroxiredoxin, putative, expressed	266	28926	10.5097
6	LOC_Os06g42000	peroxiredoxin, putative, expressed	233	23688.3	9.2059
7	LOC_Os07g15670	peroxiredoxin, putative, expressed	253	27684.6	9.8545
7	LOC_Os07g44440	peroxiredoxin, putative, expressed	221	24232.6	5.3618
7	LOC_Os07g44430	peroxiredoxin, putative	256	27785.3	6.8544

*A, amino acids; B, molecular weight; C, isoelectric point.

Table 3. Identification of rice homologs of peroxiredoxins in *A. thaliana*

Locus Id (Os*)	Homolog (At*)	Nomenclature	Identitity/Similarity (%)	No. of aa* compared
LOC_Os01g16152	AT3G06050	Type-2 PrxF	73/84	201
LOC_Os01g24740	AT1G65980	Type-2 PrxB	42/59	77
LOC_Os01g48420	AT1G65970	Type-2 PrxC	74/86	162
LOC_Os02g09940	AT1G60740	Type-2 PrxD	56/72	166
LOC_Os02g33450	AT5G06290	2-Cys Prx B	74/82	272
LOC_Os04g33970	AT3G11630	2-Cys PrxA	92/96	88
LOC_Os06g09610	AT3G26060	PrxQ	78/89	159
LOC_Os06g42000	AT3G52960	Type-2 PrxE	61/74	240
LOC_Os07g15670	AT1G21350	Antioxidant	68/78	236
LOC_Os07g44440	AT1G65990	Type-2 PrxA	27/44	83
LOC_Os07g44430	AT1G48130	1-Cys Prx	69/83	221

*Os, *Oryza sativa* L.; At, *Arabidopsis thaliana* L.; aa, amino acids.



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LOC_Os02g09940 --------------------------------MAAPTAAALSTLSTASVTSGKRFITSS----FSLSFSSR---PLATGVR---------AAGARAARRS   52
LOC_Os06g42000 --------------------------------MAAAASTLASLSATAAAAAGKRLLLSSPSRSLSLSLASRGRIAVMPHLR---------AGILSAAPRR   59
LOC_Os01g24740 ------------------------------------MNLDR----NSTPLRLLPPTSPP-----QVGFGT----TFLKWLA-------------------   32
     At.Prx2-E --------------------------------MATSLSVSRFMSSSATVISVAKPLLSP-----TVSFTAP--LSFTRSLAPNLSLKFRNRRTNSASATT   61
LOC_Os01g48420 ----------------------------------------------MAPVAVGDTLPDG-----QLGWFDG--EDKLQQVS-------------------   28
     At.Prx2-B ----------------------------------------------MAPIAVGDVVPDG-----TISFFDE--NDQLQTAS-------------------   28
LOC_Os01g16152 ---------------------------------MASALLRKATVGGSAAAAAARWASRG---LASVGSGSD-IVSAAPGVS-------------------   44
LOC_Os02g33450 --------MAACCSSLATAVSSSSAKPLAGIPPAAPHSLSLPRAPAARPLRLSASSSRSARASSFVARAGGVDDAPLVGNK-------------------   73
LOC_Os04g33970 ----------------------------------------------------------------------------------------------------
LOC_Os07g44440 -------------------------------------------------------------------MPG-----LTLGDV-------------------    9
LOC_Os07g44430 --------------------------------MDGHGDGGGQHDSQRYSRRRGCHETSAKAAQSFVAMPG-----LTIGDT-------------------   44
LOC_Os07g15670 --MSLATAAAGAQPFVRS--SSSAAAASSSRPLLAVAAARHRRPHGSLAAAAAAARRRRRRPLLQVRAARTESTGVSVGFR-------------------   77
     AT1G21350 --MAASTVVTTVTHGASSILPSRTALAVSSRRFSSFSPS--RLDFSNLRRRVNAPSPRR----LVVRAARTESAGVKLGAR-------------------   73
LOC_Os06g09610 MAFAVSTACRPSLLLPPRQRSSPPRPRPLLCTPSTAAFRRGALSATTTPTPARAALPSTTGRNRIVCGKVTTTNNYPSSCRSRRSTMMHAAAWLLLQQLS  100
         ruler 1.......10........20........30........40........50........60........70........80........90.......100

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LOC_Os02g09940 AASAST-VVATIAVGDKLPDATLSYFDPADGELKTVTVAELTAGRKAVLFAVPGAFTPTCSQKHLPGFIEKAGELHAKGVDAIACVSVNDAFVMRAWKES  151
LOC_Os06g42000 AVSASAPAAATIAVGDKLPDATLSYFDSPDGELKTVTVRDLTAGKKVVLFAVPGAFTPTCTQKHVPGFVAKAGELRAKGVDAVACVSVNDAFVMRAWKES  159
LOC_Os01g24740 ------------------------------------------------FFAA----------------------------------------VMR-----   39
     At.Prx2-E RSFATTPVTASISVGDKLPDSTLSYLDPSTGDVKTVTVSSLTAGKKTILFAVPGAFTPTCSQKHVPGFVSKAGELRSKGIDVIACISVNDAFVMEAWRKD  161
LOC_Os01g48420 -------------------------------------VHGLAAGKKVVLFGVPGAFTPTCSNQHVPGFINQAEQLKAKGVDDILLVSVNDPFVMKAWAKS   91
     At.Prx2-B -------------------------------------VHSLAAGKKVILFGVPGAFTPTCSMKHVPGFIEKAEELKSKGVDEIICFSVNDPFVMKAWGKT   91
LOC_Os01g16152 ------------------LQKARSWDEGVATNFSTTPLKDIFHGKKVVIFGLPGAYTGVCSQAHVPSYKNNIDKLKAKGVDSVICVSVNDPYALNGWAEK  126
LOC_Os02g33450 -------------------APDFDAEAVFDQEFINVKLSDYIGKKYVILFFYPLDFTFVCP-TEITAFSDRYDEFEKLNTEILGVSIDSVFSH-LAWVQT  152
LOC_Os04g33970 --------------------------------------------------------MFVSS-AEITAFSDRYEEFEKINTEVLGVSIDSVIS--------   35
LOC_Os07g44440 -------------------VPDLELDTTHG----KIRLHDFVGDAYVIIFSHPADFTPVCT-TELSEMAGYAGEFDKRGVKLLGFSCDDVESH-KDWIKD   84
LOC_Os07g44430 -------------------VPNLELDSTHG----KIRIHDFVGDTYVILFSHPGDFTPVCT-TELAAMAGYAKEFDKRGVKLLGISCDDVQSH-KDWIKD  119
LOC_Os07g15670 -------------------APQFELPEPLTG---KLWTLDDFEGNPALLVMFVCNHCPFVK-HLKKDIAKLTSFYMEKGLAAVAISSNSIVTHPQDGPDY  154
     AT1G21350 -------------------APNFELPEPLTG---NLWKLEDFELYPSLLVMFICNHCPFVI-HLKKDIVKLCNFYMKKGLAVVAISSNSVVTHPQDGPEF  150
LOC_Os06g09610 PAAARVARAADDDSSVQVSKGSAAPNFTLRDQDGRAVSLSKFKGRPVVVYFYPADETPGCT-KQACAFRDSYEKFKKAGAEVIGISGDDAASHKEFKKKY  199
         ruler .......110.......120.......130.......140.......150.......160.......170.......180.......190.......200

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LOC_Os02g09940 LGLGDA----DVLLLSDGNLELTRALGVEMDLSDKPMGLGVRSRRYALLADDGVVKVLNLEEG-GAFTTSSAEEMLKAL---------------------  225
LOC_Os06g42000 LGVGD-----EVLLLSDGNGELARAMGVELDLSDKPAGLGVRSRRYALLAEDGVVKVLNLEEG-GAFTTSSAEEMLKAL---------------------  232
LOC_Os01g24740 -----------VLLLSHSNGELAHALGVELDLPDKPAGHDVRSRCYAFLVEDGVVKVLNLKEG-VAFTTSSIEEMLKAL---------------------  106
     At.Prx2-E LGIND-----EVMLLSDGNGEFTGKLGVELDLRDKPVGLGVRSRRYAILADDGVVKVLNLEEG-GAFTNSSAEDMLKAL---------------------  234
LOC_Os01g48420 YPENK-----HVKFLADGLGTYTKALGLELDLSEK--GLGIRSRRFALLADNLKVTVANIEEG-GQFTISGAEEILKAL---------------------  162
     At.Prx2-B YPENK-----HVKFVADGSGEYTHLLGLELDLKDK--GLGVRSRRFALLLDDLKVTVANVESG-GEFTVSSADDILKAL---------------------  162
LOC_Os01g16152 LQAKD-----AIEFYGDFDGSFHKSLDLEVDLSAA--LLGRRSHRWSAFVDDGKIKAFNVEVAPSDFKVSGAEVILDQI---------------------  198
LOC_Os02g33450 DRKSGGLGDLKYPLISDVTKSISKSFGVLIPDQG------IALRGLFIIDKEGVIQHSTINNLAIGRSVDETMRTLQALQYVQ--DNPDEVCPAGWKPGD  244
LOC_Os04g33970 -----------------------KSFGVLIPDQG------IALRGLFIIDKEGVIQHSTINNLAIGRSVDETLRTLQALQYVQ--ENPDEVCPAGWKPGE  104
LOC_Os07g44440 IEAYKPGRRVGFPIVADPDREAIRQLNMIDADEKDTAGGELPNRALHIVGPDKKVKLSFLFPACTGRNMAEVLRATDALLTAA--RHR-VATPVNWKPGE  181
LOC_Os07g44430 IEAYKPGNRVTYPIMADPSREAIKQLNMVDPDEKDSNGGHLPSRALHIVGPDKKVKLSFLYPACVGRNMDEVVRAVDALQTAA--KHA-VATPVNWKPGE  216
LOC_Os07g15670 IAEEAKLYKYSFPYLYDESQEVAKAFRAVCTPEFYLFKKDGRRPFELFYHGQFDDSRPSNNVPVTGRDLSRAIDCALSGQELPFVPKPSVGCSIKWHP--  252
     AT1G21350 MAEDAKVFKYPFPYLYDESQEVAREFGAVCTPEFFLYKKDGRRPFELVYHGQFDDSRPSSNSPVTGRDLSLAIDLSLSCQPIPSNQKPSVGCSIKWHPET  250
LOC_Os06g09610 KLP--------FTLLSDEGNKVRKEWGVPADLFG-----TLPGRQTYVLDKNGVVQYIYNNQFQPEKHIGETLKILQSL---------------------  265
         ruler .......210.......220.......230.......240.......250.......260.......270.......280.......290.......300

LOC_Os02g09940 ---------------------------------------  225
LOC_Os06g42000 ---------------------------------------  232
LOC_Os01g24740 ---------------------------------------  106
     At.Prx2-E ---------------------------------------  234
LOC_Os01g48420 ---------------------------------------  162
     At.Prx2-B ---------------------------------------  162
LOC_Os01g16152 ---------------------------------------  198
LOC_Os02g33450 KSMKPD---PKGSKEYFAAI-------------------  261
LOC_Os04g33970 KSMKPD---PKDSKEYFASI-------------------  121
LOC_Os07g44440 RVVIPPGVSDEEAKARFPAGFETAQLPSNKCYLRFTQVD  220
LOC_Os07g44430 RVVIPPGVSDDEAKEKFPQGFDTADLPSGKGYLRFTKVG  255
LOC_Os07g15670 ---------------------------------------  252
     AT1G21350 KS-------------------------------------  252
LOC_Os06g09610 ---------------------------------------  265
         ruler .......310.......320.......330.........
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Figure 1. Amino acid alignment of peroxiredoxins (Prx) in rice. The rice proteins are aligned with the canonical Arabidopsis Prx2-B and Prx2-E. The conserved cysteine residues are indicated by arrows on top of the alignment. Note the sequence conservation between the newly identified LOC_Os07g15670 and AT1G21350. The rice locus Ids are identified on left and amino acid positions on right. The alignment was made with ClustalX.

Figure 1. The Type-2 Prx proteins are characterized by the presence of catalytic cysteine (Cys) residues (**Fig. 1**). The alignment of rice Prx proteins shows that the Cys residue is well conserved in members like LOC_Os02g09940 (Type-2 PrxD), LOC_Os06g42000 (Type-2 Prx E), LOC_Os01g48420 (Type-2 Prx C), LOC_Os01g16152 (Type-2 Prx F), LOC_Os02g33450 (2-Cys Prx B), LOC_Os07g44440 (Type-2 Prx A), LOC_Os07g44430 (1-Cys Prx) and LOC_Os06g09610 (PrxQ) (**Fig. 1**). However, LOC_Os01g24740 (Type-2 PrxB) and LOC_Os04g33970 (2-Cys PrxA) which contain a chloroplast precursor do not have the catalytic Cys residues (**Fig. 1**). The newly identified LOC_Os07g15670 and AT1G21350 with annotations "peroxiredoxin, putative, expressed" and "antioxidant/oxidoreductase" respectively do not have catalytic Cys residues as well (**Fig. 1**).

Taken together, the results demonstrate that like Arabidopsis, the Prx constitute a small gene family in rice. However, the functional role of Prx in rice is not clearly understood.

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