Multiple abiotic stress responsive rice cyclophilin (OsCYP-25) mediates a wide range of cellular responses

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Abbreviations: ABA, abscisic acid; DEAD, aspartate-glutamate-alanine-aspartate; OsCYP-25, Oryza sativa cyclophilin; PPIase, peptidyl prolyl isomerase; qRT-PCR, quantitative real-time polymerase chain reaction

Cyclophilins (CYP), a member of immunophilin group of proteins, are more often conserved in all genera including plants. Here, we report on the identification of a new cyclophilin gene OsCYP-25 (LOC_Os09 g39780) from rice which found to be upregulated in response to various abiotic stresses viz., salinity, cold, heat and drought. It has an ORF of 540 bp, encoding a protein of 179 amino acids, consisting of PPIase domain, which is highly conserved. The OsCYP-25 promoter analysis revealed that different cis-regulatory elements (e.g., MYBCORE, MYC, CBFHV, GT1GSCAM4, DRECRTCOREAT, CCAATBOX1, WRKY71OS and WBOXATNPR1) are involved to mediate OsCYP-25 response under stress. We have also predicted interacting partners by STRING software. In interactome, protein partners includes WD domain containing protein, the 60S ribosomal subunit biogenesis protein, the ribosomal protein L10, the DEAD-box helicase, the EIF-2α, YT521-B protein, the 60S ribosomal protein and the PPR repeat domain containing protein. The in silico analysis showed that OsCYP-25 interacts with different proteins involved in cell growth, differentiation, ribosome biogenesis, RNA metabolism, RNA editing, gene expression, signal transduction or stress response. These findings suggest that OsCYP-25 might perform an important function in mediating wide range of cellular response under multiple abiotic stresses.

Introduction

Multiple abiotic stress factors viz., water deficit, water logging, high salinity, extreme temperature, high radiation, chilling injury, heavy metal toxicity and pH cause negative impact on plant growth and productivity by impairing proteins, lipids, DNA function and reducing genome stability.1 Abiotic stresses are assumed to be the main cause of yield reduction in various crops.2 4 The estimated potential yield losses due to abiotic stresses, namely, drought, salinity, high temperature, low temperature and other factors were reported as 17, 20, 40, 15 and 8%, respectively.5 5 Rice production necessitates to be doubled to overcome the increasing demands of the population.6 In light of these facts, the study of new genes responsive to abiotic stress conditions is still required.

The ability of plants to cope with high stress surroundings in their environment, involves a combination of phenotypic plasticity and genetic adjustment.7 8 All plants can sense and transmit stress stimuli signals to trigger different cell signaling cascades involved in ion channels regulation, kinase function, hormones like salicylic acid, ethylene, jasmonic acid, and abscisic acid action and pathways related reactive oxygen species (ROS) scavenging. These signaling events altogether induce expression of defense genes that finally lead to the entire defense reaction response.9 Although multiple reports support the genetic, molecular and physiological bases of how plants act in response to particular stress, it still remains poorly understood.10

Rice (Oryza sativa L.) is the dominant cereal crop in the world, since it is the most widely consumed staple food for more than three billion people, over half the world’s population.11 Nevertheless, rice plants are highly sensitive to high salt, drought and suboptimal temperature regimes.12 Since it has a comparatively small genome with respect to other cereals, a huge germplasm collection, broad array of genetic resources, and an effective transformation system, rice considered as a model cereal system.13 In light of this fact, the immediate attention is urgently required to find out the innovative genes responsive to different abiotic stress conditions to reduce yield loss. Previously, 28 members of cyclophilin gene family in rice have been described.14

The cyclophilin, a ubiquitous protein, involved in a wide range of cellular processes viz., cell division, transcriptional regulation, protein trafficking, cell signaling, pre-mRNA splicing, molecular chaperoning and stress tolerance.14 Cyclophilins, in general, contain a single PPIase domain. Nevertheless, there are few unusual domains viz., WD40 repeat containing domain (in CyP71 from Arabidopsis, LOC_Os08 g44330 from Rice), Leu Zipper and

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Table 1. Rice cyclophilin gene members and their putative orthologous genes from maize, Arabidopsis and Poplar

| S.No. | Rice locus ID   | Gene name     | Sub-cellular location | Maize orthologous | Arabidopsis orthologous | Poplar orthologous |
|-------|----------------|---------------|-----------------------|-------------------|-------------------------|--------------------|
| 1     | LOC_Os01 g02080 | OsCYP-1       | Chloroplast           | GRMZM2G132811     | AT1G74070               | POPTR_0012s05520   |
| 2     | LOC_Os02 g02890 | OsCYP-2       | Cytosol               | GRMZM2G162388     | AT4G34870               | POPTR_0009s13270   |
| 3     | LOC_Os01 g40050 | OsCYP-3       | Cytosol               | GRMZM2G036720     | AT4G33060               | POPTR_0018s0790    |
| 4     | LOC_Os02 g02900 | OsCYP-4       | Cytosol               | Not reported      | -                       | Not reported        |
| 5     | LOC_Os01 g18210 | OsCYP-5       | Chloroplast           | GRMZM2G063244     | AT3G62030               | POPTR_0002s18610   |
| 6     | LOC_Os02 g10970 | OsCYP-6       | Cytosol               | GRMZM2G006107     | AT3G63400               | POPTR_0006s26710   |
| 7     | LOC_Os02 g30624 | OsCYP-7       | Cytosol               | GRMZM2G159675     | AT2G43810               | POPTR_0008s07780   |
| 8     | LOC_Os02 g52360 | OsCYP-8       | Cytosol               | Not reported      | -                       | Not reported        |
| 9     | LOC_Os03 g10400 | OsCYP-9       | Cytosol               | GRMZM2G007486     | AT5G67530               | POPTR_0005s19290   |
| 10    | LOC_Os03 g59700 | OsCYP-10      | Chloroplast           | GRMZM2G057329     | AT2G38730               | POPTR_0001s06720   |
| 11    | LOC_Os05 g01270 | OsCYP-11      | Chloroplast           | GRMZM2G076544     | AT5G13120               | POPTR_0001s13480   |
| 12    | LOC_Os06 g94000 | OsCYP-12      | Cytosol               | GRMZM2G397044     | AT1G01940               | POPTR_0002s15070   |
| 13    | LOC_Os06 g11320 | OsCYP-13      | Cytosol               | GRMZM2G239306     | AT2G15790               | POPTR_0004s1570    |
| 14    | LOC_Os06 g45900 | OsCYP-14      | Cytosol               | Not reported      | -                       | Not reported        |
| 15    | LOC_Os06 g45910 | OsCYP-15      | Cytosol               | GRMZM2G416190     | AT1G53720               | POPTR_0005s10020   |
| 16    | LOC_Os06 g49470 | OsCYP-16      | Lumen                 | Not reported      | -                       | Not reported        |
| 17    | LOC_Os06 g49480 | OsCYP-17      | Mitochondria          | GRMZM2G084521     | AT1G56085               | POPTR_0001s25880   |
| 18    | LOC_Os07 g08190 | OsCYP-18      | Lumen                 | GRMZM2G070807     | AT3G63400               | POPTR_0002s04800   |
| 19    | LOC_Os07 g29390 | OsCYP-19      | Mitochondria          | GRMZM2G139210     | AT3G66654               | POPTR_0008s10530   |
| 20    | LOC_Os08 g05050 | OsCYP-20      | Cytosol               | Not reported      | -                       | Not reported        |
| 21    | LOC_Os08 g19610 | OsCYP-21      | Chloroplast           | GRMZM2G085885     | AT5G35100               | POPTR_0006s20430   |
| 22    | LOC_Os08 g44330 | OsCYP-22      | Cytosol               | GRMZM2G049525     | AT3G44600               | POPTR_0009s14870   |
| 23    | LOC_Os08 g44520 | OsCYP-23      | Cytosol               | Not reported      | AT2G36130               | POPTR_0016s07640   |
| 24    | LOC_Os09 g36670 | OsCYP-24      | Lumen                 | GRMZM2G158237     | AT4G34960               | POPTR_0004s17920   |
| 25    | LOC_Os09 g39780 | OsCYP-25      | Lumen                 | GRMZM2G170397     | AT3G56070               | POPTR_0078s00220   |
| 26    | LOC_Os10 g06630 | OsCYP-26      | Cytosol               | GRMZM2G401848     | -                       | Not reported        |
| 27    | LOC_Os10 g15370 | OsCYP-27      | Mitochondria          | Not reported      | -                       | Not reported        |
| 28    | LOC_Os11 g38990 | OsCYP-28      | Lumen                 | GRMZM2G078638     | AT1G26940               | POPTR_0010s01660   |

phosphatase binding domain (in thylakoid lumen cyclophilin TLP40)\(^{15}\) which play an important role in gene repression or gene silencing via interacting with other proteins.\(^{16,17}\) The WD40 repeat is a short structural motif of ~40 amino acids, usually terminating in a tryptophan-aspartic acid (WD) dipeptide. Cyclophilin 40 (CyP40) from mammal possesses a tetratricopeptide (TPR) domain that interacts with HSP-90, representing its essential role in signaling.\(^{18}\) The expression level of cyclophilin gene family under various abiotic stresses viz., salinity, cold, heat and drought and its correlation with different model genomes have also been defined.\(^{14}\)

### Results

**Genome sequence analysis of rice cyclophilin (OsCYP-25).**

Genomic sequence analysis of rice revealed 28 rice cyclophilin gene members. The cellular localization of all predicted rice cyclophilin gene members and their putative orthologous genes from maize, Arabidopsis and Poplar are described in Table 1. The genomic sequence of cyclophilin protein (OsCYP-25) (LOC_Os09 g39780) was received from the Rice Genome Annotation Project funded by NSF (http://rice.planbiology.msu.edu/). It represents an open reading frame (ORF) of 540 bp, to encode for a protein of 179 amino acids. It has an isoelectric point (pI) of 7.8 and the molecular mass of 19.19 kDa. The protein domain search, performed in the NCBI Conserved Domain Database (NCBICDD; http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml), revealed entire protein possesses the PPlase activity and so called PPlase domain-2 (9–172) of which few residues of complete protein sequence, highlighted as PPlase activity and so called PPlase activity and so called PPlase activity, are highly specific for PPlase activity (Fig. 1A). This gene is localized on chromosome 9 of rice and putatively expressed in two spliced form (LOC_Os09 g39780.1 and LOC_Os09 g39780.2) (Fig. 1B). The human cyclophilin A (PDB-ICWA) was used as template to generate model for cyclophilins from rice (LOC_Os09 g39780) using ExPasy swiss model web server (http://swissmodel.expasy.org). For visualization and editing of PDB models, molecular graphics visualization program PyMoL was used. It found to have a similar structural
pattern with 8 β-pleated sheets, 2 α-helices and β-loop region (Fig. 1C).

Finding the similarity index of rice cyclophilin (OsCYP-25) with other genera. The comparative study of amino acid sequences of OsCYP-25 was performed using the UniProt BlastP Service (http://www.uniprot.org/blast/), which revealed a 87.36 and 74% similarities with CyP-S from Sorghum sp and CyP-P from poplar (Liriodendron sp) (Table 2). Moreover, the rice cyclophilin (OsCYP-25) also showed considerable amino acid sequence similarity of 86.78, 85 and 82.84% with the cyclophilin from Zea mays (CyP-Z), Brachypodium sp (CyP-B), and Arabidopsis thaliana (CyP-A), respectively (Table 2).

In silico analysis of OsCYP-25 promoter region. The different stress and hormone responsive cis-regulatory elements were predicted in 1.0 kb upstream promoter region of OsCYP-25 (Fig. 2). The MYBCORE, MYC, CBFHV, GT1GMSCAM4, DRECRTCOREAT, and CCAATBOX1 elements in full length OsCYP-25 promoter region are abiotic stress-responsive which respond to dehydration, salt and heat stress conditions (Table 3). On the other hand, few hormone-responsive elements viz., WRKY71OS and WBOXATNPR1 of the promoter were examined to be involved in various stress response mediated through gibberelic acid, ABA and salicylic acid (Table 3).

Searching the predicted protein-protein interaction for rice cyclophilin (OsCYP-25). The STRING computer service (http://string-db.org/) was used to find out the predicted protein-protein interaction for OsCYP-25 rice cyclophilin. The results are graphically represented in Figure 3. The OsCYP-25 protein was expected to interact with ten different proteins: a WD domain containing protein (G-β-repeat domain), the 60S ribosome subunit biogenesis protein, the ribosomal protein L10, the DEAD-box ATP-dependent RNA helicase, the EIF-2 α, the hypothetical Protein, the expressed protein, the YT521-B, the 60S ribosomal protein-related and the PPR repeat domain containing protein (Fig. 3).

Transcript profile of OsCYP-25 under stress by quantitative real time PCR. The OsCYP-25 gene transcript is highly upregulated in response to major abiotic stresses like salt, heat, cold, and drought (Fig. 4A). The upregulation is also reported in drought and cold stress. The expression analysis of OsCYP-25...
associated with PPIase catalysis, of which three of these namely His-61, Arg-62 and Phe-120, are found to be extremely important for PPIase activity. Further, phylogenetic distance among plant cyclophilins according to average distance using percentage identity is also evident. Its potent role in protein folding and molecular chaperoning has clearly been illustrated. The rice cyclophilin (OsCYP-25) showed a homology of 60–70% with the human cyclophilin A (PDB-ICWA) indicating OsCYP-25 might be involved in various cellular functions such as mRNA processing, protein degradation, chaperoning arginine kinase folding and signal transduction. The CyP-S and CyP-P, possessing peptidyl prolyl cis-trans isomerase activity, played an important role in transcriptional regulation and cell signaling. The CyP-Z, CyP-B and CyP-A involved in signal transduction, protein trafficking and molecular chaperoning.

A number of cis-acting elements in the promoter regions were indispensable for transcriptional regulation of defense-related gene expression during biotic and abiotic stresses. The cis-acting elements have been identified from the stress- and hormone response-related gene promoters of different plant species. Here, in silico study of OsCYP-25 promoter relies on the fact that predicted cis-elements are similar with those showing similar stress response and certainly have a common function. We justified that the function of predicted cis-elements which are highly correlated with similar finding of several reports. These predicted cis-elements and their trans factors might mediate gene regulatory network functioning with respect to various abiotic stresses in a similar fashion of previous findings. This study suggests that OsCYP-25 promoter is a versatile and stress-responsive that functions under multiple abiotic stresses. Cyclophilins apart from playing an important role in protein folding may perform specific functions via interacting partner proteins in larger multi-component complexes. The search for their interacting partners under high stress plant response and thereby gene interference will provide in-depth understanding of their physiological roles and potential function in stress alleviation. Multiple studies have been focused to identify the interacting proteins and to elucidate their effects on peptidyl prolyl

| Species      | UniProt Acc. No. | % similarity | Putative length (aa) | Putative function                                                                 |
|--------------|------------------|--------------|----------------------|----------------------------------------------------------------------------------|
| Arabidopsis  | AT3G56070        | 82.84        | 169                  | rotamase cyclophilin 2 (ROC2) exhibiting peptidyl-prolyl cis-trans isomerase activity involved in signal transduction |
| Brachypodium | Bradi4g38880     | 85           | 175                  | peptidyl-prolyl cis-trans isomerase CYP19-3-like                                  |
| Maize        | GRMZM2G170397    | 86.78        | 174                  | Peptidyl-prolyl cis-trans isomerase                                               |
| Poplar       | POPTR_0070s00220 | 74           | 172                  | Peptidyl-prolyl cis-trans isomerase                                               |
| Sorghum      | Sb02g012590      | 87.36        | 174                  | Peptidyl-prolyl cis-trans isomerase                                               |

Table 2. Percentage of similarity between OsCYP-25 and related cyclophilins of other plant species

Discussion

Cyclophilins, a member of immunophilin group of proteins, are ubiquitous in all genera including plants. It is characterized by diverse forms and wide range of cellular functions viz., cell division, transcriptional regulation, protein trafficking, protein folding, cell signaling, pre-mRNA splicing, molecular chaperoning and stress tolerance. Here we report the response of OsCYP-25 from rice (LOC_Os09 g39780) against various abiotic stresses. The OsCYP-25 domain PPIase-1 contains peptidyl prolyl cis-trans isomerase activity that catalyzes the cis-trans isomerisation process of proline residues. The amino acid sequence of OsCYP-25 is highly similar (similarity score 71.51%) to OsCYP2 and other cyclophilins of various plant species where seven residues (His-61, Arg-62, Phe-120, Trp-128 and His-33) expression during biotic and abiotic stresses. The cis-acting elements have been identified from the stress- and hormone response-related gene promoters of different plant species. Here, in silico study of OsCYP-25 promoter relies on the fact that predicted cis-elements are like with those showing similar stress response and certainly have a common function. We justified that the function of predicted cis-elements which are highly correlated with similar finding of several reports. These predicted cis elements and their trans factors might mediate gene regulatory network functioning with respect to various abiotic stresses in a similar fashion of previous findings. This study suggests that OsCYP-25 promoter is a versatile and stress-responsive that functions under multiple abiotic stresses. Cyclophilins apart from playing an important role in protein folding may perform specific functions via interacting partner proteins in larger multi-component complexes. The search for their interacting partners under high stress plant response and thereby gene interference will provide in-depth understanding of their physiological roles and potential function in stress alleviation. Multiple studies have been focused to identify the interacting proteins and to elucidate their effects on peptidyl prolyl...
cyclophilin plays a significant role in abiotic stress tolerance.

Previously, it has already been reported that cyclophilin plays a significant role in abiotic stress tolerance. 

Cyclophilin (CYP20-3) from Arabidopsis was reported to be involved in light as well as other abiotic stress tolerance.

It has been also reported that OsCYP20-2 play vital role in response to various abiotic stresses.

Recently we have done structural analysis of abiotic stress induced cyclophilin gene from Piriformospora indica. Here we have shown that OsCYP-25 gene transcript is upregulated in response to various abiotic stresses ascertaining its various functions directly or indirectly involved in cellular response to multiple abiotic stresses.

| Cis regulatory element | Element number | Core sequence | Role |
|-----------------------|----------------|--------------|------|
| MYBCORE               | 2              | CNGTTR       | In response to dehydration |
| GT1GMSCAM4            | 3              | GAAAAA       | Salt stress and biotic stress responsive element |
| WRKY71OS              | 2              | TGAC         | Involved into Gibberelic acid and ABA mediated pathways |
| CCAATBOX1             | 2              | CCAAT/GGTTA  | In response to heat shock |
| CBHV                  | 2              | RYCGAC       | Binding site of DRE binding proteins; in response to dehydration |
| DRECRTCORATE          | 1              | RCCGAC       | In response to drought and light |
| MYC                   | 6              | CANNTG       | In response to drought |
| WBOXATNPR1            | 1              | TTGAC/ACTG   | In response to Salicylic acid |

**Table 3.** The predicted different stress-responsive cis-elements present in OsCYP-25 promoter region using PLACE database analysis

![Figure 3. Schematic representation of the putative interaction of OsCYP-25 with other proteins. STRING program (http://string-db.org/) was used for the bioinformatic prediction of protein-protein interaction.](image)
multiple amino acid sequence alignment using Clustal W 2.0 program (http://www.ebi.ac.uk/clustalw). The pairwise amino acid sequence identity between OsCYP-25 and with respective subunits of Arabidopsis, Brachypodium, maize, Sorghum and poplar was calculated using ClustalW2 (EMBL-EBI). The protein domain search, performed in the NCBI Conserved Domain Database (NCBI-CDD; http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) to highlight the specific PPIase region. The human cyclophilin A (PDB-ICWA) was used as a template to generate model for cyclophilins from Rice (LOC_Os09 g39780) and were created using ExPasy swiss model web server (http://swissmodel.expasy.org). The comparative study of amino acid sequences of OsCYP-25, performed by using the UniProt BlastP Service (www.uniprot.org/blast/), revealed an 87.36 and 74% similarities with CyP-S from Sorghum sp and CyP-P from poplar (Liriodendron sp).

Identification of cis-regulatory elements in OsCYP-25 promoter. PLACE (http://www.dna.affrc.go.jp/PLACE/) were used to analyze the cis-acting regulatory elements and to analyze the OsCYP-25 promoter sequences (http://rice.plantbiology.msu.edu). To validate stress-responsive nature of OsCYP-25 promoter, predicted cis-regulatory elements were analyzed for their different role in various abiotic stress responses. In silico predictions of protein-protein interactions. The protein-protein interaction for OsCYP-25 rice cyclophilin was made using STRING computer service (http://string-db.org/). The predicted protein-protein interactions for OsCYP-25 were examined individually for their role in various stress response. Quantitative real-time PCR. The expression profiling of OsCYP-25 under different abiotic stress conditions was determined by quantitative real-time PCR. qRT-PCR reactions were performed by methods described previously using 5'-TTACAAGGGT TCTGCCTTCC-3' and 5'-CAAACCTGTC GCCGTAGAT-3' primer pair. Rice α-tubulin gene was used as control transcript. The qRT-PCR experiment repetitive three times and in each experiment three technical replicates were used for each time point of treatment. Relative gene expression was calculated using the 2−ΔΔCT values following Livak's method.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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