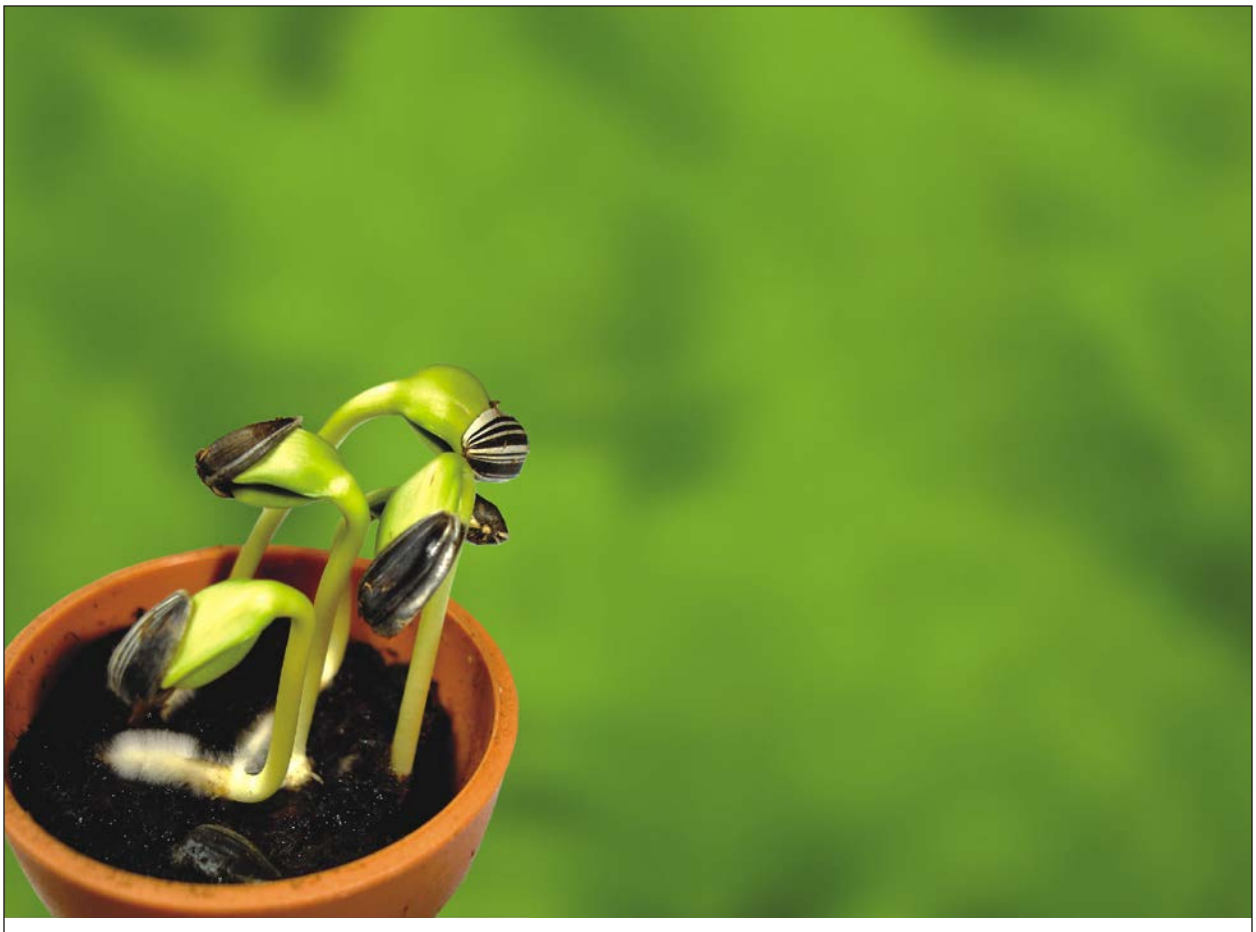

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Research Article

Differential Expression of Cyclophilin1 and Cyclophilin2 Genes under Salinity Stress in Some Native Rice Cultivars of North Kerala, India

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Abstract

Salinity is a major environmental constraint influencing growth and development of almost all the crop plants including rice leading to yield reduction throughout the world. Cyclophilins (CYP), members of immunophillin group of proteins, have been highly conserved throughout evolution and appear to be ubiquitous. They are involved in a variety of mechanisms including the regulation of salt stress in plants. As an initial step to investigate the regulation and function of two cyclophilins in rice, we isolated RNA that encodes two genes, designated OsCYP1 and OsCYP2, two rice cyclophilin genes so as to study their differential expression under salinity stress. Differential expression of the two genes in which both of them were progressively up-regulated in response to salinity stress up to a certain level of NaCl concentration in the growth medium shows the importance of these genes as indicators and regulators of salt stress in rice.

Key words: Abiotic stress, cyclophilin, native rice cultivars, *Oryza sativa*, soil salinity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Survival, growth and yield potential of diverse crop plants are badly impacted by rapid changes in environmental circumstances caused by both biotic and abiotic stresses. Abiotic stresses act as primary cause of crop yield losses throughout the world and pose one of the major threats to sustainable food production by various crop plants (Bray, 1997; Agarwal *et al*., 2006). Plants often encounter rapid environmental changes and adapt through modulation of various physiological, biochemical and molecular mechanisms. Stress is perceived and conducted through various signal transduction pathways which affect regulatory elements of stress inducible genes involved in the synthesis and alteration of various classes of proteins including transcription factors, enzymes, molecular chaperones, ion channels, transporters, etc., ultimately resulting in stress tolerance responses (Knight and Knight, 2001; Chen *et al*., 2002). Soil salinity is one of the important limiting factors for plant growth and development. Being a salt sensitive cereal crop, high salinity may impose delayed seed germination, slow seedling growth and reduced rate of seed setting leading to decreased yield in rice (Abdullah *et al*., 2001). These disorders are generally due to the combined effects of ion imbalance, hyperosmotic stress and oxidative damage caused by the reactive oxygen species formed. In the early period, rice can rapidly experience a salt stress signal via plasma membrane receptors in root cells and can quickly initiate an intracellular signal that modulates gene expression to elicit an adaptive response (Hasegawa *et al*., 2000).

Cyclophilins are the family of ubiquitous proteins (Galat, 1999) existing in all subcellular compartments of organisms ranging from bacteria to higher plants and animals, involved in a wide variety of biological processes including protein trafficking, folding and maturation (Lang *et al*., 1987; Shieh *et al*., 1989; Takahashi *et al*., 1989; Schonbrunner *et al*., 1991; Price *et al*., 1994; Ferreira *et al*., 1996), receptor complex stabilization (Leverson and Ness, 1998), apoptosis (Lin and Lechleiter, 2002), receptor signalling (Brazin *et al*., 2002; Yurchenko *et al*., 2002), RNA processing (Krzywicka *et al*., 2001) and spliceosome assembly (Bourquin *et al*., 1997; Mortillaro and Berezney, 1998; Horowitz *et al*., 2002). Cyclophilins are originally recognised and purified abundant intracellular cytosolic proteins with high affinity for the immune suppressive drug Cyclosporin A (CsA), a fungal metabolite with potent immunosuppressive activity (Handschumacher *et al*., 1984; Heitman *et al*., 1992). They belong to a family of immunosuppressant receptors called immunophilins, include the Fk506-Binding Proteins (FKBPs) (Harding *et al*., 1989) and the parvulins (Dolinski and Heitman, 1997). Immunophilins possess peptidyl prolyl cis trans isomerase (PPIase) activity, catalyzing the rotation of X-Propeptide bonds from a *cis* to *trans* conformation, a rate limiting step in protein folding (Brandts *et al*., 1975) and are of widespread importance since over 90% of proteins contain trans prolyl imide bonds (Stewart *et al*., 1990). Cyclophilins have been identified in a number of organisms including man (Haendler *et al*., 1989).

Different CYP isoforms in Arabidopsis occurring in cytosol, nucleus, mitochondria and chloroplasts were found involved in various cellular processes (Romano *et al*., 2004a, b, 2005; Fu *et al*., 2007; Dominguez-Solis *et al*., 2008). In addition to this, involvement of cyclophilins in the maintenance of PSII integrity, regulation and stress responsive pathways has been reported (Kumari *et al*., 2009). Cyclophilins play potent roles in the assembly of luminal proteins (Schubert *et al*., 2002) and are mainly involved in coordination of cell polarity and root cell proliferation (Grebe *et al*., 2000; Jackson and Soll, 1999). According to Zoratti and Szabo (1995), many cyclophilins were also found to be localized in mitochondria and mainly involved in secretory pathways, involved in mitochondrial permeability which ultimately leads to apoptosis.

Cyclophilin expression has been shown to be induced by both biotic and abiotic stresses including $HgCl_2$ (Marivet *et al*., 1992), viral infection, ethephon, salicylic acid, salt stress, heat shock and cold shock (Marivet *et al*., 1994, 1995; Scholze *et al*., 1999; Godoy *et al*., 2000), light (Chou and Gasser, 1997; Luan *et al*., 1994a), drought, wounding, fungal infection, abscisic acid and methyl jasmonate (Godoy *et al*., 2000; Kong *et al*., 2001; Sharma and Singh, 2003). Cyclophilins are classified into mainly two groups, single-domain and multiple-domain families. Single-domain cyclophilins have only the cyclophilin catalytic domain and their average length is found to be 172 amino acids. Multiple domain cyclophilins have other functional domains in addition to the cyclophilin catalytic domain. Their average length is 550 aminoacids. The other domains are expected to play roles in determining specific functions (Galat, 1999).

Plant cyclophilins have been identified from plants like tomato, maize, oil seed rape (Gasser *et al*., 1990) and Arabidopsis (Hayman and Miernyk, 1994; Chou and Gasser, 1997) and they occur either in endoplasmic reticulum (Jackson and Soll, 1999; Grebe *et al*., 2000) or in chloroplasts (Lippuner *et al*., 1994; Peltier *et al*., 2002; Schubert *et al*., 2002). Both chloroplastic and cytosolic cyclophilins have been identified in plants. Different roles such as stress response, developmental regulation and protein folding have been proposed for plant cyclophilins (Luan *et al*., 1994b;

Marivet *et al*., 1994; Kern *et al*., 1995). Thus far, large families of CYP genes have been identified in various organisms. Human genome was found to comprise 16 CYP genes (Galat, 2003), whereas, *Arabidopsis thaliana* revealed 29 CYP genes (Romano *et al*., 2004a). In yeast, CYPs were shown to play an essential role in the recovery of cells when subjected to heat shock treatment (Sykes *et al*., 1993). Wide distribution and ubiquitous nature of CYPs signify their fundamental and important role in plant survival. Though diverse functions of CYPs have been suggested in plants (Deng *et al*., 1998; Berardini *et al*., 2001; Gullerova *et al*., 2006; Oh *et al*., 2006; Nigam *et al*., 2008), their physiological significance and the molecular basis of stress responsive expression are still largely unknown. Several cyclophilins have been reported in rice and they are encoded by a small gene family (Buchholz *et al*., 1994; Trivedi *et al*., 2013). OsCYP1 and OsCYP2 two related but distinct rice cyclophilins (Buchholz *et al*., 1994). Reports show that in rice, many of the cyclophilin genes show differential expression during abiotic stress conditions as compared with the control conditions (Ruan *et al*., 2011; Trivedi *et al*., 2013).

Under the above conditions, in the present experiment studied the expression of two rice cyclophilin genes; OsCYP1 and OsCYP2 in some native rice cultivars of North Kerala, India under varying salinity stress.

MATERIALS AND METHODS

Germination of seeds and growing of plant materials: The experiment was conducted in the experimental rainout poly house of Department of Botany, University of Calicut, Kerala, India located at 11°35'N latitude and 75°48'E longitude in the first crop season of 2013. Seven native cultivars of rice including five cultivars collected from one of the saline rice habitats of North Kerala namely Orthadian, Chovvarian, Kuttusan, Kuthiru and Orkazhama and two native rice cultivars namely Kunhutty and Veliyan collected from one of the non-saline rice habitats of North Kerala were used for the study. Enough number of healthy and mature caryopses taken from single plant per cultivar were washed in running tap water to remove infected and unfilled grains and dust particles. The seeds were soaked in distilled water, allowed to germinate in 10 cm diameter petri dishes covered with lid under room temperature. The water was changed every day. The seeds started to germinate from the third day onwards. On the 10th day, required numbers of germinated seedlings were transferred to coloured plastic pots of 25 cm diameter filled with paddy soil mixed with enriched compost in 3:1 ratio. Two seedlings were initially planted per pot and after establishment of the seedlings the smaller among the two were removed. The plants were maintained in the experimental poly house of the Department under wetland conditions, always maintaining 3 cm of water above the soil level. The soil was fertilized with 1g N: P: K =18: 18: 18 per pot at fortnightly intervals starting from the 30th day. Weeding was done manually whenever required. Plants were grown in randomized block design with three replications.

Experimental treatments: The experimental treatment was started from the 45th day onwards using aqueous solutions of sodium chloride as detailed in Table 1. Progressive salt stress was applied mimicking the variation in salt content in the saline rice farms of the area.

Total RNA isolation and cDNA synthesis: Total RNA was isolated from 100 mg of fresh clean leaf of rice plants from all the treatments including control using Trizol reagent (Invitrogen, Life Technologies, USA) according to the manufacturer's instructions. Enough precautions were taken for the RNA isolation process. The RNA was dissolved in 50 µL sterile RNase-free water and quantified by spectrophotometry. The RNA concentration and purity were determined at 260 and 280 nm (A_{260}/A_{280}). The RNA was purified further using RNase free DNase (Qiagen Inc., CA, USA) by following the manufacturer's instructions. First strand of cDNA was synthesized from 2 µg of total RNA using oligo $(dT)_{12-18}$primers (Invitrogen, Life Technologies, USA) and MultiScribe Reverse Transcriptase (Applied Biosystems, USA) following manufacturer's instruction. Two microgram of RNA was transcribed reversely in a 20 µL reaction volume using oligo $(dT)_{12-18}$ primer. Reactions were incubated at 25°C for 10 min, 37°C for 120 min and then 85°C for 5 min. The resultant cDNA product was stored at -20°C until the subsequent PCR reactions.

Primer design: Gene specific primers of the genes OsCYP1 and OsCYP2 for amplification were taken from NCBI database "Pick Primers" modified using Primer 3.0 software. Primer sequence for β-actin was adopted from Ruan *et al*. (2011) (Table 2). All primers were synthesized by Sigma.

Semi quantitative RT-PCR: Twenty five microliter of PCR mixture (Fermentas, Lithuania) contains 0.05 U μL^{-1} TaqDNA polymerase, reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP), specific forward and reverse primers 1.0 µM each and 200 ng of the cDNA as the template DNA for the PCR reactions.

Table 1: Details of salinity treatment applied in the case of the different rice cultivars

Sl No.	Treatments
T1	Control
T2	10 mM (0.91 dS m^{-1}) on 45th day
T3	10 mM (0.91 dS m^{-1}) on 45th day and 30 mM (2.74 dS m^{-1}) on 53rd day
T4	10 mM (0.91 dS m^{-1}) on 45th day, 30 mM (2.74 dS m^{-1}) on 53rd day and 50 mM (4.57 dS m^{-1}) on 61st day
T5	10 mM (0.91 dS m^{-1}) on 45th day, 30 mM (2.74 dS m^{-1}) on 53rd day, 50 mM (4.57 dS m^{-1}) on 61st day and70 mM (6.39 dS m^{-1}) on 69th day
T6	10 mM (0.91 dS m^{-1}) on 45th day, 30 mM (2.74 dS m^{-1}) on 53rd day, 50 mM (4.57 dS m^{-1}) on 61st day, 70 mM (6.39 dS m^{-1}) on 69th day and 100 mM (9.13 dS m^{-1}) on 77th day
T7	10 mM (0.91 dS m^{-1}) on 45th day, 30 mM (2.74 dS m^{-1}) on 53rd day, 50 mM (4.57 dS m^{-1}) on 61st day, 70 mM (6.39 dS m^{-1}) on 69th day, 100 mM (9.13 dS m^{-1}) on 77th day and 200 mM (18.26 dS m^{-1}) on 85th day

Table 2: Details of primer sequences, amplicon size and annealing temperatures of the primers used in the PCR

Primer names	Sequence (5'-3')	Amplicon size	Annealing temperatures
OsCYP1	F-TCGACATCCTCATCGGCAAG	550	52.5
	R-AATCAGTTGGCGTGGTCGT		
OsCYP2	F-GCCTTTCGCCAGTATCAGTC	210	59.0
	R-CAGATCCAACTCCACCGAAT		
β-actin	F-GACCTTGCTGGGCGTGAT	175	56.0
	R-GTCATAGTCCAGGGCGATGT		

The expression of β-actin gene was used as an internal control for determining the RT-PCR amplification efficiency among different reactions. The primers and corresponding annealing temperature of each primer for gene-specific transcript amplification is given in Table 2. For the subsequent amplification, 200 ng of each reverse transcription reaction product was used as a template. The amplification of genes was optimized to initial denaturation at 95°C for 5 min and 40 cycles of amplification (each of 95°C for 30 sec, appropriate annealing temperature 72°C for 45 sec), followed by final extension at 72°C for 5 min. The primer pairs yield PCR products of 550, 210 and 175 in the case of the genes OsCYP1, OsCYP2 and β-actin, respectively. Two microliter of the amplified PCR product mixed with 2 µL of gel loading dye (6X) (HiMedia) was taken for loading on the gel. The amplified reaction products were separated/resolved, detected on 1.2% (w/v) agarose gel, stained with ethidium bromide and 100 bp DNA ladder was used as molecular weight marker (Invitrogen Trackit 100 BP DNA, Life Technologies, USA) to measure the sizes of the amplified products. The bands of interest were excised using a sterile surgical blade and eluted for re-amplification using Fermentas (Genetix Biotech Asia) gel extraction kit. The re-amplified PCR products were checked on agarose gels once again to confirm their molecular weights and a selected few of these validated products were sequenced (SciGenom Labs Pvt. Ltd., Kochi, Kerala). The identity of the sequences was determined by comparing the sequence with the sequences available in the NCBI nucleotide databases (Accession Numbers: OsCYP1- L29471.1; OsCYP2- L29470.1) (Buchholz *et al*., 1994) using Basic Local Alignment Search Tool (BLAST) algorithm.

Data analysis: The experiment was repeated for three times and the resolved bands in the agarose gel were analysed using gel documentation system (Cell BioSciences, USA). The filters were exposed and the images were captured at -20°C and analysed using Alphaimager software. The fold changes in expression level relative to the control were expressed.

RESULTS

For exploring the expression of the cyclophilin genes OsCYP1 and OsCYP2 in rice in response to salinity stress, semi quantitative PCR was used. Semi quantitative PCR is one of the methods to identify and understand differential gene expression. Expression analysis of the two genes in seven different growth conditions/treatments was conducted by semi quantitative PCR. To assess gene expression by relative semi-quantitative assay, co-amplified internal controls might be established as reference genes. A structural protein β-actin encoding gene was used as the reference gene in this semi quantitative PCR (Fig. 1). As such, it is assumed that basal expression levels of this actin mRNA are constant and do not change with the experimental conditions. Changes in both the gene transcripts were observed in all the genotypes starting from 10 mM salt treatment onwards. Increase in the intensity/fold change in the relative concentrations in the PCR amplified products corresponds to the mRNA produced and corresponding quantity of cDNA formed.

Expression pattern of OsCYP1 in different salt concentrations: Each rice cultivar responded to different salt concentrations in a different way. The results presented in Fig. 2-6 show that the OsCYP1 and OsCYP2 genes are

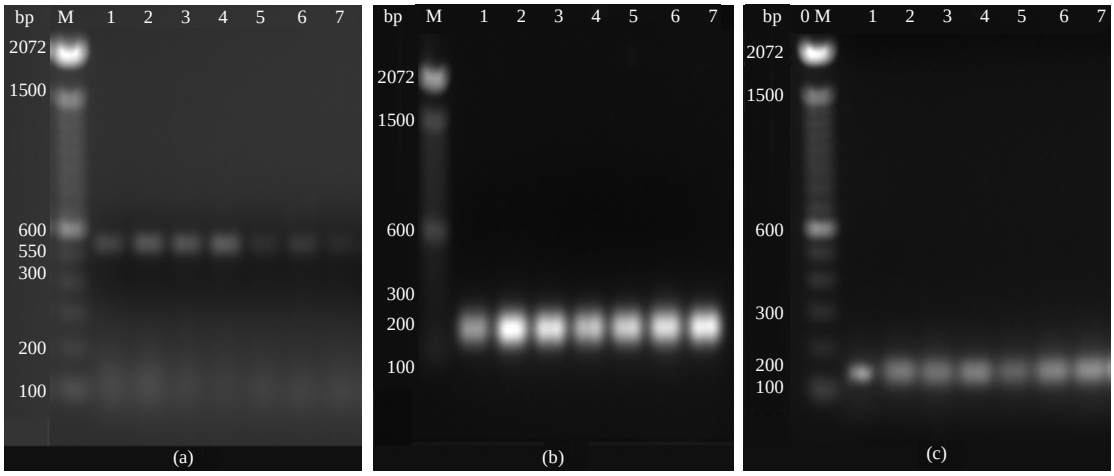

Fig. 1(a-c): PCR amplification of (a) OsCYP1, (b) OsCYP2 and (c) β-actin genes, 1: Orthadian, 2: Chovvarian, 3: Kuttusan, 4: Kuthiru, 5: Orkazhama, 6: Kunhutty and 7: Veliyan

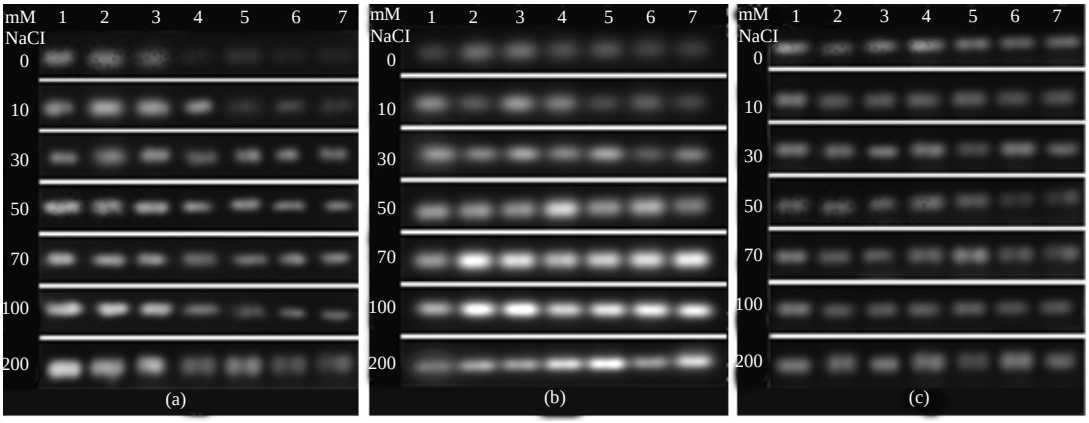

Fig. 2(a-c): PCR amplification of (a) OsCYP1, (b) OsCYP2 and (c) β-actin genes under different salt concentrations, 1: Orthadian, 2: Chovvarian, 3: Kuttusan, 4: Kuthiru, 5: Orkazhama, 6: Kunhutty and 7: Veliyan

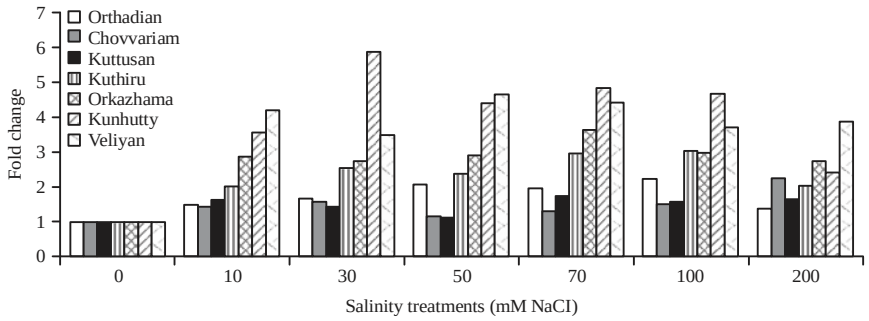

Fig. 3: Fold change of OsCYP1

differentially expressed in the rice leaves in relation to rise in salinity stress. The sequential change in the relative intensity of the gene amplified was induced by salt stress in all the varieties studied. In all the cases the expression of both the

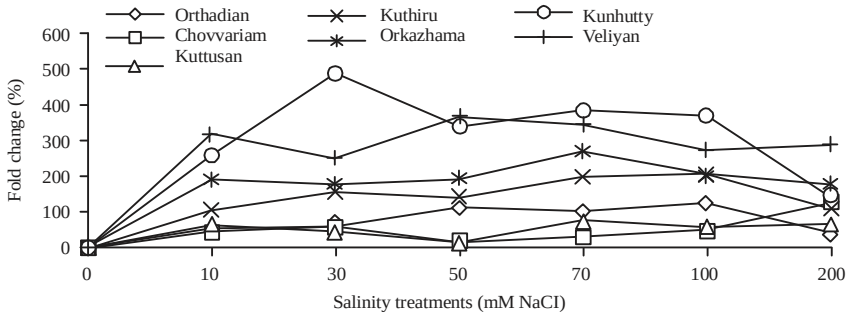

Fig. 4: Percentage of variation in fold change of OsCYP1

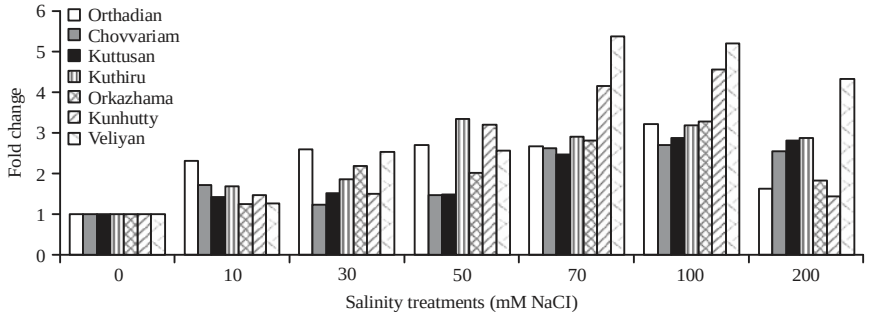

Fig. 5: Fold change of OsCYP2

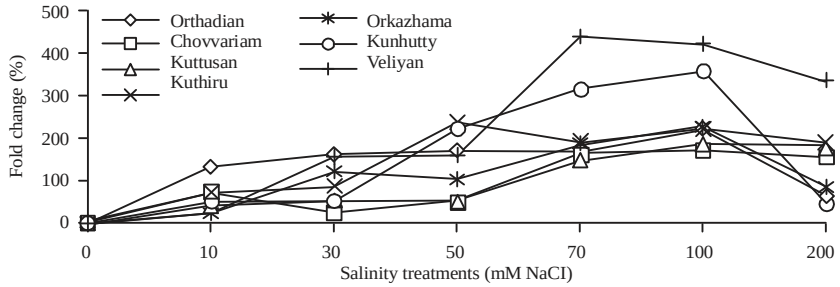

Fig. 6: Percentage of variation in fold change of OsCYP2

genes was positively up-regulated while increasing the salt concentrations. In Orthadian, the expression was maximal at 100 mM followed by 50 and 70 mM of NaCl. In the case of Chovvarian, the relative expression of OsCYP1 was maximal at 200 mM salinity level. In Kuttusan, from 10-50 mM concentration of NaCl the relative expression was decreasing and from 50 mM it was again increasing. Kuthiru showed the maximal expression at a salt concentration of 70 mM followed by 100 mM. Orkazhama showed maximal OsCYP1 gene expression at 70 mM followed by 100 and 50 mM NaCl stress. The expression of OsCYP1 was maximal in Kunhutty at 30 mM and its level got gradually decreased. In Veliyan, the maximum expression was at 50 mM followed by 10 mM NaCl stress (Fig. 3 and 4).

Expression pattern of OsCYP2 in different salt concentrations: In the case of OsCYP2 gene, the expression pattern was a little bit different from that of OsCYP1. The cultivars Orthadian, Chovvarian, Kuttusan, Orkazhama and Kunhutty showed the highest expression at 100 mM, Veliyan at 70 mM and Kuthiru at 50 mM concentration of NaCl (Fig. 2). Irrespective of the cultivars used for the study, OsCYP2

showed diverse expression pattern in the experimental conditions (Fig. 5 and 6). Up-regulation was seen up to a particular salt level beyond which there was down regulation of the gene. In rice, up-regulation of the cyclophilin gene OsCYP2, in response to salt stress has been reported by Ruan *et al.* (2011). The cultivars collected from both the saline as well as non-saline rice tracts showed up-regulation of the cyclophilin genes under study in response to progressive salt stress up to a particular level of salt concentration.

DISCUSSION

Cyclophilins, an affiliate of immunophilin group of proteins, ubiquitous in nature and found in all genera are remarkably conserved during their evolutionary processes (Romano *et al.*, 2005). These are characterized by diverse forms of existence and doing wide range of cellular functions. The expression profile of Arabidopsis cyclophilin genes revealed that 13 out of 30 genes were found to be up-regulated in senescence stage and 5 of them highly up-regulated (Trivedi *et al.*, 2012). *In silico* analysis has revealed a positive correlation between rice cyclophilin protein family and plant stress response (Trivedi *et al.*, 2012). It is also reported that cyclophilins play a potent role in abiotic stress tolerance (Kim *et al.*, 2012; Sekhar *et al.*, 2010). The present study revealed the potential relationship between the transcription of the cyclophilin genes OsCYP1 and OsCYP2 and stress response.

The cellular roles of cyclophilins in different biological processes like DNA transcription (Obata *et al.*, 2005), apoptosis (Sorrentino *et al.*, 2013), fertility (Atchison and Means, 2004), hormone signalling (Lavy *et al.*, 2012), chromatin remodelling process (Li and Luan, 2010) and disease resistance (Gong *et al.*, 2011; Marra *et al.*, 2006) have also been reported. Cyclophilins play an important role in protein folding. They 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 (Kumari *et al.*, 2013; Gullerova *et al.*, 2006).

OsCYP2 shows circadian rhythm expression as time goes. OsCYP2 expression is not only specific to salt stress but is ubiquitous in the response of rice seedlings to other types of stresses including drought, heat and cold (Ruan *et al.*, 2011). Here in our result it is clearly noted that both the cyclophilins (OsCYP1 and OsCYP2) are up-regulated due to the exposure to salinity stress. The up-regulation of OsCYP2 in response to various stresses such as high salinity, drought, heat, oxidative stress and hypoxia have been reported (Kumari *et al.*, 2009; Matsumura *et al.*, 1999). Therefore, we speculate that OsCYP1 and OsCYP2 may function as key integrators in response to multiple stresses.

Major abiotic stresses such as drought, salinity and low and extreme temperatures have been found to cause extensive damage to the growth, yield and productivity of almost all the crop plants worldwide. For the development of crops with inbuilt tolerance to multiple stresses, it is imperious to prospect for exotic abiotic stress tolerance genes from resistant species acclimated to diverse environmental conditions. Priyanka *et al.* (2010) revealed that northern blot analysis in stressed pigeon pea plants, using CcCYP as a probe showed intense hybridization signals because of enhanced transcript levels under drought, salt, heat and cold stresses compared to weak signals in the unstressed plants, indicating the stress responsive nature of CcCYP gene of *Cajanus cajan*. Other workers have also reported that different CYP genes of maize, bean (Marivet *et al.*, 1992), *Solanum commersonii* (Meza-Zepeda *et al.*, 1998), *Thellungiella halophila* (Chen *et al.*, 2007) and *Solanum tuberosum* (Godoy *et al.*, 2000) were found to show increased transcript levels during abiotic stresses such as drought, salinity and extreme temperatures.

Sharma and Singh (2003) reported that drought tolerant sorghum cultivars exhibited higher levels of induced PPIase activity as compared to susceptible genotypes when subjected to drought stress. Moreover, enhanced levels of PPIase activity under stress conditions might regulate the expression of other stress tolerance genes involved in signal transduction (Harrar *et al.*, 2001). Higher PPIase activity was noticed in CcCYP transgenic plants than that of controls, under stress conditions, indicating that the *Cajanus cajan* CYP functions as an efficient chaperone (Priyanka *et al.*, 2010). The CYPs of *Phaseolus vulgaris* (Kumar *et al.*, 2015), *Vicia faba* (Luan *et al.*, 1994a) and *Thellungiella halophila* (Chen *et al.*, 2007) were found to show characteristic ability to act as efficient chaperones and they were found to play active roles in the proper folding, assembly and transport of nascent proteins besides protecting them from proteolytic degradation and protein combination under severe stress conditions. The Plases have also been concerned with chromatin remodelling and cell cycle progression events and the regulation of protein kinases by controlling the activity or stability of crucial regulatory proteins (Nigam *et al.*, 2008).

Under salt stress, OsCYP2 is likely to up-regulate the activities of antioxidant enzymes (SOD, CAT and APX) at post-translation level to control H_2O_2 levels, resulting in the

reduction of MDA levels, which may ultimately avoid oxidative damage of photosystems. The CYPs are ubiquitous chaperon proteins that have an intrinsic peptidyl prolyl *cis/trans* isomerase activity, participating in protein folding and are structurally conserved throughout evolution, found in bacteria to plants and animals (Galat, 1999). Rice OsCYP2 gene introduced into CYP2-yeast mutant revealed complementation and also contributed to the growth of wild type yeast under various stresses like salinity, high temperature and osmotic and oxidative stresses (Kumari *et al*., 2009). Over expression of ThCYP gene in yeast and tobacco cells was found to confer increased tolerance against salt stress (Chen *et al*., 2007). Similarly, the pigeon pea CYP (CcCYP) gene plays a crucial role in conferring tolerance to multiple abiotic stresses. It is also suggested that the OsCYP-25 gene transcript is highly up-regulated in response to major abiotic stresses such as salt, heat, cold and drought (Trivedi *et al*., 2013).

In the present experiment, OsCYP1 and OsCYP2, two rice cyclophilin genes were separated and their expression was identified by semi quantitative polymerase chain reaction. OsCYP1 and OsCYP2 have peptidyl prolyl cis-trans isomerase (PPIase or rotamase) activity that is specifically inhibited by cyclosporine A (Kumari *et al*., 2009). Moreover, OsCYP2 lacks introns, contains an AT-rich region at the 5′ end of the transcript, suggesting that OsCYP2 is likely to be preferentially translated during stress conditions (Buchholz *et al*., 1994). OsCYP1 and OsCYP2 could respond to multiple environmental stresses such as high salt, drought, heat and oxidative stress. Kumari *et al*. (2009) reported that heterologous expression of OsCYP2 was enough to enhance ability of *E. coli* cells to survive, to complement the yeast mutant lacking native OsCYP2 and to improve the growth of wild type yeast under abiotic stresses. There is a previous report that significant differential changes in transcript abundance of OsCYP2 were noted in shoots of salt sensitive (IR64) and tolerant (Pokkali) rice cultivars at different developmental stages under normal and salinity stress conditions (Kumari *et al*., 2009).

Since the cultivars such as Orthadian, Chovvarian, Kuttusan, Kuthiru and Orkazhama are native to a saline rice tract, they show the highest expression of OsCYP1 and OsCYP2 in the saline conditions. Chandramohanan and Mohanan (2012) reported that rice cultivars such as Kuthiru and Orkazhama performed well under conditions of moderate salt stress. One of our previous works has revealed that Veliyan, a cultivar originally collected from a non saline rice tract has no significant yield reduction under salt stress. However, early induction of flowering has been observed under salt stress (Joseph and Mohanan, 2013). This may be due to the effect of salinity stress on the up-regulation of the genes responsible for flowering response in rice plants. The present study establishes that rice cyclophilin genes OsCYP1 and OsCYP2 are up-regulated during abiotic stresses such as salinity. The mechanism which is targeted by rice cyclophilin proteins to bring about multiple abiotic stress protection has not been worked out and further characterization is still needed. The gene family plays multitude of roles in stress conditions as well as in different developmental stages making it one of the strong candidates for crop improvement studies especially under stressful environments.

REFERENCES

Abdullah, Z., M.A. Khan and T.Z. Flowers, 2001. Causes of sterility in seed set of rice under salinity stress. J. Agron. Crop Sci., 187: 25-32.

Agarwal, P.K., P. Agarwal, M.K. Reddy and S.K. Sopory, 2006. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep., 25: 1263-1274.

Atchison, F.W. and A.R. Means, 2004. A role for Pin1 in mammalian germ cell development and spermatogenesis. Front. Biosci., 9: 3248-3256.

Berardini, T.Z., K. Bollman, H. Sun and R.S. Poethig, 2001. Regulation of vegetative phase change in *Arabidopsis thaliana* by Cyclophilin 40. Science, 291: 2405-2407.

Bourquin, J.P., I. Stagljar, P. Meier, P. Moosmann and J. Silke *et al*., 1997. A serine/arginine-rich nuclear matrix cyclophilin interacts with the C-terminal domain of RNA polymerase II. Nucleic Acids Res., 25: 2055-2061.

Brandts, J.F., H.R. Halvorson and M. Brennan, 1975. Consideration of the possibility that the slow step in protein denaturation reactions is due to cis-trans isomerism of proline residues. Biochemistry, 14: 4953-4963.

Bray, E.A., 1997. Plant responses to water deficit. Trends Plant Sci., 2: 48-54.

Brazin, K.N., R.J. Mallis, D.B. Fulton and A.H. Andreotti, 2002. Regulation of the tyrosine kinase Itk by the peptidyl-prolyl isomerase Cyclophilin A. Proc. Natl. Acad. Sci. USA., 99: 1899-1904.

Buchholz, W.G., L. Harris-Haller, R.T. DeRose and T.C. Hall, 1994. Cyclophilins are encoded by a small gene family in rice. Plant Mol. Biol., 25: 837-843.

Chandramohanan, K.T. and K.V. Mohanan, 2012. Kaipad rice farming in north Kerala: An indigenous saline resistant organic farming system. Indian J. Traditional Knowl., 11: 185-189.

Chen, A.P., G.L. Wang, Z.L. Qu, C.X. Lu, N. Liu, F. Wang and G.X. Xia, 2007. Ectopic expression of *ThCYP1*, a stress-responsive cyclophilin gene from *Thellungiella halophila*, confers salt tolerance in fission yeast and tobacco cells. Plant Cell Rep., 26: 237-245.

Chen, W., N.J. Provart, J. Glazebrook, F. Katagiri and H.S. Chang *et al*., 2002. Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stress. Plant Cell, 14: 559-574.

Chou, I.T. and C.S. Gasser, 1997. Characterization of the cyclophilin gene family of *Arabidopsis thaliana* and phylogenetic analysis of known cyclophilin proteins. Plant Mol. Biol., 35: 873-892.

Deng, W., L. Chen, D.W. Wood, T. Metcalfe and X. Liang *et al*., 1998. Agrobacterium VirD2 protein interacts with plant host cyclophilins. Proc. Natl. Acad. Sci., 95: 7040-7045.

Dolinski, K. and J. Heitman, 1997. Peptidyl-Prolyl Isomerases. In: Guidebook to Molecular Haperones and Protein-Folding Catalysts, Gething, M.J., J. Sambrook and S. Tooze (Eds.). Oxford University Press, London, UK., ISBN-13: 9780191547270, pp: 359-369.

Dominguez-Solis, J.R., Z. He, A. Lima, J. Ting, B.B. Buchanan and S. Luan, 2008. A cyclophilin links redox and light signals to cysteine biosynthesis and stress responses in chloroplasts. Proc. Natl. Acad. Sci. USA., 105: 16386-16391.

Ferreira, P.A., T.A. Nakayama, W.L. Pak and G.H. Travis, 1996. Cyclophilin-related protein RanBP2 acts as chaperone for red/green opsin. Nature, 383: 637-640.

Fu, A., Z. He, H.S. Cho, A. Lima, B.B. Buchanan and S. Luan, 2007. A chloroplast cyclophilin functions in the assembly and maintenance of photosystem II in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA., 104: 15947-15952.

Galat, A., 1999. Variations of sequences and amino acid compositions of proteins that sustain their biological functions: An analysis of the cyclophilin family of proteins. Arch. Biochem. Biophys., 371: 149-162.

Galat, A., 2003. Peptidylprolyl cis/trans isomerases (immunophilins): Biological diversity-targets-functions. Curr. Top. Med. Chem., 3: 1315-1347.

Gasser, C.S., D.A. Gunning, K.A. Budelier and S.M. Brown, 1990. Structure and expression of cytosolic cyclophilin/peptidyl-prolyl cis-trans isomerase of higher plants and production of active tomato cyclophilin in *Escherichia coli*. Proc. Natl. Acad. Sci. USA., 87: 9519-9523.

Godoy, A.V., A.S. Lazzaro, C.A. Casalongue and B. San Segundo, 2000. Expression of a *Solanum tuberosum* cyclophilin gene is regulated by fungal infection and abiotic stress conditions. Plant Sci., 152: 123-134.

Gong, Y.H., M. Chen, Y. Xu, N. Dong, Z. Sang, J. Liu and Y. Yuan, 2011. Subtractive hybridization analysis of gastric diseases-associated *Helicobacter pylori* identifies peptidyl-prolyl isomerase as a potential marker for gastric cancer. FEMS Microbiol. Lett., 320: 103-109.

Grebe, M., J. Gadea, T. Steinmann, M. Kientz and J.U. Rahfeld *et al*., 2000. A conserved domain of the Arabidopsis GNOM protein mediates subunit interaction and cyclophilin 5 binding. Plant Cell, 12: 343-356.

Gullerova, M., A. Barta and Z.J. Lorkovic, 2006. AtCyp59 is a multidomain cyclophilin from *Arabidopsis thaliana* that interacts with SR proteins and the C-terminal domain of the RNA polymerase II. RNA, 12: 631-643.

Haendler, B., R. Keller, P.C. Hiestand, H.P. Kocher, G. Wegmannn and N.R. Movva, 1989. Yeast cyclophilin: Isolation and characterization of the protein, cDNA and gene. Gene, 83: 39-46.

Handschumacher, R.E., M.W. Harding, J. Rice, R.J. Drugge and D.W. Speicher, 1984. Cyclophilin: A specific cytosolic binding protein for cyclosporin A. Science, 226: 544-547.

Harding, M.W., A. Galat, D.E. Uehling and S.L. Schreiber, 1989. A receptor for the immuno-suppressant FK506 is a *cis-trans* peptidyl-prolyl isomerase. Nature, 341: 758-760.

Harrar, Y., C. Bellini and J.D. Faure, 2001. FKBPs: At the crossroads of folding and transduction. Trends Plant Sci., 6: 426-431.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol., 51: 463-499.

Hayman, G.T. and J.A. Miernyk, 1994. The nucleotide and deduced amino acid sequences of a peptidyl-prolyl *cis-trans* isomerase from *Arabidopsis thaliana*. Biochim. Biophys. Acta (BBA)-Gene Struct. Expression, 1219: 536-538.

Heitman, J., N.R. Movva and M.N. Hall, 1992. Proline isomerases at the crossroads of protein folding, signal transduction and immunosuppression. New Biol., 4: 448-460.

Horowitz, D.S., E.J. Lee, S.A. Mabon and T. Misteli, 2002. A cyclophilin functions in pre mRNA splicing. EMBO J., 21: 470-480.

Jackson, K. and D. Soll, 1999. Mutations in a new Arabidopsis cyclophilin disrupt its interaction with protein phosphatase 2A. Mol. Gen. Genet., 262: 830-838.

Joseph, E.A. and K.V. Mohanan, 2013. A study on the effect of salinity stress on the growth and yield of some native rice cultivars of Kerala state of India. Agric. For. Fish., 2: 141-150.

Kern, G., D. Kern, F.X. Schmid and G. Fischer, 1995. A kinetic analysis of the folding of human carbonic anhydrase II and its catalysis by cyclophilin. J. Biol. Chem., 270: 740-745.

Kim, S.K., Y.N. You, J.C. Park, Y. Joung, B.G. Kim, J.C. Ahn and H.S. Cho, 2012. The rice thylakoid lumenal cyclophilin OsCYP20-2 confers enhanced environmental stress tolerance in tobacco and Arabidopsis. Plant Cell Rep., 31: 417-426.

Knight, H. and M.R. Knight, 2001. Abiotic stress signalling pathways: Specificity and cross-talk. Trends Plant Sci., 6: 262-267.

Kong, H.Y., S.C. Lee and B.K. Hwang, 2001. Expression of pepper cyclophilin gene is differentially regulated during the pathogen infection and abiotic stress conditions. Physiol. Mol. Plant Pathol., 59: 189-199.

Krzywicka, A., J. Beisson, A.M. Keller, J. Cohen, M. Jerka-Dziadosz and C. Klotz, 2001. *KIN241*: A gene involved in cell morphogenesis in *Paramecium tetraurelia* reveals a novel protein family of cyclophilin-RNA interacting proteins (CRIPs) conserved from fission yeast to man. Mol. Microbiol., 42: 257-267.

Kumar, M.S., S.S. Chakravarthy, P.R. Babu, K.Y. Rao and V.D. Reddy, 2015. Classification of cytochrome P450s in common bean (*Phaseolus vulgaris* L.). Plant Syst. Evol., 300: 211-216.

Kumari, S., P. Singh, S.L. Singla-Pareek and A. Pareek, 2009. Heterologous expression of a salinity and developmentally regulated rice cyclophilin gene (*OsCyp2*) in *E. coli* and *S. cerevisiae* confers tolerance towards multiple abiotic stresses. Mol. Biotechnol., 42: 195-204.

Kumari, S., S. Roy, P. Singh, S.L. Singla-Pareek and A. Pareek, 2013. Cyclophilins: Proteins in search of function. Plant Signal. Behavior., 10.4161/psb.22734

Lang, K., F.X. Schmid and G. Fischer, 1987. Catalysis of protein folding by prolyl isomerase. Nature, 329: 268-270.

Lavy, M., M.J. Prigge, K. Tigyi and M. Estelle, 2012. The cyclophilin DIAGEOTROPICA has a conserved role in auxin signaling. Development, 139: 1115-1124.

Leverson, J.D. and S.A. Ness, 1998. Point mutations in v-Myb disrupt a cyclophilin-catalyzed negative regulatory mechanism. Mol. Cell, 1: 203-211.

Li, H. and S. Luan, 2010. AtFKBP53 is a histone chaperone required for repression of ribosomal RNA gene expression in *Arabidopsis*. Cell Res., 20: 357-366.

Lin, D.T. and J.D. Lechleiter, 2002. Mitochondrial targeted cyclophilin D protects cells from cell death by peptidyl prolyl isomerization. J. Biol. Chem., 277: 31134-31141.

Lippuner, V., I.T. Chou, S.V. Scott, W.F. Ettinger, S.M. Theg and C.S. Gasser, 1994. Cloning and characterization of chloroplast and cytosolic forms of cyclophilin from *Arabidopsis thaliana*. J. Biol. Chem., 269: 7863-7868.

Luan, S., M.W. Albers and S.L. Schreiber, 1994a. Light-regulated, tissue-specific immunophilins in a higher plant. Proc. Natl. Acad. Sci. USA., 91: 984-988.

Luan, S., W.S. Lane and S.L. Schreiber, 1994b. pCyP B: A chloroplast-localized, heat shock-responsive cyclophilin from fava bean. Plant Cell, 6: 885-892.

Marivet, J., P. Frendo and G. Burkard, 1992. Effects of abiotic stresses on cyclophilin gene expression in maize and bean and sequence analysis of bean cyclophilin cDNA. Plant Sci., 84: 171-178.

Marivet, J., M. Margis-Pinheiro, P. Frendo and G. Burkard, 1994. Bean cyclophilin gene expression during plant development and stress conditions. Plant Mol. Biol., 26: 1181-1189.

Marivet, J., P. Frendo and G. Burkard, 1995. DNA sequence analysis of a cyclophilin gene from maize: Developmental expression and regulation by salicylic acid. Mol. Gen. Genet., 247: 222-228.

Marra, R., P. Ambrosino, V. Carbone, F. Vinale and S.L. Woo *et al*., 2006. Study of the three-way interaction between *Trichoderma atroviride*, plant and fungal pathogens by using a proteomic approach. Curr. Genet., 50: 307-321.

Matsumura, H., S. Nirasawa and R. Terauchi, 1999. Technical advance: Transcript profiling in rice (*Oryza sativa* L.) seedlings using Serial Analysis of Gene Expression (SAGE). Plant J., 20: 719-726.

Meza-Zepeda, L.A., M.M. Baudo, E.T. Palva and P. Heino, 1998. Isolation and characterization of a cDNA corresponding to a stress-activated cyclophilin gene in *Solanum commersoni*. J. Exp. Bot., 49: 1451-1452.

Mortillaro, M.J. and R. Berezney, 1998. Matrin CYP, an SR-rich cyclophilin that associates with the nuclear matrix and splicing factors. J. Biol. Chem., 273: 8183-8192.

Nigam, N., A. Singh, C. Sahi, A. Chandramouli and A. Grover, 2008. SUMO-conjugating enzyme (Sce) and FK506-binding protein (FKBP) encoding rice (*Oryza sativa* L.) genes: Genome-wide analysis, expression studies and evidence for their involvement in abiotic stress response. Mol. Genet. Genomics, 279: 371-383.

Obata, Y., K. Yamamoto, M. Miyazaki, K. Shimotohno, S. Kohno and T. Matsuyama, 2005. Role of cyclophilin B in activation of interferon regulatory factor-3. J. Biol. Chem., 280: 18355-18360.

Oh, K., M.G. Ivanchenko, T.J. White and T.L. Lomax, 2006. The *Diageotropica* gene of tomato encodes a cyclophilin: A novel player in auxin signaling. Planta, 224: 133-144.

Peltier, J.B., O. Emanuelsson, D.E. Kalume, J. Ytterberg and G. Friso *et al*., 2002. Central functions of the lumenal and peripheral thylakoid proteome of *Arabidopsis* determined by experimentation and genome-wide prediction. Plant Cell, 14: 211-236.

Price, E.R., M. Jin, D. Lim, S. Pati, C.T. Walsh and F.D. McKeon, 1994. Cyclophilin B trafficking through the secretory pathway is altered by binding of cyclosporin A. Proc. Natl. Acad. Sci. USA, 91: 3931-3935.

Priyanka, B., K. Sekhar, T. Sunita, V.D. Reddy and K.V. Rao, 2010. Characterization of Expressed Sequence Tags (ESTs) of pigeonpea (*Cajanus cajan* L.) and functional validation of selected genes for abiotic stress tolerance in *Arabidopsis thaliana*. Mol. Genet. Genomics, 283: 273-287.

Romano, P.G.N., A. Edvardsson, A.V. Ruban, B. Andersson, A.V. Vener, J.E. Gray and P. Horton, 2004a. *Arabidopsis* AtCYP20-2 is a light-regulated cyclophilin-type peptidyl-prolyl cis-trans isomerase associated with the photosynthetic membranes. Plant Physiol., 134: 1244-1247.

Romano, P.G.N., P. Horton and J.E. Gray, 2004b. The *Arabidopsis cyclophilin* gene family. Plant Physiol., 134: 1268-1282.

Romano, P., J. Gray, P. Horton and S. Luan, 2005. Plant immunophilins: Functional versatility beyond protein maturation. New Phytol., 166: 753-769.

Ruan, S.L., H.S. Ma, S.H. Wang, Y.P. Fu, Y. Xin *et al*., 2011. Proteomic identification of OsCYP2, a rice cyclophilin that confers salt tolerance in rice (*Oryza sativa* L.) seedlings when overexpressed. BMC Plant Biol., Vol. 11. 10.1186/1471-2229-11-34

Scholze, C., A. Peterson, B. Diettrich and M. Luckner, 1999. Cyclophilin isoforms from *Digitalis lanata*. Sequences and expression during embryogenesis and stress. J. Plant Physiol., 155: 212-219.

Schonbrunner, E.R., S. Mayer, M. Tropschug, G. Fischer, N. Takahashi and F.X. Schmid, 1991. Catalysis of protein folding by cyclophilins from different species. J. Biol. Chem., 266: 3630-3635.

Schubert, M., U.A. Petersson, B.J. Haas, C. Funk, W.P. Schroder and T. Kieselbach, 2002. Proteome map of the chloroplast lumen of *Arabidopsis thaliana*. J. Biol. Chem., 277: 8354-8365.

Sekhar, K., B. Priyanka, V.D. Reddy and K.V. Rao, 2010. Isolation and characterization of a pigeonpea cyclophilin (*CcCYP*) gene and its over-expression in *Arabidopsis* confers multiple abiotic stress tolerance. Plant Cell Environ., 33: 1324-1338.

Sharma, A.D. and P. Singh, 2003. Effect of water stress on expression of a 20 kD cyclophilin-like protein in drought susceptible and tolerant cultivars of sorghum. J. Plant Biochem. Biotechnol., 12: 77-80.

Shieh, B.H., M.A. Stamnes, S. Seavello, G.L. Harris and C.S. Zuker, 1989. The *ninaA* gene required for visual transduction in *Drosophila* encodes a homologue of cyclosporin A-binding protein. Nature, 338: 67-70.

Sorrentino, G., M. Mioni, C. Giorgi, N. Ruggeri and P. Pinton *et al*., 2013. The prolyl-isomerase Pin1 activates the mitochondrial death program of p53. Cell Death Differ., 20: 198-208.

Stewart, D.E., A. Sarkar and J.E. Wampler, 1990. Occurrence and role of *cis* peptide bonds in protein structures. J. Mol. Biol., 214: 253-260.

Sykes, K., M.J. Gething and J. Sambrook, 1993. Proline isomerases function during heat shock. Proc. Natl. Acad. Sci. USA., 90: 5853-5857.

Takahashi, N., T. Hayano and M. Suzuki, 1989. Peptidyl-prolyl *cis-trans* isomerase is the cyclosporin A-binding protein cyclophilin. Nature, 337: 473-475.

Trivedi, D.K., S. Yadav, N. Vaid and N. Tuteja, 2012. Genome wide analysis of *Cyclophilin* gene family from rice and Arabidopsis and its comparison with yeast. Plant Signal. Behav., 7: 1653-1666.

Trivedi, D.K., M.W. Ansari and N. Tuteja, 2013. Multiple abiotic stress responsive rice cyclophilin: (OsCYP-25) mediates a wide range of cellular responses. Commun. Integr. Biol., Vol. 6, No. 5. 10.4161/cib.25260

Yurchenko, V., G. Zybarth, M. O'Connor, W.W. Dai and G. Franchin *et al*., 2002. Active site residues of cyclophilin A are crucial for its signaling activity via CD147. J. Biol. Chem., 277: 22959-22965.

Zoratti, M. and I. Szabo, 1995. The mitochondrial permeability transition. Biochim. Biophys. Acta (BBA)-Rev. Biomembr., 1241: 139-176.