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Genome-wide Study and Expression Profiles of a Promising Negative Regulator of Abiotic Stress Gene Family *OsDHSRP* in *Oryza sativa*

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Abstract

Rice is the global staple food, but the crop has been gradually impeded by many environmental constraints such as drought, floods, salinity, heat, and cold. Generally, plants adapt to environmental cues by altering physiological conditions primarily by modulating the expression of several stress-responsive genes. *OsDHSRP1* is an E3-ubiquitin ligase whose expression is highly stimulated by salinity, heat, and drought conditions, and it acts as a negative modulator by boosting reactive oxygen species (ROS) production, thereby causes damages to vital cellular organelles. In the present study, we are reporting genome-wide prediction, structural, evolutionary characterization, and expression analysis of *OsDHSRP* gene family of *Oryza sativa* under diverse abiotic stress conditions. A total of 15 *OsDHSRP* genes were discovered in *Oryza sativa* genome, which contains a C3HC4 zinc finger conserved domain. The elucidation of intron/exon and motif patterns provides structural aspects of these genes. *Cis*-regulatory analysis and transcription factor prediction studies revealed their roles and interactions with genes involved in multiple abiotic variables. Expression analysis of *OsDHSRP* genes by RT-qPCR revealed that *OsDHSRP1* is associated with cold, drought and salt stress conditions, suggesting the role of *OsDHSRP1* during diverse abiotic stresses. This study provides further insights into the regulation of expression of *OsDHSRP* genes for developing climate resilient crops.

Key words: Abiotic stress, Climate resilient crops, E3-ubiquitin ligase, *OsDHSRP*, *Oryza sativa*

Introduction

Worlds staple food crop, Rice (*Oryza sativa*), feeding more than 3.5 billion people worldwide and provides 20% of the world's daily calorie needs. It is vital for food security, better nutrition, good health, and global food stability (1). Abiotic factors, which include heat or temperature, flooding, dry conditions and salinity or nutritional deficiency, may hinder the germination process, seedling establishment, vegetative expansion, delayed flowering, panicle advancement, pollen infertility results in loss of productivity (2). Stress complications at the time of grain filling may adversely affect milling, cause chalkiness, alter carbohydrate content, and cooking characteristics (3). Combined abiotic factors, have a higher influence on the cultivation of rice than a single stressor (4). The consequences of abiotic stresses on the fields of agriculture are unclear, however the FAO reports that it impacts around 96.5% of rural land globally (5). As the world's population grows,

addressing the adverse effects of abiotic stress on crops is crucial for ensuring food security (6). Stress is a complicated and multigenic process that affects crop growth and development as well as yield. It has been found that abiotic stresses, especially temperature, drought, and saline soils, account for >60% of agricultural production losses globally (7,8). Stress caused by salinity primarily impedes growth of plants by causing oxidative damage, ionic disequilibrium, and osmotic stress (9). Heat stress occurs when temperatures rise above a certain threshold for an extended period, causing lasting impairment to plant growth (10). However, plants adapt to adverse conditions through molecular and cellular plasticity (11). Plant adaptations during abiotic stress involves various molecular mechanisms, notably sensing, signalling, expression of genes, synthesis of proteins, and post-translational alterations (12). High temperature alters metabolic pathways that impact the integrity of RNA and protein. Heat shock proteins (Hsps), ubiquitin ligases, and helicases all play vital functions in the regulation of RNA and protein metabolism during thermal stress (Nagaraju et al. 2021). Overall, plant's adaptation to abiotic stresses is modulated by an array of molecular networks which involves a variety of genes, signalling pathways, metabolic reactions, transcription and processing, perception, transmission of signals, processing, and protein synthesis and modifications (14).

In response to abiotic stressors, a number of proteins regulate cellular functions by biochemical or molecular mechanisms. Raffeiner et al. (15) noticed that breakdown of proteins is vital for regulatory functions as protein synthesis and conformational changes. In plants, the ubiquitin-proteasome system (UPS) plays a key role in the regulation of protein function via ubiquitin-mediated degradation. The UPS is a type of post-translational modification (PTM) mechanism that ligates ubiquitin to specific proteins, directing them to undergo breakdown. The UPS system is a diverse and precise mechanism to sustain physiological equilibrium in plants, enabling them to respond quickly to environmental stimuli (16). Hence, understanding the proteins or genes involved in UPS system is crucial. The E3 family of ubiquitin ligases comprises a broad family of enzymes that engage in the ubiquitination cascade along with the E1 and E2 ubiquitin activating and conjugating enzymes respectively. The Arabidopsis, rice, and maize genomes are estimated to comprise around 1100 genes encoding E3-ubiquitin ligases (17). All eukaryotes contain a highly conserved peptide comprised of 76 amino acids that make up ubiquitin (18). Three monomeric types of E3-ubiquitin ligases-RING, HECT, and U-box form an intermediate during ubiquitination (19). One superfamily of E3 ligases includes the Really Interesting New Gene (RING), homologous to the E6-AP carboxyl terminus (HECT), and U-box ligase, while the other superfamily includes cullin4-damaged-specific DNA binding protein1 (CUL4-DDB1), anaphase promoting complex (APC), and skp1-cullin-F-box (SCF) ligases (20). The ubiquitin-proteasome system is required for several plant hormone signaling processes, particularly E3 ubiquitin ligases that may detect and initiate signal transduction (21).

The expression of *OsDHSRP* gene indirectly elevates levels of methylglyoxyl making the plant more susceptible. *In vitro* assays carried out so far show that *OsDHSRP1* overexpressed plants had lower survival rates under drought and salt treatments in comparison with untransformed control plants. No difference in survival rates was observed among control and transgenic plants under ABA stress which reveals as it functions through ABA independent pathway. Along with ubiquitin assay, RT-qPCR analysis in *A. thaliana* confirms that *OsDHSRP* is reducing the activity of other positive regulators under stress conditions (22). Therefore, it is important to modulate of expression of these negative regulators which will enhance tolerance to multiple environmental variables. *OsDHSRP1,* an E3 ubiquitin ligase is one of such potential negative regulators. Other similar E3 ubiquitin ligases are also involved in stress adaptations such as *HOS1*, *AtATL78*, and *AtATL80* in Arabidopsis inversely regulate the plant's tol-

erance to cold stress (23). E3 ubiquitin ligases are also upregulated when exposed to heat stress. Heat stress resistance was enhanced by *AtSAP5* (24). *Oryza sativa* salt-, drought-, and ABA-induced RING finger protein 1 (*OsSADR1*) is a RING-type E3-ubiquitin ligase that is significantly increased at the transcriptional level by these three environmental cues (25). Overexpression of the RING E3-ubiquitin ligase RING domain-containing protein 1 (*OsRDCP1*), enhances rice tolerance towards an acute water crisis and is transcriptionally upregulated by water stress (26). *OsDIS1* adversely impacts the response to drought stress by ubiquitinating and degrading numerous stress-related proteins. Reduced drought tolerance is caused by overexpressing *OsDIS1* in rice, whereas increased drought tolerance and survival rates are caused by disrupting *OsDIS1* gene (27). In rice, a comprehensive analysis of *OsDHSRPs* has not been reported so far. We therefore predicted gene family members of *OsDHSRP* in rice genome and predicted gene structure, evolutionary analysis, conserved domains and motifs, synteny, transcription factor and miRNA using the tools of bioinformatics. Furthermore, we quantified transcript abundance of *OsDHSRP* gene members in various tissues of *Oryza sativa* under diverse abiotic stress conditions by RT-qPCR. The results obtained in this study will provide further directions for targeting these genes to develop climate resilient crops to thrive in multiple abiotic stress conditions.

Material and Methods

In silico identification, and characterization of *OsDHSRP* gene homologues

To find out the number of *OsDHSRP* genes in the rice genome, similar protein orthologs from *Arabidopsis thaliana* (*AT4G31450*) (22) were employed for BlastP against the *Oryza sativa* genome in the Phytozome database (http://www.phytozome.net/) (28). The hits were selected on the basis of a threshold E value of less than 1E-5. Then the data of *OsDHSRP* genes were submitted to the motif finder tool to predict the conserved motif in protein sequences. Only the genes with the C3HC4 Zinc Finger domain were picked from the results, while the rest were deemed redundant.

Gene structure prediction, subcellular localization, conserved motif analysis and physical mapping

The genomic and coding sequences of predicted *OsDHSRP* genes were retrieved and submitted to the Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn/index.php) to find out the Intron/Exon number and distribution. Furthermore, WOLFPSORT (https://www.genscript.com/wolf-psort.html) server was used to forecast the proteins' subcellular location (29). Parameters such as molecular weight, GRAVY (grand average of hydropathy), isoelectric point, aliphatic indices of proteins were predicted using ProtParam software (https://web.expasy.org/protparam/). MEME (http://meme-suite.org/tools/meme), (30) tool was employed to predict conserved motifs found in *OsDHSRP* proteins with parameters such as 10 number of motifs and 6 to 20 widths. The physical mapping of these genes was carried out by online Phenogram server (https://ritchielab.org/).

The evolutionary relationship and collinearity analysis

Mega version 11.0 was employed to align the amino acid sequences of the DHSRP proteins from *Oryza sativa*, *Sorghum bicolor*, *Zea mays*, *Triticum*, and *Arabidopsis* (http://www.megasoftware.net). The evolutionary tree was built by Mega Version 7.0 with neighbour joining method and 1000 bootstrap replicates (31). Synteny analysis between *Oryza sativa vs Zea mays* was carried out using TB tool and *Miscanthus sinensis vs Oryza sativa*; *Panicum virgatum vs Oryza sativa*; *Setaria italica vs Oryza sativa* were performed using phytozome database Synteny tool. The Ka/Ks calculator (http://services.cbu.uib.no/tools/kaks) was employed to determine the synonymous and non-synonymous substitution ratio and the time of evolution (MYA) of *OsDHSRP* paralog genes.

Promoter analysis of OsDHSRP genes, miRNA and transcription factor prediction

Promoter sequences (1500 bp upstream of the coding region) of all candidate genes were retrieved from Phytozome database. *Cis*-regulatory elements located in promoter regions were predicted using PLANT CARE tool (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). The miRNAs that target *OsDHSRPs* in order to regulate their expressions were predicted using psRNA target tool (https://www.zhaolab.org/psRNATarget/) with all default settings. The results were visualised as a network with Cytoscape tool. The transcription factor binding sites in all *OsDHSRP* genes were predicted using plant transcriptional factors database (PTFDB) (http://planttfdb.gao-lab.org/), and network was generated by Cytoscape tool (https://cytoscape.org/).

Prediction of protein structure and analysis of protein-protein interactions

The 2-dimensional structures of potential *OsDHSRP* proteins were determined using SOPMA server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) (32) and the 3-dimensional protein structures were predicted using Swiss model server (https://swissmodel.expasy.org/). The protein structure verification server (PSVS) (https://saves.mbi.ucla.edu/) was used to evaluate predicted structures and stability and analysed by Ramachandran plot. For the analysis of protein-protein interactions (PPI) of these homologs, STRING data base was employed (https://string-db.org/).

Gene ontology, Coexpression and in silico expression analysis

Gene ontology enrichment of *OsDHSRP* genes was performed using PANNZER2.0. The co-expression analysis for *OsDHSRP1* gene was performed using ATTED-2 tool (https://atted.jp/gene_coexpression/). The base line expression data (FPKM, Acc. Numbers) of all predicted *OsDHSRP* genes were retrieved from Rice Expression Database (RED) (https://ngdc.cncb.ac.cn/databasecommons/database/id/2029). Additionally, differential expression data of these genes were retrieved from the Gramene Database Expression Atlas (https://www.gramene.org/) and analysed by heat map built with TB tools.

Plant material and stress conditions

Oryza sativa cultivar MTU1010 was chosen for this study, and the germplasm was collected from IIRR, Rajendranagar, Hyderabad. Seeds were allowed to germinate on wet Petri dishes containing tissue paper in dark for 7 days at normal room temperature. After germination, seeds were grown in wet tissue culture containers under the light for a 16h photoperiod and 8h dark at a temperature of 25°C and 70% humidity and maintained in a plant growth chamber. After 15 days, plantlets were exposed to different abiotic stresses for a period of 16h, including 200 mM mannitol for induction of drought stress, and 200 mM NaCl for salinity stress. Furthermore, cold stress was imposed by incubating plantlets at 4°C and heat stress at 45°C (22). Corresponding control plants (untreated) were maintained simultaneously under identical conditions.

Quantitative real time PCR analysis of OsDHSRP genes

Total RNA was extracted from leaf and stem tissues of rice plants treated with different stresses along with untreated controls by Trizol method. The quality of RNA was determined using Eppendorf bio-photometer. Using prime script first strand cDNA synthesis kit (Takara), 500 nano grams of RNA was converted into first strand cDNA. According to *in silico* expression data, three *OsDHSRP* genes were considered for RT-qPCR analysis. Unique primers for *EF1-alpha*, *OsDHSRP1*, *OsDHSRP2*, and *OsDHSRP8* were designed using NCBI primer blast tool (Table 1). 2X SYBR premix Ex Taq (Tli RNase H Plus, Takara, Japan) master mix with gene specific primers was used for determination of relative transcript abundance of *OsDHSRP* homologues. RT-qPCR was carried out at 95°C for 1 min followed by 95°C for 10 sec for 40

cycles, 60°C for 30 sec, along with other default settings in ABI7500 Realtime PCR system (Applied Biosystems, Foster City, CA, USA). Gene expression of *OsDHSRP* genes in both treated and untreated samples was normalised with the reference gene *EF1-alpha*. Sample analysis was carried out using two biological and three technical replicates. By employing the comparative $2^{-\triangle\triangle Ct}$, relative expression of *OsDHSRP* genes were determined (Livak et al., 2001) and transcript abundance was represented. The average fold expression of genes was calculated by paired t- test using GraphPad prism software (https://www.graphpad.com/scientific-software/prism/www.graphpad.com/scientific software/prism/).

Table.1: Primers designed using NCBI primer blast.

S.NO	GENE	PRIMER SEQUENCE 5'-3'	LENGTH
1	*OsDHS-RP1-FP*	AAGGGGCTTAAGTG-GAACGG	20
2	*OsDHS-RP1-RP*	CTGAACCTAGCAC-CCCAAGG	20
3	*OsDHS-RP2-FP*	GAAGCAATGC-CAGAATGCGT	20
4	*OsDHS-RP2-RP*	GCGCCAGTATTCAGT-GGAGT	20
5	*OsDHS-RP8-FP*	CCAGCCTTT-GACTCCTCGTT	20
6	*OsDHS-RP8-RP*	CTTGGTAACGG-CAGTCTTGC	20
7	*OsEF-1A-FP*	GGGTCGTGTT-GAGACTGGAG	20
8	*OsEF-1A-RP*	CACGTACCCAC-GCTTCAGAT	20

Results and Discussion

Identification and characterization of OsDHSRP genes

The ortholog of characterized *OsDHSRP* protein domain from *Arabidopsis thaliana* was utilized as a query sequence for BlastP search against *Oryza sativa* genome for the identification of *OsDHSRP* homolog genes. Among all the hits, 15 genes were identified as potential candidates as *OsDHSRP* gene family members. All these 15 sequences containing C3HC4 zinc finger domain have been named as *OsDHSRP1*, *OsDHSRP2*, *OsDHSRP3*, and so on. The chromosomal location of these genes has been shown in the Table 2. Characterization of genes was carried out and the protein with the longest peptide sequence has been found as OsDHSRP4 (709 amino acids) and the protein with the shortest one as OsDHSRP6 (197 amino acids). The molecular weight of candidate proteins was analysed, all predicted OsDHSRP proteins ranged in between 20 kDa to 76 KDa. Protein parameters analysed include pI which ranged from 3.85 to 9.33, and GRAVY from -0.068 to + 0.325 (Table 3). Gene structure prediction showed that among 15 *OsDHSRP* genes, *OsDHSRP4*, *OsDHSRP8*, *9*, *1*, *11*, *13*, and *14* have 4/5 introns and exons, while *OsDHSRP2*, *3*, *10* have 3/4 introns and exons; *OsDHSRP6, 12, 15* have no introns (Fig. 1b). The subcellular localization of OSDHSRP homologs were investigated; *OsDHSRP1, OsDHSRP2, OsDHSRP3, OsDHSRP4, OsDHSRP7, OsDHSRP10, OsDHSRP12, OsDHSRP13, OsDHSRP14* have been localized in the nucleus; *OsDHSRP6, OsDHSRP8, OsDHSRP9, OsDHS*RP15 in the chloroplast; *OsDHSRP5* and *OsDHSRP11* in the vacuole.

Table.2: Characterisation of OsDHSRP homologues genes in rice.

Gene Name	Transcript ID	START END	Chr	CDS bp	Introns/ Exons	Domain	Localization
>Os-DHS-RP1	>LOC_ Os02g05692	2791241..2795471	2	580	4,5	ZINCFINGERC3HC4	NUCLEUS
>Os-DHS-RP2	>LOC_ Os08g14320	8582060..8584105	8	1050	3,4	ZINCFINGERC3HC4	NUCLEUS
>Os-DHS-RP3	>LOC_ Os04g51400	30439466..30442859	4	1104	3,4	ZINCFINGERC3HC4	NUCLEUS

>Os-DHS-RP4	>LOC_Os04g10680	5802054..5811151	4	2130	4,5	ZINCFINGERC3HC4	NUCLEUS
>Os-DHS-RP5	>LOC_Os04g55510	33022715..33028387	4	2004	5,6	ZINCFINGERC3HC4	VACUOLE
>Os-DHS-RP6	>LOC_Os01g53500	30730644..30731238	1	594	0,1	ZINCFINGERH2	CHLORO-PLAST
>Os-DHS-RP7	>LOC_Os01g06590	3099427..3105235	1	1506	5,6	ZINCFINGERC3HC4	NUCLEUS
>Os-DHS-RP8	>LOC_Os01g49770	28582842..28586527	1	606	4,5	ZINCFINGERC3HC4	CYTO-PLASM
>Os-DHS-RP9	>LOC_Os01g47740	27303110..27312308	1	1575	4,5	ZINCFINGERC3HC4	CHLORO-PLAST
>Os-DHS-RP10	>LOC_Os03g07790	3960942..3966307	3	753	3,4	ZINCFINGERC3HC4	NUCLEUS
>Os-DHS-RP11	>LOC_Os06g48040	29059686..29063780	6	1740	4,5	ZINCFINGERC3HC4	VACUOLE
>Os-DHS-RP12	>LOC_Os12g05270	2346442..2347276	12	834	0,1	ZINCFINGERC3HC4	NUCLEUS
>Os-DHS-RP13	>LOC_Os05g48970	28085119..28092428	5	1503	4,5	ZINCFINGERC3HC4	NUCLEUS
>Os-DHS-RP14	>LOC_Os05g47670	27317412..27320555	5	1095	4,5	ZINCFINGERC3HC4	NUCLEUS
>Os-DHS-RP15	>LOC_Os03g57410	32736205..32737399	3	657	0,1	ZINCFINGERH2	CHLORO-PLAST

Table.3: Protein parameters of OsDHSRP Homologs

Gene Name	Protein length (A.A)	Protein Molecular Weight(kDa)	GRAVY	PI
OsDHSRP1	580	64.278	-0.757	7.94
OsDHSRP2	349	38.586	-0.85	9.33
OsDHSRP3	367	41.0323	-0.886	8.82
OsDHSRP4	709	77.5573	-0.65	6.09
OsDHSRP5	667	72.641	-0.619	5.92
OsDHSRP6	197	20.5115	0.325	7.57
OsDHSRP7	501	54.6499	-0.579	8.59
OsDHSRP8	201	23.2885	-0.649	6.6
OsDHSRP9	524	57.7528	-0.591	6.1
OsDHSRP10	250	28.678	-0.665	5.91
OsDHSRP11	579	64.7518	-0.858	6.94
OsDHSRP12	277	29.126	-0.218	3.85

OsDHSRP13	500	54.536	-0.413	5.86
OsDHSRP14	364	39.6005	-0.606	9.2
OsDHSRP15	208	20.88264	-0.068	6.28

pI- isoelectric point; GRAVY-Grand average of hydropathicity index

The ring H2 domain and zinc finger C3HC4 domain are conserved domains found in OsDHSRP homologs

The biological function of these genes can be related to motifs found; conserved domain prediction shows all gene homologs have evolved together. Using MEME tool, a total of 10 motifs were identified in *OsDHSRP* genes. Out of 10 motifs found, motifs 1, 2, 3 are conserved across all *OsDHSRP* genes. Majority of motifs are limited to specific members of the *OsDHSRP* gene family. Motif 4 was found only in *OsDHSRP2* and 3, motif 10 in *OsDHSRP8* and 14, motif 5 and 7 in *OsDHSRP1* and 11 and motif 9 is exclusively located in *OsDHSRP9* and 13. Within an evolutionary clade, paralog gene pairs like *OsDHSRP4, OsDHSRP5; OsDHSRP8, OsDHSRP4; OsDHSRP2, OsDHSRP3;* show similar motif distribution pattern among them (Fig.1c). The results of the motif pattern and the conserved domain analysis of candidate genes showed that ring H2 domain and zinc finger C3HC4 domain are conserved among all genes and located towards C-terminal end of peptide (Fig. 1c). The pattern of zinc finger C3HC4 domain was similar in all proteins. The zinc finger C3HC4 domain (Fig. 1d) is present in all *OsDHSRP* gene homologs. This suggests that all predicted genes belong to *OsDHSRP* gene family. The identical motif patterns among genes of same groups correlates with evolutionary relationships.

OsDHSRP genes exhibit close evolutionary relation with Sorghum bicolor

Identification of conserved sequences among related species provides evolutionary constraints and importance of protein function. The evolutionary relationship among *OsDHSRP* genes was analysed and phylogenetic tree was constructed (Fig. 1a) and all candidate genes were grouped into 4 clades. A total of 6 pairs of paralogs were observed among 15 genes. *OsDHSRP6* and *OsDHSRP11* is a paralog pair from group 1, *OsDHSRP4* and *OsDHSRP5* from group 2, *OsDHSRP2* and *OsDHSRP3*; *OsDHSRP1* and *OsDHSRP12* from group 3. *OsDHSRP15* and *OsDHSRP19* is a paralog pair from group 4. The phylogenetic analysis of OsDHSRP proteins among closely related species such as *Sorghum bicolor*, *Zea mays*, *Arabidopsis thaliana*, *Triticum aestivum*, and *Oryza sativa* showed 4 ortholog pairs with Sorghum. The pairs are *OsDHSRP2* and *SbDHSRP10*; *OsDHSRP4* and *SbDHSRP13*; *OsDHSRP14* and *SbDHSRP4*; *OsDHSRP13* and *SbDHSRP7*. There is one ortholog pair related with *Z. mays* and *A. thaliana* which includes *OsDHSRP15* and *ZmDHSRP7*; *OsDHSRP10* and *AtDHSRP14* respectively. Based on above results it is clear that *Oryza sativa OsDHSRP* proteins are closely related with *Sorghum bicolor.*

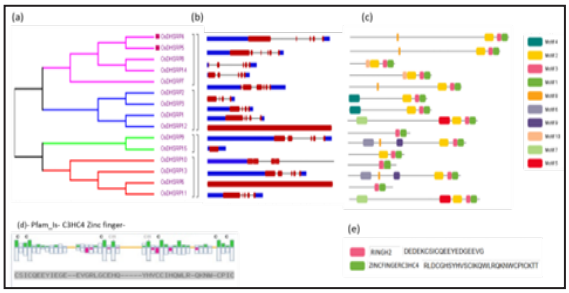

Figure 1 – a) The phylogenetic tree was constructed by MEGA v11.0 with the NJ method. b) Structures of the 15 putative OsDHSRP genes where red colour indicates exon, blue colour is UTRs and grey line is introns. c) Motif structure of homologous proteins. The different motifs are designated by different colours. d) Structure of C3HC4 zinc finger domain. e) Conserved domains and their sequence which are present in all homologs.

OsDHSRP genes show collinearity among Oryza sativa, Zea mays, Miscanthus sinensis, Panicum virgatum, and Seteria viridis

Synteny analysis helps understanding the gene divergence and rearrangements across species. Genomes were analysed using TB tools and no syntenic regions have been found between *O. sativa* and *A. thaliana* for *OSDHSRP* homologs. The collinearity between *Z. mays* and *O. sativa* chromosome 1 shows 2, 3, 4 homologs each with *Z. mays*, and chromosome 3, chromosome 6, chromosome 8 respectively. *O. sativa* chromosome 2 shows 2 homologs with *Z. mays* chromosome 5. *O. sativa* chromosome 3 shows 2, 1, 1, 1 homolog each with *Z. mays* chromosome 1, chromosome 7 and chromosome 9 respectively. Rice chromosome 2, shows 2 homologues each with *Z. mays* chromosome 10 and chromosome 2, chromosome 5 shows 1, 2, 3 homologs with *Z. mays* chromosome 3, chromosome 6 and chromosome 8 respectively. Rice chromosome 6 shows 2 homologues with *Z. mays* chromosome 5. *O. sativa* chromosome 8 shows 2, 1, homologs with *Z. mays* chromosome 10 and 2; and chromosome 12 shows one homologue with *Z. mays* and chromosome 4 (Fig. 2a). Chromosome 1 of rice showed homology with *Miscanthus* chromosome 5 and chromosome 6. Rice chromosome 2 showed one homology each with *Miscanthus* chromosome 7 and chromosome 8; and chromosome 3 showed 2, 2 homologies with *Miscanthus* chromosome 1 and chromosome 2. Rice chromosome 4 showed 1, 2 homologies with *Miscanthus* chromosomes 11 and 12 respectively. Chromosome 5 of rice showed homology each with *Miscanthus* chromosome 16 and chromosome 17; and chromosome 6 showed one homology each with *Miscanthus* chromosomes 18 and 19 respectively; and rice chromosome 8 exhibited one homology with *Miscanthus* chromosome 13. Synteny analysis of *Oryza sativa* chromosome 1 showed 4 homologs with *Panicum* chromosome 5. Rice chromosome 2 displayed 2 homologies with *Panicum* chromosome1; and chromosome 3 showed 4 homologs with *Panicum* chromosome 9. Also, rice chromosome 4 showed 4 homologs with *Panicum* chromosome 7; and chromosome 5 showed 2 homologues with *Panicum* chromosome 3; and chromosome 6 displayed 2 homologs with *Panicum* chromosome 4. Rice chromosome also showed 2 homologues with *Panicum* chromosome 6. In collinearity mapping of genes among *Oryza sativa* and *Setaria italic*, most of the homology was found in the scaffold region of *Setaria italica* (Fig. 3).

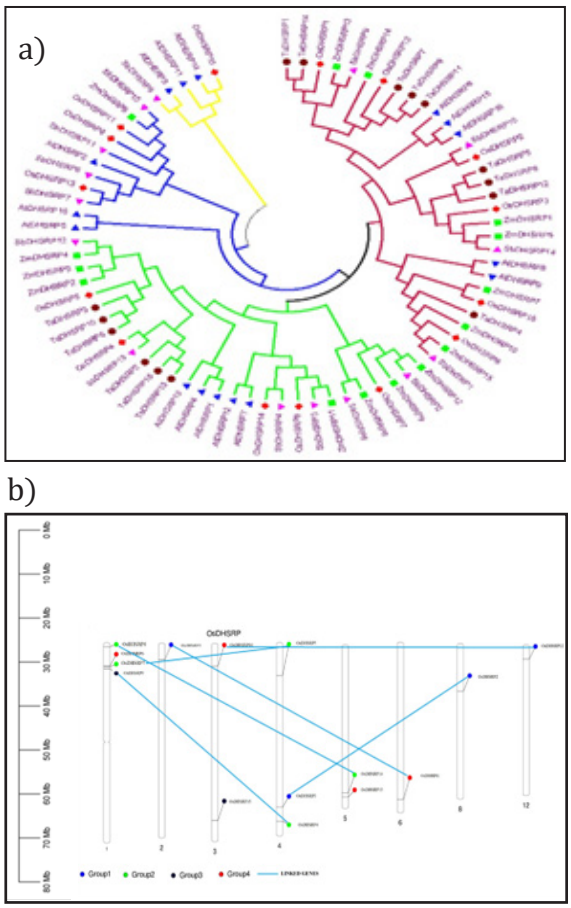

Figure 2a. The phylogenetic tree constructed using Mega 11.0 by bootstrap method. The colour of branches indicates four different groups. 2b. Distribution of *OsDHSRP* genes on the chromosomes of rice genome. Here, genes with same colour belongs to same evolutionary groups and genes connected by blue lines are linked ones.

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Conserved protein sequences among related species and gene paralogs that undergo purifying or stabilizing selection

Multiple sequence alignment with protein sequences of *OsDHSRP1*, *OsDHSRP5*, *Zm00001d014609*, *sobic.004G042100.1. P, Traes_7AL_ADB7BCC89.1*, *AT5G10650.1* was analysed using Mega 11.0 by ClustalW and visualised through Snap gene viewer. The alignment showed conserved blocks among all these genes, suggesting that these *OsDHSRP* genes are conserved across diverse species (Fig.1 supplementary material). According to Ka/Ks annotated evolutionary tree, 6 paralog pairs were observed. The paralog pair *OsDHSRP14*, *OsDHSRP18* has the lowest Ka/Ks value. All paralogs displayed Ka/Ks ratio less than one, which explains specific purifying selection. MYA was calculated to predict the duplication distance. The estimation of divergence time for 6 pairs showed that gene duplication occurred between 11.2 -71.8 MYA. The Ka/KS values, MYA values, paralog pair are given in Table 4.

Table.4: Ks-synonymous substitution; Ka-non-synonymous substitution;T(MYA)-Evolution time in Million years ago. Time calculated based on T=Ks/2x where x is $6.56x10^{-9}$ formula.

GENE PARALOGS	KA	KS	KA/KS	MYA
OsDHSRP7,OsDHSRP5	0.39945	0.51475	0.7760078	30445884.15
OsDHSRP1,OsDHSRP11	0.093260335	0.1746	0.5341371	7108257.241
OsDHSRP10,OsDHSRP12	0.1471	0.4224	0.3482481	11211890.24
OsDHSRP3,OsDHSRP2	0.363	0.4433	0.8188586	27667682.93
OsDHSRP9,OsDHSRP4	0.3731	0.4914	0.7592593	28437500
OsDHSRP14,OsDHSRP8	0.082964555	0.2423	0.3424043	6323517.912

Cis-regulatory elements associated with abiotic stress were found in OsDHSRP homologs and OsDHSRP genes are more abundant on chromosome 1

The predicted *cis*-regulatory elements found in promoter regions of *OsDHSRP* homologs include light-responsive elements (LRE), drought-responsive MYBs, *cis*-elements associated with meristem expression, circadian cycle, cell cycle regulation, MYB binding site, low temperature-responsive elements (LTRE), endosperm-specific negative regulation, and elements with seed-specific regulation. Plant hormone related elements such as auxin, methyl jasmonate, gibberellin, salicylic acid and abscisic acid-responsive and defence and stress-responsive elements implicated in biotic stress were also noticed. Some abiotic stress elements like drought and salt, low temperature, and metal-responsive elements were detected. Most of the elements include abscisic acid-responsive elements (ABRE) which determine their function in abiotic stress response (Fig. 4a). The presence of LRSs specifies that they are regulated by light. Metal-responsive elements suggest that the genes are involved in heavy metal stress tolerance. Based on this data, *OsDHSRP* genes may play key roles under drought, salinity, heat, cold and metal stresses. Physical mapping of *OsDHSRP* genes on *Oryza sativa* genome revealed that these genes are scattered across all chromosomes and most of the genes are localized on chromosome 1 of *Oryza sativa* (Fig. 2b).

The protein structure reveals OsDHSRP as a functional E3-ubiquitin ligase

Enzymatic activity, ligand binding sites, active sites, and interactions between *OsDHSRP* proteins determine their biological function. The 2-D structure prediction showed different forms of proteins including beta turns, extended strands, alpha helices, and random coils. The proportion distribution of these are tabulated In Table 5 (Fig. 5b). The 3-dimensional structure of *OsDHSRP* proteins were predicted using Swiss model server. The models were select-

ed based on maximum percentage of identity with standard PDB structures. The selected structure PDB ID, protein ID, percentage identity are tabulated (Table 1). 3D structures are shown in Figure 3, supplementary material. The template 6W9a.1.B-E3 ubiquitin ligase, ubiquitin conjugating enzyme E2 showed the highest structural similarity with most of the homologs including *OsDHSRP2*, *OsDHSRP5*, *OsDHSRP9*, *OsDHSRP1*, and *OsDHSRP12.* Template ID 6W9a.2.B-E3 ubiquitin ligase RLIM exhibited similarity with *OsDHSRP3*, *OsDHSRP9*, and *OsDHSRP15.* Furthermore, template 6W7z.1.B-E3 ubiquitin ligase, E2 ubiquitin conjugating enzyme D2, showed similarity with *OsDHSRP4*, *OsDHSRP6*, *OsDHSRP7*, *OsDHSRP10*, *OsDHSRP14*, and *OsDHSRP13*. Using string database, the proteins which interact with *OsDHSRP1* protein was analysed. Metabolic processes involved with *OsDHSRP1* protein can be understood by protein interactions. The results suggest that *OsDHSRP1* protein interacts with ARF-16, MP-ARF11 auxin-responsive element that precisely binds to DNA sequence 5'-TGTCTC-3' was found in the auxin-responsive promoter elements (Aux REs). Additionally, other interacting proteins include BHLH 156, a transcription factor which is involved in negative regulation of iron acquisition genes for mugineic acid (MA) family. Phyto siderophores biosynthesis, S-adenosylmethionine cycle and iron transport (Fig. 5a), *Os03g0383800* SAP domain containing protein, *Os06g0666400*, *Os02g0173200*, *Os04G0663100*, *Os01g0687600*, have been found as uncharacterized proteins. Another protein *Os07g0626200* appeared like Acinus protein. The results of string interaction analysis among the 15 candidate genes showed that they are non-interactive with each other.

Table.5: Secondary structure of *OsDHSRP* protein homologs.

Gene/ protein Name	Alpha helix %	Extended Stand %	Beta turn %	Random coil%
>*OsDHSRP1*	25.69	7.59	2.76	63.97
>*OsDHSRP2*	24.64	12.89	6.3	56.16
>*OsDHSRP3*	33.51	11.17	5.45	49.86
>*OsDHSRP4*	19.46	8.32	2.96	69.25
>*OsDHSRP5*	16.49	9	3.3	71.21
>*OsDHSRP6*	36.04	12.69	6.09	45.18
>*OsDHSRP7*	16.17	8.18	2.4	73.25
>*OsDHSRP8*	42.79	2.99	1.49	52.74
>*OsDHSRP9*	12.79	11.07	2.1	74.05
>*OsDHSRP10*	23.56	21.15	8.17	47.12
>*OsDHSRP11*	22.45	5.7	2.94	68.91
>*OsDHSRP12*	12.88	15.52	11.19	44.4
>*OsDHSRP13*	15.2	9.8	3.4	71.6
>*OsDHSRP14*	37.64	6.87	6.32	49.18
>*OsDHSRP15*	14.4	17.6	4.4	63.6

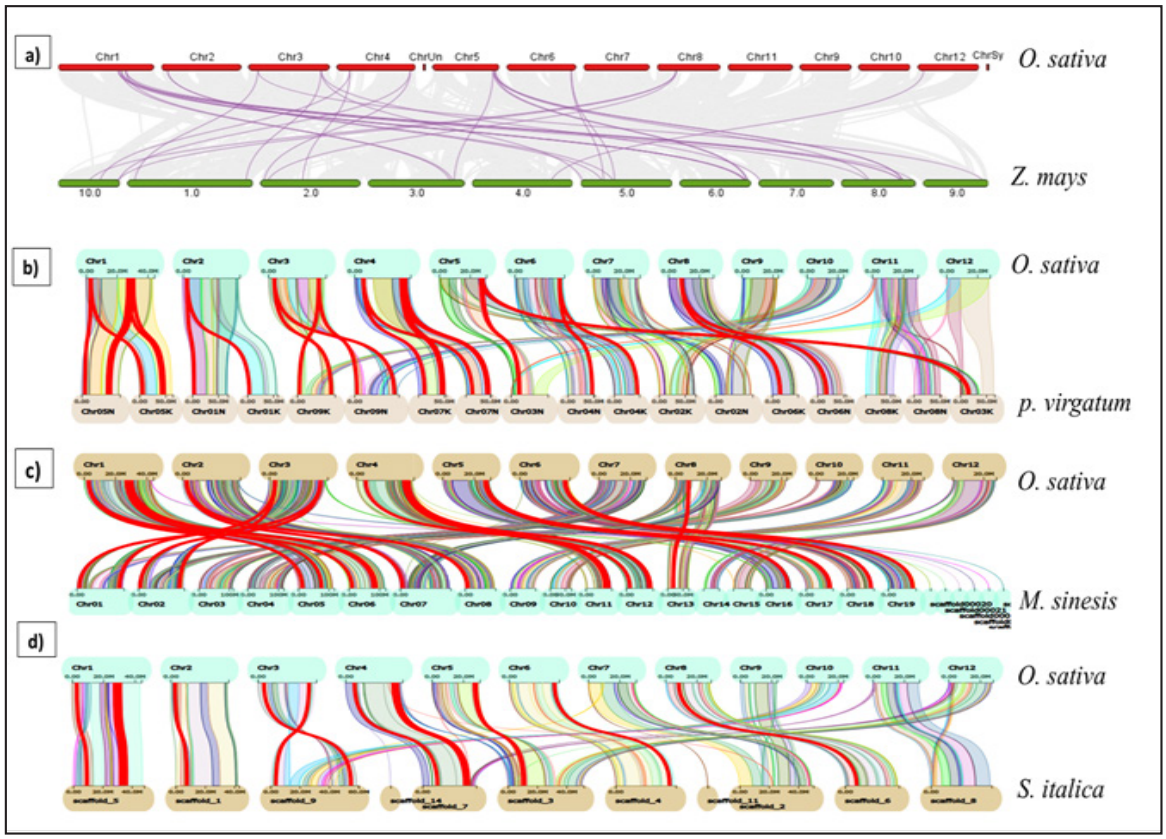

Figure 3a. It shows collinearity among *Z. mays* and *O. sativa* species were purpled coloured lines indicates collinearity of genes. 3b. Synteny map of *O. sativa* and *P. virgatam* where red lines indicate collinearity among them. 3c. Synteny of *O. sativa* with *M. sinesis* were red lines shows collinearity among homologous genes. 3d. Collinearity of genes among *O. sativa* and *S. italica* v2.2. Red colour lines show collinearity among homologous genes in two species of b, c, d.

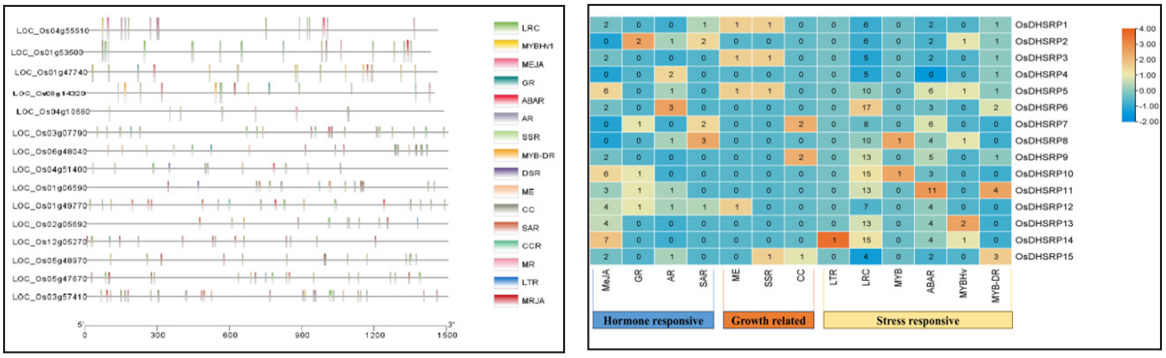

Figure 4- a) The distribution of cis regulatory elements present in 15 *OsDHSRP* gene homologs. Each colour indicates a specific promoter element. b) Cis regulatory elements in the promoter of each *OsDHSRP* gene are classified according to their probable activities, which include hormone-responsive, environmental stress-related, and growth related.

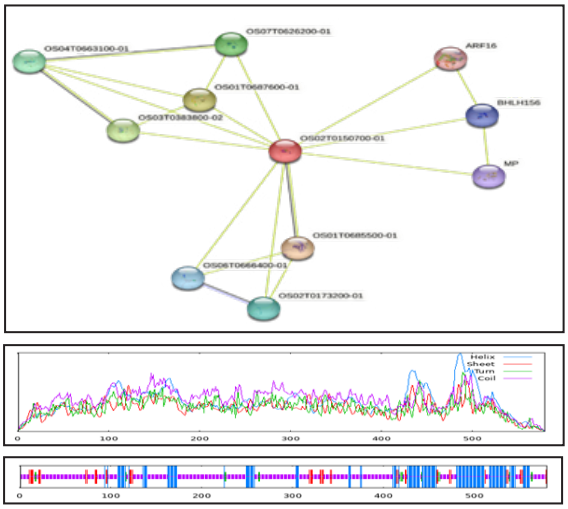

Figure 5a. Protein -protein interactions of *OsDHSRP1* protein from String Database. 5b. 2D structure of *OsDHSRP1* protein. The distribution of helix, sheet, turn, coils were indicated with different colours.

miRNA targets

Abiotic stress-related transcription factors (TFs) bind to regulatory regions of *OsDHSRP* genes, trigger the stress-responsive expression. Gene expression of *OsDHSRP* genes rely on the binding of specific transcriptional factors. Our analysis revealed that candidate genes are regulated by 35 distinct types of transcriptional factors. Among them, MYB, WOX, FAR1, EIL, TALE, Trihelix, CAMTA, C2H2, LBD, ERF, MYB-related, CPP, HD-zip, HSF, BZIP, GATA, SBP, MIKC-MADS, AP2, NAC, WRKY, C3H, YABBY, RAV, bHLH, G2-like are mostly abiotic stress-related transcriptional factors and are involved in plant growth and development (Fig. 6a) (34, 35,36). Dof, C2H2, YABBY, GATA, C3H are zinc domain containing proteins whereas other transcriptional factors are related in plant growth and development and some are hormone-responsive transcription factors. The miRNA identified can be used to target these *OsDHSRP* homologous genes and regulate their expression patterns. *OsDHSRP1* shows 6 miRNA targets which include *O. sativa* miRN2282, miR444c, miRN2222, miR528, miR440, and miRN2376 (Fig. 7). miR444c and miR528 are drought stress-related and miR444c is over expressed under stress (37). Additionally, the miR5508 has been noticed which is a potential target to regulate the expression of both *OsDHSRP13* and *OsDHSRP9* genes.

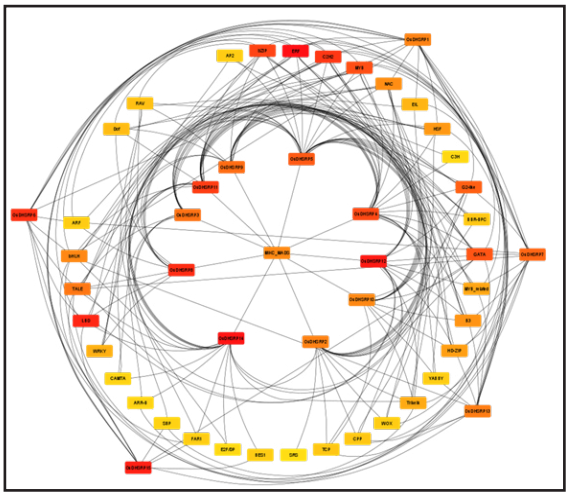

Figure 6. Network designed by Cytoscape tool of Predicted Transcriptional factors of *OsDHSRP* homologs genes. Presence of transcriptional factors such as bHLH, YABBY, MYB, NAC, WRKY, ARF determines *OsDHSRP* homologs are stress responsive.

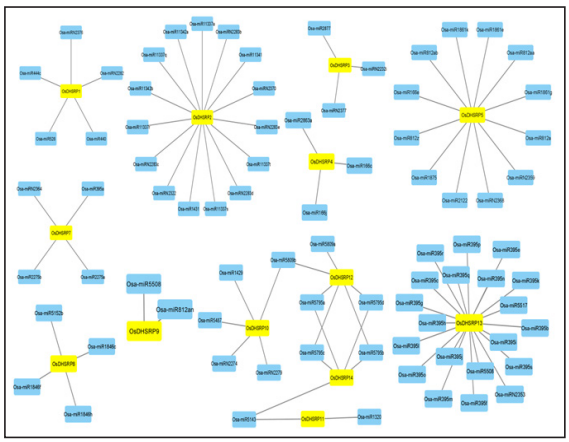

Figure 7 – The Network showing predicted MiRNAs of 15 *OsDHSRP* genes, Here yellow colour indicates genes and blue are predicted miRNAs of the specific gene. miRNAs can be used to target *OsDHSRP* homolog genes.

OsDHSRP genes show metal ion binding and ubiquitin ligase activity

Understanding the molecular function of candidate genes and their participation in biological functions in plants was carried out by GO studies. The GO terms were analysed for all the candidate genes and results showed most of the genes exhibit similar functions with ubiquitin protein ligase activity-GO:0061630, metal ion binding, GO:0046872, and ligase activity, GO:0016874 by *OsDHSRP1*, *OsDHSRP2*, *OsDHSRP3*, *OsDHSRP4*, *OsDHSRP5*, *OsDHSRP9*, *OsDHSRP8*, *OsDHSRP10*, *OsDHSRP11*, *OsDHSRP13*, and *OsDHSRP14.* While the gene *OsDHSRP10* showed acyl transferase activity, GO:0016746 alongside with above functions, *OsDHSRP15* showed only acyl transferase activity. *OsDHSRP7* has only the ubiquitin protein ligase activity and metal ion binding site without ligase activity. *OsDHSRP6* has transferase activity, GO:0016740 along with metal ion binding. On the other hand, *OsDHSRP14* has an additional helicase activity of GO:0004386 but *OsDHSRP12* showed zinc ion binding ubiquitin, GO:0008270 and protein ligase activity.

E3 ubiquitin ligases are expressed together with OsDHSRP1 gene

Identifying other genes that are likely to function together with *OsDHSRP1* gene and participate in related cellular functions helps to know their co-ordination during stress response which further, lays foundation for manipulating gene expression in plant. Co-expression analysis for *OsDHSRP1* gene was carried out and the results are presented as a network which was created using Cytoscape tool (Fig. 2 supplementary material). Several genes were co-expressed along with *OsDHSRP1* which includes *Os01g0936200*. This gene has feruloyl esterase A activity, an enzyme which shows the highest rank with *OsDHSRP1* expression. Several other E3 ubiquitin protein ligases such as *RHB1A*, *AIRP2*, *atg7*, *AIRP2-LIKE*, *CHIP*, *At3g022g0* were also expressed. Furthermore, ubiquitin receptor *RAD23d* is expressed along with *OsDHSRP1* gene.

In silico expression patterns of OsDHSRP genes

In silico expression data (38) reveals that the expression of *OsDHSRP1* gene is high in root and embryo tissues compared to other tissues/organs (Fig. 4a, supplementary material) and low in leaf blade, endosperm, and reproductive leaf sheath. Among the organs, comparatively, expression of *OsDHSRP1* is more in leaf than in stem (Fig. 4b supplementary material). Under sodium dihydro-phosphate treatment at different time periods (Fig. 5c supplementary material) (39), *OsDHSRP1*, *OsDHSRP2*, and *OsDHSRP4* exhibited strong expression patterns. When rice plants were exposed to heavy metal stress (cadmium sulphate) (40) (Fig. 5a Supplementary material), *OsDHSRP1* exhibited moderate expression. Moreover, *OsDHSRP7* and *OsDHSRP9* displayed the highest expression under low temperature stress (Zhang et al., 2012) (Fig. 5b supplementary material). Additionally, *OsDHSRP1* exhibited a moderate expression, whereas *OsDHSRP10* and *OsDHSRP8* displayed the highest expressions.

Upregulation of OsDHSRP1 gene under various abiotic stress treatments

Analysing gene expression provides insights into whether specific transcripts are upregulated or downregulated under specific stress conditions. As per RT-qPCR analysis, *OsDHSRP1* exhibited a strong expression pattern in leaf tissues under cold stress, along with drought and salt stresses. Compared to leaf tissues, expression of *OsDHSRP1* and *OsDHSRP8* gene was high in stem tissue under salt stress. The expression of *OsDHSRP1* gene was low in untreated plant tissues like stem and leaf in comparison with abiotic stress conditions. When exposed to stress, the relative expression of *OsDHSRP1* was 18.74, 12.81, and 11.27-folds in cold-treated leaves, drought-treated leaves, and salt-exposed stem tissues respectively (Fig. 8). *OsDHSRP2* gene shows high expression levels (fold change) under heat in the leaf tissue. The relative expression of this gene also suppressed in untreated

plants than the plants subjected to stress. *OsDHSRP8* gene showed strong expression under saline conditions in stem tissues and lower expression in drought-stressed leaf tissue and cold conditions. Overall, the expression of *OsDHSRP* homologs displayed high expression patterns under various abiotic stresses in comparison with control (without any stress) conditions.

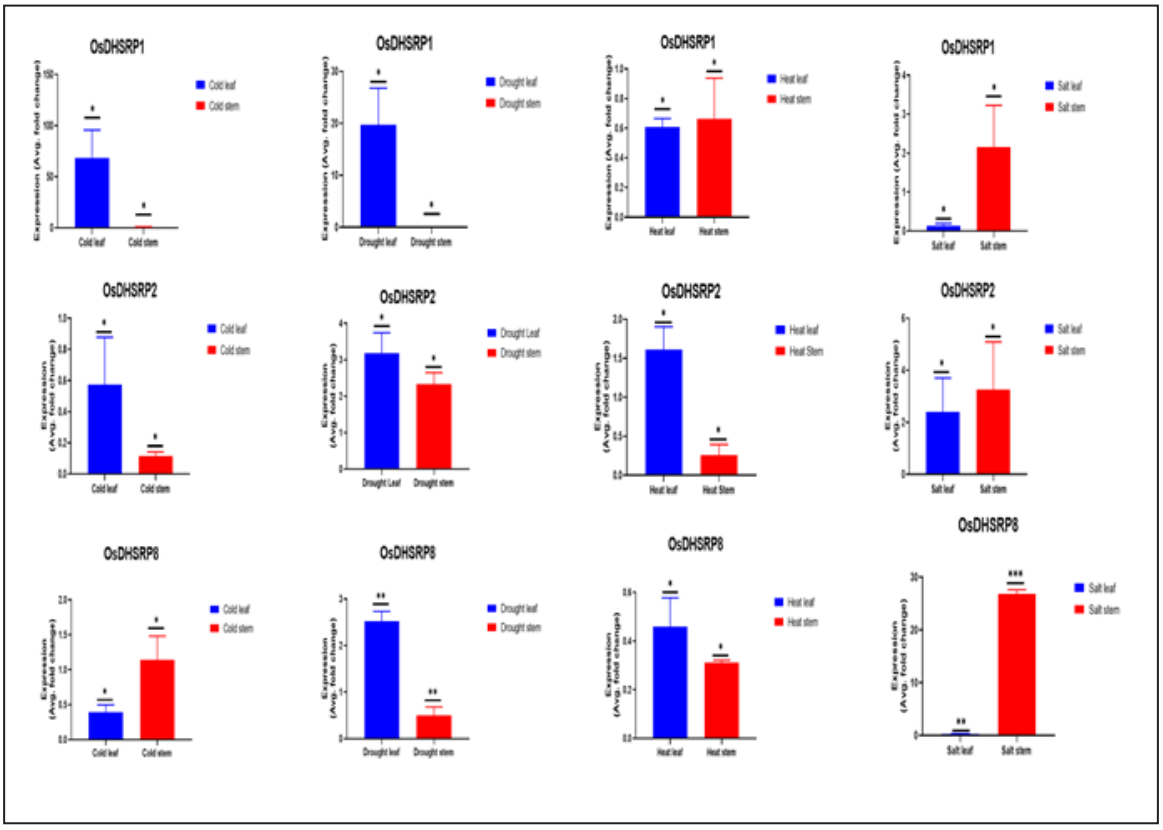

Figure 8. Graphs showing expression (average fold change) patterns of *OsDHSRP* homologs done by RT-qPCR analysis. y-axis indicates expression values while x-axis indicates sample type. Blue colour represents leaf tissues and red colour represents stem tissue. *Indicates *P* value<0.05, ** indicates *P*<0.001 and ***indicates *P*<0.0001. error bars represent standard deviation.

Various environmental variables exert an impact on overall agricultural yields (41,42). Among abiotic stresses, drought, salinity, and temperature are three critical and devastating environmental concerns that severely impair crop productivity (43,44). *OsDHSRP* gene is a potential negative regulator of abiotic stress in rice. In the current research, we employed a genome-wide analysis approach to examine the chromosomal localization of *OsDHSRP* genes, their evolutionary connections, gene architecture, *cis*-acting elements, and *in silico* and *in vitro* gene expression profiles of *OsDHSRP* genes under diverse stress conditions in rice. The *OsDHSRP1* locus encodes a drought, heat, and salt-induced ring finger protein possessing E3 ubiquitin ligase functions. Major function of this gene is to sustain protein stability and refold of proteins. In plants experiencing abiotic stress, it functions as an antagonistic regulator. It renders plants more susceptible to stress by boosting the levels of reactive oxygen species (ROS)

at the cellular level (45). *In vitro* ubiquitination assays were performed in rice plants, and mediated ubiquitination was validated using the Ni-NTA purification technique (22). Its expression is strongly driven by elevated temperatures and it blocks the glyoxylase pathway by degrading the *OsGLY1-11.2* via the 26S proteasome system, thereby increasing methylglyoxal levels in plant cells. The plant becomes more susceptible to a range of abiotic stresses, particularly heat, as ROS and methyl glyoxyl (MG) levels rise (45). In plants, environmental factors such as salt, drought, heat, and abscisic acid (ABA) drive the expression of the *OsDHSRP* genes. The ligase function of the gene is mediated by the ring H2 domain. E3 ligases interact with various proteins and degrade their substrates via the proteasome complex. *In vitro* ubiquitination experiments revealed *OsDHSRP* interacts with two target proteins; an acyl carrier protein (ACP) OsACP1 and OsGLYI-11.2. The over expression of the *OsDHSRP* gene was tested in Arabidopsis plants. The rate of germination was observed in control and transgenic plants treated with 0.1-5 mM ABA, 0-200 mM NaCl and 0-200 mM mannitol for a period of seven days. The data highlight that control plants have threefold higher survival rates than transgenic plants, suggesting that the *OsDHSRP* gene functions as a negative regulator under stress. In transgenic plants, the rate of germination was significantly lower in contrast to control plants with increasing salt and mannitol concentrations, but no significant variation was detected among transgenic and control plants subjected to ABA stress. This demonstrates that the gene can be implicated in ABA-independent pathways. In Arabidopsis, overexpression of *OsDHSRP1* also resulted in impaired heat tolerance and reduced recovery abilities (22). RT-qPCR analysis in transgenic *A. thaliana* over expressing *OsDHSRP1* gene was used to find out the variations in the expression of other stress-responsive genes (22). Transgenic lines displayed down regulation of other stress related genes such as NAC and salt overly sensitive (SOS) pathway genes under salt stress, MYB2 and bZIP under ABA stress, heat shock proteins in high temperature stress, and DREB2A and 2B under drought stress. These findings reveal that overexpression of the *OsDHSRP1* gene suppresses other stress-responsive genes. The results infer that it acts as a negative regulator in response to heat, salinity, and drought, but there are no substantial changes in ABA stress (22). Under normal conditions, both control and transgenic plants exhibited equal levels of methyl glyoxal. However, under stress, transgenic plants exhibit excessive MG accumulation; inferring that MG levels are associated with *OsGLY11.2* gene expression because it plays critical roles in glyoxylate pathway (46). The results indicate that down regulation or degradation causes an increase in MG levels. In the current study, 15 *OsDHSRP* gene homologs have been identified in *Oryza sativa* genome, all of which comprised the signature zinc finger domain C3HC4 type along with ring H2 domain. The C-terminal end of the motif contains conserved domains. Phylogenetic study revealed that these 15 genes are divided into four distinct clades and these are closely associated with *Triticum aestivum* and *Sorghum bicolor*. Amongst the closely related species, *Zea mays* and *A. thaliana* have been noticed. The gene architectures of 15 homologs are comparable within the group, and it is worth noting that *OsDHSRP12* and *OsDHSRP6* possess only one exon without any introns or UTRs. All selected *OsDHSRP* proteins contain C3HC4 domain and are also validated as E3 ubiquitin ligases according to gene ontology studies. Ubiquitin-mediated modifications of proteins regulate a wide range of physiological activities, including growth and development, hormonal, stress-related responses, and cellular divisions. It is known that E3 ligases play critical roles in ubiquitin-protease cascade in plants (17).

Our results revealed that *OsDHSRP1* proteins have residues among *S. bicolor*, *Zea mays*, and *Triticum aestivum*, indicating that these proteins are highly conserved among the species. This is consistent with phylogenetic analysis results. *OsDHSRP* genes show four

paralog pairs with *S. bicolor* and one with maize and Arabidopsis. Despite having many paralogs, there is no genetic collinearity between sorghum and rice, although *Zea mays* exhibits it. The identified promoter elements are consistent with the reported function of the gene as stress-responsive since findings include LREs, MYB-drought-responsive, low temperature-, metal-, and various plant hormone-responsive elements. This prediction suggests that the gene is implicated in low temperature or cold, drought, and heavy metal stresses. The existence of MeJA promoter elements implies that the gene plays a role in a variety of biotic stress responses also (47). Numerous transcription factors, such as AP2/ERF, NAC, WRKY, MYB, and bHLH, are widely recognised as environmental stress regulators (48,49). The key TF families WRKY, MADS-box, and MYB regulate both growth and developmental processes, biotic and abiotic stress responses, and a range of plant developmental aspects through specific and/or cross-talk modulation across multiple TFs (50). TFs such as *OsIDS1*, *OsWRKY1*, and *OsWRKY45-2* are negative regulators involved in the salt stress response by suppressing the expression of their target genes (51). bHLH is crucial to salt stress regulation in rice. Numerous regulons have been thoroughly examined in rice, including DREB, AREB, and NAC (52). As a result, understanding transcriptional regulatory networks is critical for finding out the way genes function and respond to abiotic stresses. ARF, bHLH, SAP domain containing proteins showed interactions with *OsDHSRP1* protein. Auxin response factors (ARFs), important in auxin transport and signaling elements, regulate rice developmental processes, such as root formation, tiller and leaf angulation, floral organ growth, and size of grain. An N-terminal A20 domain and a C-terminal AN1 domain form the two zinc-finger domains that collectively make a group of proteins known as stress associated proteins (SAPs) have been discovered (53). These proteins have been found to regulate immunological signaling and a variety of other reactions in animals, including those to biotic and abiotic stressors (54). Similar to their animal counterparts, SAPs were recently found as new E3 ubiquitin ligases. *AtMBP1*, a negative regulator of ABA and stress signaling, is ubiquitinated and targeted for degradation by SAP5 in Arabidopsis (55). *OsSAP7* has also been found to have E3 ligase activity (56). *OsDHSRP1* and its homologs show ubiquitin protein ligase activity and metal ion binding site.

The miRNA prediction allows us to identify the potential target genes. *OsDHSRP1* gene has been shown to be the target of rice micro RNAs such as miR528, miRN2282, miRN2222, miRN2376, and miR444c. Few of novel miRNAs were discovered in rice such as miR444c.1, c.2, d, e, and few members which are likely to be highly conserved among monocot plants such as maize, switchgrass, wheat, sugarcane and sorghum (57), but not in Arabidopsis. Probable targets of two miRNAs (miR1432 and miR444d) include calcium signaling. Ca^{2+} acts as a second messenger and a signal transducer when plants are exposed to abiotic stimuli (58). In plants, elevated temperatures may be responsible for the variations in the heat-induced repression of miR5508 expression patterns in the rice cultivars (59). Further, it has been found in wheat that the heat-responsive miRNAs, miR528 and miR9662 modulate the stress-responsive mitochondrial proteins (60).

Expression *OsDHSRP1* gene using RT-qPCR revealed that its expression is higher in comparison with in silico expression data. The activity is highly expressed in leaf tissues compared to stem. The relative fold expression of *OsDHSRP1* gene is the highest in leaf tissue under cold stress. In both stem and leaf, the relative fold changes are high under cold treatment than in untreated control plants. *In silico* expression data shows high expression of *OsDHSRP8* gene in 48 hours but comparatively low in 12-hour cold treated plants (61). In RT-qPCR analysis, *OsDHSRP8* shows similar fold change in untreated and cold treated plants after 16 hours. The expression levels of *OsDHSRP1* and *OsDHSRP8* genes are similar at

12-hour cold exposure but *OsDHSRP1* expression is higher than *OsDHSRP8* under cold treatment in both leaf and stem tissues. The overall expression of *OsDHSRP1* is relatively higher under various treatments compared to untreated control plants. The increase in expression levels of *OsDHSRP1* regulates various physiological functions, such as increase in the accumulation of methylglyoxal levels and down regulates the expression of other stress responsive genes such as heat shock proteins, NAC and DREB2A and DREB2B which may make the plants susceptible to stress conditions. Higher expression levels of this gene also degrade two proteins, *OsACP1* and *OsGLY11.2* by ubiquitination through 26S proteasome mediated system (22). This shows that *OsDHSRP1* gene is a potential negative regulator during abiotic or environmental stresses.

Conclusion

In the present research, a total of 15 *DHSRP* genes were identified in *Oryza sativa* genome. All *DHSRPs* were grouped into four clades based on phylogenetic analysis, indicating the existence of four ancestor genes. *OsDHSRP* gene homologs exhibited similar characteristics in terms of gene structure, conserved domains, and motifs. The protein parameters and predicted protein structures provide the structural features of *OsDHSRP* genes. These candidate genes from rice have Ka/Ks ratios lower than 1, indicating that strong purifying selection was successfully applied to the genes during the course of evolution. Identification of *cis*-acting elements and transcription factor prediction might be useful to understand the gene regulation under stress. The expression by RT-qPCR and regulation of *OsDHSRP* genes offer overexpression or suppression and thus improve abiotic stress tolerance in rice. This can be aided by the prediction of miRNAs and the understanding of expression profiles under distinct abiotic stress conditions.

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Author contribution statement

Anjana Priyadarshani K and Srinivas Naik K have designed the experiments and the structure of the article and prepared the first draft. All others have added lateral text in the manuscript and refined it. Prashanth B and Viswanadha Naik J have prepared the figures. Viswanadha Naik J, Ashok Kumar K have added lateral text and figures in the manuscript. Srinivas Naik K, Prashant S, Ashok Kumar K, Viswanadha Naik J, Prashanth B, Anjana Priyadarshani K have revised the manuscript. All authors have approved it.

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Declaration of competing interest

The authors state that they do not have any known financial conflicts of interest.

Data availability

The data are available with the corresponding author K. Srinivas Naik.

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