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Genome-wide analysis of Rice OsDHSRP gene family and their Expression profiles under different Abiotic Stresses

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Abstract

Rice serves as a principal food class globally and the crop have been gradually impeded by environmental constraints such as drought, floods, salinity, heat, and cold. Plants adapt their physiological operations in response to external stimuli via signal transduction, thereby modulates gene expression and triggers a range of cellular responses. To feed the current population expansion, it is necessary to develop crops which are able to withstand climate change is highly desirable. 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 ROS production. The genome-wide comprehensive and expression analysis of OsDHSRP1 in rice have still not been reported. In the current research, we are providing genome wide prediction, structural, evolutionary characterization, and expression analysis of OsDHSRP gene family of *Oryza* under diverse abiotic stresses. A total of 15 OsDHSRP genes were discovered in *Oryza* genome, which contains C3HC4 zinc finger conserved domain. The elucidation of Intron/Exon and motif patterns provide structural aspects of these genes. Cis-regulatory analysis and Transcription factor prediction studies revealed their roles and interaction with genes involved in multiple abiotic variables. Expression evaluation of OsDHSRP genes by q-RT PCR revealed that OsDHSRP1 exhibited strong expression trends in leaflets during cold stress followed by drought and salt stress conditions, suggests the role of OsDHSRP1 under diverse abiotic stress circumstances in *Oryza*. This study provides further insights into regulation of expression of OsDHSRP genes for developing climate resilient crops.

Key Message

We discovered 15 OsDHSRP genes in Oryza genome.

In silico characterization of these genes and proteins revealed structural and functional aspects.

Promoter, transcription factor, miRNA prediction provides insights into modulation of OsDHSRPs.

Expression profiling of OsDHSRPs revealed up-regulation of genes, helps in regulation of genes to enhance abiotic stress tolerance.

INTRODUCTION

Abiotic stresses exert an adverse effect on rice yield and productivity, particularly in the in context of changing ecological conditions. Abiotic factors, which includes heat or temperature, flooding, dry conditions and salinity or nutritional deficiency, may induce undesirable climates that hinder the germination process, seedling establishment, vegetative expansion, flowering, panicle advancement, grain filling results in loss of productivity (Jeyasri et al., 2021). These variables negatively influence the cultivation of rice, development, and quality of grains (Beena et al., 2021b; Anie et al., 2022; Stephen et al., 2022). Declining supply of rice as a result of climate change will have significant socioeconomic effects because rice is the world's main primary food crop. Diverse abiotic stressors trigger physical as well as biochemical fluctuations in agricultural crops (Manikanta et al., 2020; Ali et al., 2022; Manikanta et al., 2022). Combined abiotic factors, have a higher influence on the cultivation of rice than single stress. (Pandey et al., 2017). These stresses exert physiological implications that influence rice yield worldwide (Ramu et al., 2016; Ali et al., 2022). Abiotic stress complications at the time of grain filling may adversely affect milling, chalkiness, carbohydrate content, and cooking characteristics (Lanning and Siebenmorgen, 2013).

Sedentary crops experience severe environmental stress, which hampers overall productive potential. Stresses including drought, salt, and abnormal temperatures may restrict growth and decrease agricultural yield (Yasin et al. 2020). As the world's population grows, addressing the adverse effects of abiotic stress on crops is crucial for ensuring food security (Pareek et al. 2020; Chaudhry and Sidhu 2022). 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 (FAO 2021).

Impact of Diverse Abiotic Stress on Growth and Development of Rice

Stress is a complicated and multigenic process that affects crop growth and development of plants, as well as yield. However, plants adapt to adverse conditions through molecular and cellular plasticity (Narendra and Sarvajeet 2016; Imran et al. 2021). Plant adaptations for abiotic stress involve various molecular mechanisms, notably sensing, signalling, expression of genes, synthesis of proteins, and post-translational alterations (Zhang et al. 2022). Discovering vital genes and their pathways and signalling processes will be essential for enhancing plant resilience to stress (Narendra and Sarvajeet, 2016).

Abiotic stress factors including severe drought, salt, and temperature are responsible to significant loss of yield in global agricultural cultivation. The latest research found that abiotic stresses, especially temperature, drought, and salty soils, account for 50% of agricultural production losses globally (Kajla et al 2015).

The salinization of the soil represents an adverse environmental element that impacts the germination of seeds, growth of crops, and output (Van Zelm et al., 2020). Excessive salt accumulation affects around 900 million hectares of agricultural land worldwide, with irrigated land accounting for roughly one-third. The crisis is escalating as a consequence of climate change and the impact of anthropogenic activities, costing $27.3 billion annually (Qadir et al., 2014; Shahid et al., 2018).

Drought is a devastating environmental stress that causes massive loss of yield in crops, particularly rice (53–92%), wheat (57%), and maize (63–87%) (Daryanto et al. (2016). Globally, reduced precipitation and dynamic rainfall patterns causes frequent drought stress (Cheng et al., 2021). Drought stress hampers the growth of crops and yield throughout germination to maturity (Cui et al., 2020; Taraneh et al., 2020; Wasaya et al., 2021). As a consequence, drought stress adversely affects plant development, water-nutrient communications, moreover, photosynthesis causes a large decline in productivity (Ansari et al., 2019; Mubarik et al., 2021).

Plants have developed diverse molecular and cellular defence mechanisms for mitigating drought stress. Plants employ an extensive network of signals and receptors to communicate adaptations to stress throughout root, shoot, and leaf tissues under circumstances of drought. ABA enhances drought stress tolerance by activating hydrogen peroxide (H 2 O 2), a primary ROS in guard cells. Drought stress triggers oxidative strain in crops, resulting in elevated Superoxide and APX function and decreased CAT activity (Nahar et al, 2018; Panda et al, 2021; Aslam et al, 2022). Takahashi et al. (2020) found that ABA-dependent and ABA-independent regulation systems facilitate stomatal cells maintain equilibrium during drought stress. The latest evidence points out that non-coding RNA (Yasin et al. 2020) and peptides might serve as signalling molecules in drought-induced adaptations (Kim et al. 2021).

Rice is intolerant to soil salt, which affects 25–30% of areas with irrigation, resulting in about 1 billion hectares of salty land (Shahid et al., 2018). The presence of salt in soil or water may restrict crop development and yield, specifically in dry or semi-arid areas (Chourasia et al. 2022). Salt exposure leads to retarded germination of seeds and the set, sterile spikes, and decreased its leaf dried matter, surface area of leaves, tillers number, grains per panicle, and the viability of pollen (Reshma et al., 2021). Stress caused by salinity primarily impedes growth of plants by causing oxidative damage, ionic disequilibrium and osmotic stress (Basu et al, 2021). Premature senescence of leaves is frequently driven by salinity stress, which leads to a surge in Na + and Cl— levels and a consequent decrease in K + and Ca 2 + ions (Fahad et al, 2015; Ganapati et al, 2022). Plants produce excessive amounts of reactive oxygen species (ROS) in response to salinity, affecting the metabolism and basic physiological mechanisms (Miller et al, 2010; Das and Roychoudhury, 2014). Numerous plant hormone signalling mechanisms regulate resilience to stress while promoting development amid high-salinity circumstances, enabling plants to thrive.

Temperature is a critical environmental factor as it impacts plants geographical dispersion, output, and development. Plants in general require optimum temperature ranges for their growth to maintain normal physiological function and development, which could be severely impaired when the temperature ranges are exceeded or decreased. Heat stress occurs when temperatures rise above a certain threshold for an extended period of time, causing lasting impairment to plant growth (Wahid et al. 2007). Slight rises in temperature have adverse effects on primary crops which includes corn, wheat, and rice at the time of flowering, limiting crop yield (Imran et al. 2021). Rice, wheat, and maize yields are expected to drop by 3–8% for every one-degree Celsius rise in temperature (Paul et al., 2020).

Extreme temperatures may injure plant cell membrane lipids, enhance permeability on the membrane, alter temperature conductivity, and also impair osmoregulation, ultimately resulting in cellular death. (Mathur., et al 2014; De Pinto etal.,2015). Hot temperatures perturb the equilibrium of formation of ROS and quenching in cells, causing to the build-up of oxidants including H2O2 and MDA. This contributes to oxidative injury under heat stress (Zhao., 2021). High temperature alters metabolic pathways, that impact the integrity of RNA and protein. Hsps, ubiquitin ligases, and helicases all play vital functions in the regulation of RNA and protein metabolism during thermal stress.

Cold stress is triggered by extreme temperature decrease causes alterations in the membrane structure, electrolyte efflux, decreased protoplasmic streaming and plasmolysis. This leads to abnormal metabolism, unusual ripening of fruits, vascular browning, and increased susceptibility to decay, reduced plant growth, which can be lethal in severe conditions (Devasirvatham et al,2018). Plants withstands and adapts to cold stress mechanically by making conformational changes to the plasma membrane as of increased unsaturated lipids, increases the membrane's permeability and endurance. (Janská et al, 2010).

E3 Ubiquitin Ligases; Potential Negative regulators of Abiotic stress:

The 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 (Dong et al, 2022). In response to abiotic stressors, a number of proteins regulate cellular functions by biochemical or molecular mechanisms. According to Raffeiner et al. (2023), breakdown of proteins is vital for regulatory functions as protein synthesis and conformation. 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 ligate 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 (Xu and Xue, 2019).

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. (Smalle et al, 2004). All eukaryotes contain a highly conserved peptide comprised of 76 amino acids that make up ubiquitin. (Yang et al, 2021). Three monomeric types of E3-ubiquitin ligases—RING, HECT, and U-box—form an intermediate during ubiquitination. (Deshaies et al,2009). 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. (Zhang et al,2011). 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. (Park et al,2018). The RING E3-ubiquitin ligase RING domain-containing protein 1 (OsRDCP1), whose overexpression enhances rice tolerance towards an acute water crisis and it is transcriptionally upregulated by drought stress (Bae et al, 2011). OsDIS1 adversely impacts the response to drought stress by ubiquitinating followed by 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 (Ning et al, 2011).

OsDHSRP1 is an E3-ubiquitin ligase, its expression is strongly stimulated by salinity, heat, and drought and functions as a negative regulator of different stresses by causing degradation of various stress responsive proteins. The OsDHSRP1 gene triggers the degradation of OsGLYI-11.2 and OsACP1 targets via ubiquitination cascade and Arabidopsis homolog also found to be interacts with similar targets. (Kim et al,2021). E3 ubiquitin ligases also involved in adaptation to cold stress. HOS1, AtATL78, and AtATL80 in Arabidopsis inversely regulates the plant's tolerance to cold stress. (Suh et al ,2015). The ubiquitin-proteasome system is required for several plant hormone signaling processes, particularly E3 ubiquitin ligases that may detect and initiate signal transduction. (Kelley et al,2018). According to previous research, E3 ubiquitin ligases up regulated when exposure to heat strain. Heat stress resistance was enhanced by AtSAP5. (Kim et al,2015). Modulation of expression of these negative regulators will enhance tolerant to multiple environmental variables.

In the current research, we predicted gene family members of OsDHSRP gene in Oryza genome and providing in silico characterization including gene structure, evolutionary analysis, conserved domains and motifs, synteny, transcription factor and miRNA prediction. Furthermore, we quantified transcript abundance of OsDHSRP gene members in various tissues of Oryza under diverse abiotic stress conditions by q-RT PCR. This research will provide further directions for targeting these genes to develop climate resilience crops to thrive in multiple abiotic stress conditions.

MATERIALS AND METHODS

1. IN SILICO IDENTIFICATION, AND CHARACTERIZATION OF OsDHSRP GENE HOMOLOGUES.

To discover the OsDHSRP genes in the rice genome, the previously characterised Arabidopsis AtDHSRP protein was employed for BlastP against the *Oryza sativa* genome in the Phytozome database (http://www.phytozome.net/) (Goodstein et al, 2012). The hits were selected on the basis of a threshold E value of less than 1E-5. The potential OsDHSRP genes were submitted to the motif finder tool to predicted the conserved motif in protein sequences. The genes with the C3HC4 Zinc Finger domain were picked from the results, while the rest were deemed redundant.

2. GENE STRUCTURE PREDICTION and SUB CELLULAR LOCALIZATION.

The genomic and coding sequences of predicted OsDHSRP genes were retrieved and submitted to Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn/index.php) to find the Intron/Exon distribution. Furthermore, WOLFPSORT (https://www.genscript.com/wolf-psort.html) server is used to forecast the proteins' subcellular location. (Hu et al,2015).

3. PROTEIN PARAMETERS, CONSERVED MOTIF ANALYSIS and PHYSICAL MAPPING:

Protein parameters such as molecular weight, GRAVY (grand average of hydropathy), isoelectric point, aliphatic indexes are predicted by using ProtParam software (https://web.expasy.org/protparam/). MEME (http://meme-suite.org/tools/meme), (Bailey et al,2009) tool was employed to predict conserved motifs found in OsDHSRP genes with parameters such as 10 number of motifs and 6 to 20 widths. The physical mapping of these genes was done by online Phenogram server (https://ritchielab.org/).

4. THE EVOLUTIONARY RELATIONSHIP AND COLLINEARITY ANALYSIS

Mega version 11.0 was employed to align the amino acid sequences of the DHSRP genes 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. (Kumar et al,2016). Synteny analysis between *Oryza sativa vs Zea mays* was done using TB tool and *Miscanthus sinensis vs Oryza sativa*; *Panicum virgatum vs Oryza sativa*; *Setaria italica VS Oryza sativa* were done using gramene database Synteny tool(https://www.gramene.org/). 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.

5. PROMOTER ANALYSIS OF OsDHSRP GENES.

Promoter sequences (1500 bp upstream) of all candidate genes were retrieved from Phytozome database. Cis regulatory elements located in promoter regions were predicted by using PLANT CARE tool (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

6. miRNA AND TRANSCRIPTION FACTOR PREDICTION

The miRNA that could target OsDHSRPs in order to regulate their expression were predicted using psRNA target tool (https://www.zhaolab.org/psRNATarget/) with all default settings. The results are 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 is generated by Cytoscape tool (https://cytoscape.org/).

7. 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) (Geourjon et al,1995) and the 3-dimensional protein structures were predicted by using Swiss model server. (https://swissmodel.expasy.org/). The PSVS (protein structure verification server) (https://saves.mbi.ucla.edu/) is used to evaluate predicted structures and stability was analysed by Ramachandran plot. For the analysis of Protein-Protein interactions (PPI) of these homologs STRING data base was employed. (https://string-db.org/).

8. GENE ONTOLOGY AND CO EXPRESSION ANALYSIS

Gene ontology enrichment of OsDHSRP genes was done by using PANNZER2.0. The Co-expression analysis for OsDHSRP1 gene was performed using ATTED-2 tool (https://atted.jp/gene_coexpression/).

9. INSILICO EXPRESSION ANALYSIS

The base line expression data (FPKM, Acc No) of all predicted OsDHSRP genes were retrieved from Rice Expression Database (RED (http://expression.ic4r.org/). Additionally differential expression data (Acc. No) of these genes were retrieved from Gramene database expression atlas (https://www.gramene.org/) and analysed by heat map built with TB tools.

10. PLANT MATERIAL AND STRESS CONDITIONS:

A local variety of Oryza sativa cultivar MTU1010 was chosen for study, the germplasm was collected from IIRR – Rajendranagar, Hyderabad. Seeds were allowed to germinated on wet Petri dishes containing tissue paper in dark for 7 days at normal room temperature. After germination, seeds were shifted to light for a 16 hrs photo period and 8hr dark. After 15 days, plantlets were exposed to different abiotic stress for a period of 16hrs, including 200 mM Mannitol for induction of drought stress, 200 mM NaCl for salinity stress. Furthermore, cold stress was imposed by incubating plantlets at 4C and heat stress at 45C, corresponding control plants (untreated) were maintained.

11. QUANTITATIVE REAL TIME PCR ANALYSIS OF OSDHSRP GENES.

Total RNA extracted from leaf and stem tissues of Rice plants espoused to different stress 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 micro grams of RNA was converted into first strand cDNA.

According to In silico expression data, three OsDHSRP genes were considered for q-RT PCR analysis. Unique primers for EF1-alpha, OsDHSRP1, OsDHSRP2, OsDHSRP8 were designed using NCBI primer blast tool. (Table 5). 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. Thermal conditions for qRT-PCR analysis are carried out at 95C for 1min followed by 950c for 10 sec for 40 cycles, 60C for 30 sec, along with other default settings in ABI7500 Realtime PCR system. (Applied Biosystems, Foster City, CA). Gene expression of OsDHSRP genes in both treated and untreated samples was normalised with the reference gene EF1-alpha. For every sample analysis was carried out using two biological and three technical replicates. By employing the comparative 2^ΔΔCT technique, relative expression of OsDHSRP genes were determined and transcript abundance was represented as heatmap constructed using Tb tool.

Table 5
Primers designed using NCBI primer blast.

S.NO	GENE	PRIMER SEQUENCE 5'-3'	LENGTH
1	OsDHSRP1-FP	AAGGGGCTTAAGTGGAACGG	20
2	OsDHSRP1-RP	CTGAACCTAGCACCCCAAGG	20
3	OsDHSRP2-FP	GAAGCAATGCCAGAATGCGT	20
4	OsDHSRP2-RP	GCGCCAGTATTCAGTGGAGT	20
5	OsDHSRP8-FP	CCAGCCTTTGACTCCTCGTT	20
6	OsDHSRP8-RP	CTTGGTAACGGCAGTCTTGC	20
7	OsEF-1A-FP	GGGTCGTGTTGAGACTGGAG	20
8	OsEF-1A-RP	CACGTACCCACGCTTCAGAT	20

RESULTS

1. IDENTIFICATION AND CHARACTERIZATION OF OsDHSRP GENES.

The characterized OsDHSRP sequence from *Arabidopsis thaliana* utilized as a query sequence for BlastP search against *Oryza Sativa* genome for identification of OsDHSRP genes. Among all the hits, 15 genes were determined as potential candidates OsDHSRP gene family members. All these 15 sequences containing C3HC4 zinc finger domain which are renamed as OsDHSRP1, OsDHSRP2, OsDHSRP3, ……..OsDHSRP15 with respect to their location on pseudo chromosomes. (Table 1). Characterization of genes was done and the protein with longest peptide sequence was OsDHSRP4 (709 a.as) and protein with shortest one is OsDHSRP6 (197 a.as). The molecular weight of candidate proteins was analysed, all predicted OsDHSRP proteins ranges in between 20 kDa to 76 KDa. We analysed protein parameters include pI ranged from 3.85 to 9.33, GRAVY ranged from − 0.068 to + 0.325. (Table 2). Gene structure prediction shown that among 15 OsDHSRP genes OsDHSRP4, OsDHSRP8,9,1,11,13,14 has 4/5 intron to exon ratio; OsDHSRP2,3,10 has 3/4 intron to exon ratio; OsDHSRP6,12,15 has no introns and the intron to exon ratio was almost similar among members of same group. (Fig. 1b). The subcellular localization of OSDHSRP homologs were investigated; OsDHSRP1, OsDHSRP2, OsDHSRP3, OsDHSRP4, OsDHSRP7, OsDHSRP10, OsDHSRP12, OsDHSRP13, OsDHSRP14 are localized in nucleus; OsDHSRP6, OsDHSRP8, OsDHSRP9, OsDHSRP15 found in chloroplast; OsDHSRP5 and OsDHSRP11are localized in vacuole.

TABLE – 1

Characterisation of OsDHSRP homologues genes in rice.

Gene Name	Transcript ID	START END	Chr	CDS bp	Introns/Exons	Domain	Localization
>OsDHSRP1	>LOC_Os02g05692	2791241..2795471	2	580	4,5	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP2	>LOC_Os08g14320	8582060..8584105	8	1050	3,4	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP3	>LOC_Os04g51400	30439466..30442859	4	1104	3,4	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP4	>LOC_Os04g10680	5802054..5811151	4	2130	4,5	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP5	>LOC_Os04g55510	33022715..33028387	4	2004	5,6	ZINCFINGERC3HC4	VACUOLE
>OsDHSRP6	>LOC_Os01g53500	30730644..30731238	1	594	0,1	ZINCFINGERH2	CHLOROPLAST
>OsDHSRP7	>LOC_Os01g06590	3099427..3105235	1	1506	5,6	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP8	>LOC_Os01g49770	28582842..28586527	1	606	4,5	ZINCFINGERC3HC4	CYTOPLASM
>OsDHSRP9	>LOC_Os01g47740	27303110..27312308	1	1575	4,5	ZINCFINGERC3HC4	CHLOROPLAST
>OsDHSRP10	>LOC_Os03g07790	3960942..3966307	3	753	3,4	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP11	>LOC_Os06g48040	29059686..29063780	6	1740	4,5	ZINCFINGERC3HC4	VACUOLE
>OsDHSRP12	>LOC_Os12g05270	2346442..2347276	12	834	0,1	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP13	>LOC_Os05g48970	28085119..28092428	5	1503	4,5	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP14	>LOC_Os05g47670	27317412..27320555	5	1095	4,5	ZINCFINGERC3HC4	NUCLEUS
>OsDHSRP15	>LOC_Os03g57410	32736205..32737399	3	657	0,1	ZINCFINGERH2	CHLOROPLAST

TABLE-2

Protein parameters of OsDHSRP Homologs

pI- isoelectric point

GRAVY-Grand average of hydropathicity index

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

2. PREDICTION OF MOTIFS AND CONSERVED DOMAIN.

Using MEME tool, motifs which are found in OsDHSRP genes were predicted, a total of 10 motifs were identified. Out of 10 motifs found, motifs-1,2&3 are conserved across all OsDHSRP genes. The majority of motifs are limited to specific members of the OsDHSRP gene family. Motif 4 found only in OsDHSRP 2&3, motif 10 in OsDHSRP8&14, motif 5 and 7 in OsDHSRP 1&11 and motif 9 is exclusively located in OsDHSRP 9&13. Almost the motif pattern is comparable within the same clade. (Fig. 1c). The results of 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 N- terminal end of peptide. (Fig. 1e). 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 were belongs to OsDHSRP gene family. The identical motif patterns among genes of same groups correlates with evolutionary relationships.

3. THE EVOLUTIONARY ASPECTS AND SYNTENY ANALYSIS.

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* and *Triticum aestivum* and *Oryza sativa* showed 4 ortholog pairs with Sorghum they are OsDHSRP2 and SbDHSRP10; OsDHSRP4 and SbDHSRP13; OsDHSRP14 and SbDHSRP4; OsDHSRP13 and SbDHSRP7; There is one ortholog pair each with Zm and At 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.

Collinearity analysis of genes among Os, Zm, At. Genomes was done using TB tools and there is no syntenic regions found between Os and At for OSDHSRP homologs. The collinearity between Zm and Os shown that Os chromosome 1 shows 2, 3, 4 homologs each with Zm chromosome 3, chromosome 6, chromosome 8 respectively; Os chromosome 2 shows 2 homologs with Zm chromosome 5; Os chromosome3 shows 2,1,1,1 homolog each with Zm chromosome1, chromosome7 and chromosome9 respectively; Os chromosome shows 2,2 homologue each with Zm chromosome 10 and chromosome 2; Os chromosome 5 shows 1,2,3 Homologs with Zm chromosome 3, chromosome 6 and chromosome 8 respectively; Os chromosome 6 shows 2 homologues with Zm chromosome 5; Os chromosome 8 shows. 2. 1. Homologs with Zm chromosome 10 and 2; Os chromosome 12 shows one homologue with Zm chromosome 4. (Fig. 2a).

Synteny analysis results of *Miscanthus sinesis* versus *Oryza sativa* displayed that Os chromosome1 shows homology of 2, 2 with Ms chromosome 5 and chromosome 6; Os chromosome 2 shows one homology each with Ms Chromosome 7 and Chromosome 8; Os chromosome3 shows 2,2 homologies with Ms chromosome 1 and Ms Chromosome 2; Os chromosome 4 shows 1,2 homology with Ms chromosome 11 and chromosome 12 respectively. Chromosome 5 shows one homology each with Ms Chromosome 16 and chromosome 17; Os chromosome 6 shows 1 homology each with Ms chromosome 18 and chromosome 19 respectively; Os chromosome8 showed one homology with Ms chromosome 13.

Synteny analysis results of *Oryza sativa* versus *Panicum virgatum* revealed that Os chromosome1 Shows 4 homologs with Pv chromosome 5; Os chromosome 2 shows 2 homologies with Pv chromosome1; Os chromosome 3 shows 4 homologs with Pv chromosome 9; Os chromosome 4 shows 4 homologs with PV chromosome 7; Os chromosome 5 shows 2 homologues with Pv chromosome 3; Os chromosome 6 shows 2 homologs with Pv chromosome 4; Os chromosome a shows 2 homologues with Pv chromosome 6.

The outcome of collinearity mapping of genes among *Oryza sativa* and *Setaria italica* are most of the homology was found in scaffold region of *Setaria Italica*. (Fig. 3).

4. MULTIPLE SEQUENCE ALIGNMENT AMONG SPECIES AND Ka/Ks ANNOTATION.

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, suggests that these OsDHSRP genes are conserved across the species. (Fig. 2b).

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 tabulated. (Table 3).

TABLE – 3

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

5. PROMOTER ANALYSIS AND CHROMOSOMAL LOCATIONS.

The predicted Cis regulatory elements found in promoter regions of OsDHSRP homologs are as follows- Light responsive element, MYB drought responsive, meristem expression, circadian cycle, cell cycle regulation, MYB binding site, Low temperature responses, Endosperm specific negative regulation, seed specific regulation. Plant hormone related such as auxin, Methyl Jasmonate, gibberellin, salicylic acid and abscisic acid responsive and Defence and stress responsive elements against biotic stress. Some abiotic stress elements include drought and salt, low temperature responsive and metal responsive. Among stress related elements most of them include abscisic acid responsive element which determines their function in abiotic stress response. (Fig. 4a). The presence of light responsive elements specifies they are regulated by light. Metal responsive element suggests the genes are involved in heavy metal stress. Based on this data OsDHSRP genes may play key roles under drought, salinity and heat or cold stress. Some unique elements show diversity among gene homologs in their roles. Physical mapping of OsDHSRP genes on Oryza genome revealed that these genes are scattered across all chromosomes and most of the genes are localized on chromosome 1 of Oryza (Fig. 4b).

6. PROTEIN 2D & 3D STRUCTURE MODELING AND PPI ANALYSIS

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 were tabulated. (Table 4), (Fig. 5b).

TABLE – 4

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

The 3-dimensional structure of OsDHSRP proteins were predicted using Swiss model server. The models were selected based on maximum percentage of identity with standard PDB structures. The selected structure PDB ID, protein ID, percentage identity was tabulated. (Table 2). 3D structures are given in (Fig. 5c).

The template 6W9a.1.B- E3 ubiquitin ligase, Ubiquitin conjugating enzyme E2 showed highest structural similarity with most of the homologous including OsDHSRP2, OsDHSRP5, OsDHSRP9, OsDHSRP1, OsDHSRP12. Template ID 6W9a.2.B- E3 ubiquitin ligase RLIM shown similarity with OsDHSRP3, OsDHSRP9, OsDHSRP15. Furthermore, template 6W7z.1.B - E3 ubiquitin ligase, E2 ubiquitin conjugating enzyme D2, Showed similarity with OsDHSRP4, OsDHSRP6, OsDHSRP7, OsDHSRP10, OsDHSRP14. OsDHSRP13.

Using string database, the proteins which interact with OsDHSRP1 protein was analysed. The interpretation of result suggests that OsDHSRP1 protein interacting with ARF-16, MP-ARF11 auxin responsive element that precisely binds to DNA sequence 5'-TGTCTC-3' found in the auxin-responsive promoter elements (Aux REs). Additionally, other interacting proteins include BHLH 156- transcription factor 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, - which are uncharacterized proteins. Another protein Os07g0626200 is AcinusL protein-like; The result of string interaction analysis among the 15 candidate genes shown they are non- interactive with each other.

7. miRNA AND TRANCRIPTION FACTORS PREDICTION-

The results of transcriptional factor identification revealed that candidate genes are regulated by 35 distinct types of transcriptional factors. These includes 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). (Kaplan et al,2012; Xiao et al, 2021; Han et al,2020). 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 related responsive Tfs.

The miRNA was identified, which can be used to target these homologous genes and regulate their gene expression patterns. OsDHSRP1 shows 6 miRNA targets which includes Osa-miRN2282, Osa-miR444c, Osa-miRN2222, Osa-miR528, Osa-miR440, Osa-miRN2376. (Fig. 7). Additionally, we found that Osa-miR5508 is a potential target for both OsDHSRP13 and OsDHSRP9 genes.

8. GENE ONTOLOGY AND CO EXPRESSION ANALYSIS-

The GO terms are analysed for all the candidate genes. The results showed that in molecular function category, most of the genes exhibited similar functions that is ubiquitin protein ligase activity-GO:0061630, metal ion binding-GO:0046872, and ligase activity-GO:0016874 was displayed by OsDHSRP1, OsDHSRP2, OsDHSRP3, OsDHSRP4, OsDHSRP5, OsDHSRP9, OsDHSRP8, OsDHSRP10, OsDHSRP11, OsDHSRP13,

OsDHSRP14. The gene OsDHSRP10 showed acyl transferase activity-GO:0016746 alongside with above functions and OsDHSRP15 showed only acyl transferase activity. OsDHSRP7 has only the ubiquitin protein ligase activity and metal ion binding without ligase activity. OsDHSRP6 has transferase activity-GO:0016740 along with metal ion binding. OsDHSRP14 have an additional helicase activity of GO:0004386 and OsDHSRP12 showed zinc ion binding ubiquitin-GO:0008270 and protein ligase activity.

Co-expression analysis for OsDHSRP1 gene was done and the results were tabulated and a network was created using Cytoscape tool. (Fig. 6b). Several genes were co expressed along with OsDHSRP1 gene includes Os01g0936200 is probable feruloyl esterase A an enzyme showing highest rank with OsDHSRP1 expression. Several other E3 Ubiquitin protein ligases such RHB1A, AIRP2, atg7, AIRP2-LIKE, CHIP, At3g022g0 were also expressed. Furthermore, Ubiquitin receptor RAD23d is also expressed along with OsDHSRP1 gene.

9. INSILICO EXPRESSION PATTERN ANALYSIS

According to In silico expression data (Xia et al,2017) the expression of OsDHSRP1 gene is high in root and embryo compared to other tissues/organs. (Fig. 8e) and low in leaf blade, endosperm, and reproductive leaf sheath. Among organs comparatively, expression of OsDHSRP1 is more in leaf than in stem. (Fig. 8f).

Under sodium dihydro phosphate treatment at different time periods (Fig. 8b) (Secco et al,2013), OsDHSRP1, OsDHSRP2, OsDHSRP4 exhibited strong expression patterns.

When rice plants exposed to heavy metal stress (cadmium sulphate) (Oono et al,2014) (Fig. 8a)- OsDHSRP1 showed moderate expression. Moreover, OsDHSRP7 and OsDHSRP9 displayed highest expression under cold stress or low temperature stress. (Zhang et al, 2012) (Fig. 8c). Additionally, OsDHSRP1showed an moderate expression, whereas OsDHSRP10 and OsDHSRP8 had highest expression.

10. q-RTPCR ANALYSIS

According to qRTPCR analysis, OsDHSRP1 exhibited strong expression pattern in leaf tissue under cold stress, in contrast to drought and salt stress. Compared to leaf tissue, expression of OsDHSRP1 gene was high in stem tissue under salt stress. The comparative expression of OsDHSRP1 gene shows low in untreated plant tissues both stem and leaf in relative abiotic stress conditions. When exposed to stress, the OsDHSRP1 gene's relative expression up-regulated by 18.74, 12.81, and 11.27 -fold in cold leaves, drought leaves, and salt stem tissues, respectively. (Fig. 8d).

OsDHSRP2 Gene shows high expression levels (17.03- fold change) under salt in stem tissue. The relative expression of this gene also suppressed in untreated plants than plants subjected to stress. OsDHSRP8 gene showed strong expression (13.68 folds) under salt conditions in stem tissue and moderate expression in drought stressed leaf tissue and cold conditions. Overall, the expression of OsDHSRP homologs displayed high expression patterns under various abiotic stresses compared to natural environmental conditions.

DISCUSSION

Various environmental variables, specifically biotic and abiotic constraints, are brought about by world-wide climate change and also exert an impact overall agricultural yields (Raza et al., 2019; Farooq et al., 2022). Among abiotic stresses, Drought, salinity, and temperature are three critical and devastating environmental concerns that severely impair crop productivity (Kajla. 2015). This stress may hinder plant development as well as growth by generating species of reactive oxygen, cellular membrane injury, and declined photosynthesis rates. The resulting consequences impair biochemical, physiological, and molecular functions. Over 100 countries grow rice as a key staple and financial source. Most of the available Rice genotypes are vulnerable to external stressors, these may detrimentally impact their physiological responses, reproductive processes, and grain metabolism (Ren et al. 2005; Cruz et al. 2013). Plants will employ numerous molecular and physiological responses in order to thrive under stress conditions. Major molecular responses include expression of various stress related genes which sustain plant survival under stress, in contrary certain genes will also be expressed and acts as negative regulators and intensify damage under stress. One such gene is OsDHSRP, a potential negative regulator of abiotic stress in rice. Therefore, in the future, further research will be necessary for a deeper comprehension of the abiotic stress tolerant responses in rice. In the current research, we employed a genome-wide analysis approach to examine the chromosomal spots, evolutionary connections, gene architecture, cis-acting elements, and in silico and in vitro gene expression profiles of OsDHSRP genes under diverse stress in rice.

The OsDHSRP1 locus encodes a drought, heat, and salt-induced ring finger protein possessing E3 ubiquitin ligase function. The gene major function 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 ROS levels at the cellular level. (Huang et al, 2022). In vitro ubiquitination assays were performed in rice plants, and mediated ubiquitination was validated using the Ni-NTA purification technique (Kim). Its expression is strongly driven by elevated temperatures and 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 MG levels rise (Huang et al,2022). In plants, environmental factors such as salt, drought, heat, and abscisic acid (ABA) drives the expression of the OsDHSRP gene. The ligase function of 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: OsACP1 and OsGLYI-11.2. The over expression of the OsDHSRP gene in Arabidopsis plants, as well as the rate of germination, was observed in control and transgenic plants treated to ABA 0.1-5Mm, salt concentrations 0-200Mm Nacl, and mannitol 0-200Mm for a period of seven days. The data highlight that control plants have three folds higher survival rates than transgenic plants, suggesting that the OsDHSRP gene functions as a negative regulator under stress. Transgenic plants 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 subject to ABA stress. This demonstrates that the gene can be implicated in ABA-independent pathways.

In Arabidopsis, overexpression of OsDHSRP1 resulted in impaired heat tolerance and reduced recovery abilities (Kim et al., 2020). Previous studies of Kim et al qRT-PCR analysis of the OsDHSRP1 gene was carried out in transgenic At over expressed OsDHSRP1 gene to investigate variations in the expression of other stress responsive genes. Transgenic lines displayed down regulation of other stress related genes such as NAC and SOS under salt stress, MYB2 and bZIP under ABA stress, heat shock proteins in high temperature stress, and DRED2A and 2B under drought stress. These findings reveal that overexpression of the OsDHSRP1 gene suppresses other stress-responsive genes. As a result, it functions as a negative regulator in response to heat, salinity, and drought, but there are no substantial changes in ABA stress. (Kim et al. 2020).

Under normal conditions, both control and transgenic plants had equal methyl glyoxal levels. However, under stress, transgenic plants exhibit excessive methyl glyoxal accumulation; MG levels are associated with OsGLY11.2 gene expression because its plays critical role in glyoxylate pathway (Mustafz et al,2014), indicating that down regulation or degradation causes an increase in metal glyoxal levels. Glyoxalases show positive modulation in response to various abiotic stresses. (Belavadi et al. 2017).

Finding and understanding the genes in a cluster will enable to describe the diversity of expressed proteins and correlate with biological functions by analysing characteristics akin to conserved domains and motifs, protein structure, promoter and transcription factor analysis and gene expression patterns across diverse environmental cues.

In the current study, we predicted 15 OsDHSRP gene homologs in Oryza genome, all of which comprised the signature Zinc finger domain C3HC4 type along with ring H2 domain. The N-terminal end of the motif contains conserved domains. According to phylogenetic study, these 15 genes are divided into four distinct clades and these genes are closely associated with Triticum and Sorghum amongst closely related species such as Zm and At. 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. The vast majority of C3HC4-type RING finger proteins have been found to be validated as E3 ubiquitin ligases. Ubiquitin-mediated modifications of proteins regulates a wide range of physiological activities, including growth and development, hormonal, stress-related responses, and cellular division. Since E3 ligases plays critical roles in ubiquitin-protease cascade in plants (Smalle et al,2004).

The MSA results revealed that OsDHSRP1 proteins displayed highly conserved residues among Sorghum, Zea mays, and Triticum, indicating that these genes are highly conserved among species. This is consistent with phylogenetic analysis results, which show that OsDHSRP genes showed four paralog pairs with Sorghum and only one with Maize and Arabidopsis. Despite having many paralogs, there is no genetic collinearity between sorghum and rice, although Zea mays exhibits. The identified promoter elements are consistent with the reported function of the gene as a stress responsive since findings include light responsive elements, MYB-drought responsive, low temperature responsive, metal responsive, and various plant hormone responsive elements found in promoter regions of these genes. This prediction suggesting that gene expression is related to low temperature or cold stress, drought stress, heavy metal stress, and furthermore, genes are regulated by light (Kaur et al. (2017). The existence of MeJA promoter elements implies that the gene plays a role in a variety of biotic stress responses. (Seo et al. 2013).

Numerous transcription factors, such as AP2/ERF, NAC, WRKY, MYB, and bHLH, are widely recognised as environmental stress regulators. (Huang et al. 2012). The YABBY TFs interconnected to a GA-responsive element in the GA3ox2 promoter, which influences the feedback regulation of the biosynthesis of GA in rice (Dai et al. 2007). OsYAB4 can contribute to rice growth and development by modulating the rice GA signalling pathway (Yang et al. (2016).

The key TF families WRKY, MADS-box, and MYB regulate growth and development processes, biotic and abiotic stress responses, and a range of plant developmental aspects through specific and/or cross-talk modulation across multiple TFs (Chen et al. 2016). SNAC1 can significantly boost rice growth during both the vegetative and blooming periods. The proteins OsNAC529 and OsNAC630,31 bind to the

OsLEA3 promoter, significantly increasing plant resilience to high salt and drought stress. The transcription factor OsbHLH130 is active under drought stress, modifying the composition of the cuticular wax to promote Tolerance to plant. (Gu et al,2021). In the present work predicted TFs also associated with stress, which correlates with gene function. 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. (Cheng et al, 2021). bHLH is also crucial to salt stress regulation in Rice. Using transcription factor screening, a novel gene was discovered (BEAR1) that contributed to the rice salt responsiveness and is a potential molecular marker for breeding rice for improved salt tolerance. (Teng et al, 2022). According to previous reports Numerous regulons have been thoroughly examined in rice, including DREB, AREB, and NAC. (Todaka et al,2012). As a result, understanding transcriptional regulatory networks is critical for understanding the way genes function and respond to abiotic stress.

The miRNA prediction allows us to identify the potential targets of the genes. OsDHSRP1 gene shown to be targeted by Osa-miR528, Osa-miRN2282, Osa-miRN2222, Osa-miRN2376, Osa-miR444c. Under cadmium (Cd)-induced stress, Osa-miR444, being one of the most vital miRNAs involved in down-regulation, was believed to target MADS-box. (Ding et al, 2011). Few of novel miRNAs were discovered such as Osa-miR444c.1, c.2, d, e, and f members are likely to be highly conserved among monocot plants includes Maize, Switchgrass, Wheat, Sugarcane and Sorghum. (Sunkar et al,2005), but not in Arabidopsis and the target sequences of miR444 family were also present in soyabean and grapes. Probable targets of two miRNAs (Osa-miR1432 and Osa-miR444d) include calcium signaling. A subsequent messenger that is present everywhere, Ca2 + causes physiological alterations when exposed to environmental or abiotic stimuli. (Bouché et al,2005). In plant physiologies and/or responses to elevated temperatures may be responsible for the variations in the heat-induced repression of miR5508 expression patterns are observed in the chosen rice cultivars. (Khan et al, 2017). Further, it has been found in wheat that the heat-responsive miRNAs, miR528 and miR9662 modulate the stress-responsive mitochondrial proteins. (Ravichandran et al,2019).

ARF, bHLH, SAP domain containing proteins shown interactions with OsDHSRP1 Protein. (ARFs), important auxin signaling elements, regulate rice developmental processes, such as root formation, tiller and leaf angulation, floral organ growth, and size of grain. An auxin responsive factor-dependent provides defense against biotic (fungus and viruses) and abiotic and nutritional stressors. (Song et al, 2023). Recent research has discovered SAP proteins as novel stress regulating proteins. 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) (Giri et al, 2013). These proteins also been found to regulate immunological signaling and a variety of other reactions in animals, including those to biotic and abiotic stressors (Heyninck et al, 2005). 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. (Kang et al,2011). OsSAP7 has exposed to have E3 ligase activity. (Sharma et al, 2015).

The GO annotation shows that OsDHSRP1 and its homologs shows Ubiquitin protein ligase activity and metal ion binding. In comparison to Insilco Expression data with qRTPCR expression data, the OsDHSRP 1 gene highly expressed in leaf tissue compared to stem and similar patterns were observed in qRTPCR analysis. The relative fold expression of OsDHSRP1 gene is 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 treatment. (Zhang et al, 2012). Whereas in qRTPCR analysis, OsDHSRP8 shows similar range of fold change in untreated and cold treated plants after 16 hours. The expression levels of 1 and 8 genes are similar at 12-hour cold exposure but results of expression analysis shown OsDHSRP1 is higher than 8 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 & DREB2B which causes plants susceptible during stress conditions. Higher expression levels of this gene also degrade two proteins, OsACP1 and OsGLY11.2 by ubiquitination through 26S proteasome mediated system. (Kim et al,2020). This shows that OsDHSRP1 gene is a potential negative regulator during abiotic or environmental stresses.

CONCLUSION

In the present research we discovered a total of 15 DHSRP genes found in Oryza sativa genome. All DHSRPs grouped into four clades based on phylogenetic analysis, indicating the existence of four ancestor genes. OsDHSRP gene homologs were exhibited similar characteristics in terms of gene structure, conserved domains and motifs. The protein parameters and predicted protein structures will provide 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. To understand further about and manipulate OsDHSRP genes to generate climate-resilient crops, identification of cis-acting elements and transcription factor prediction might be advantageous. The regulation of

OsDHSRP genes will offers engineering and improvement of 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.

Declarations

Declaration of competing interest

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

Data availability statement

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

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Figures

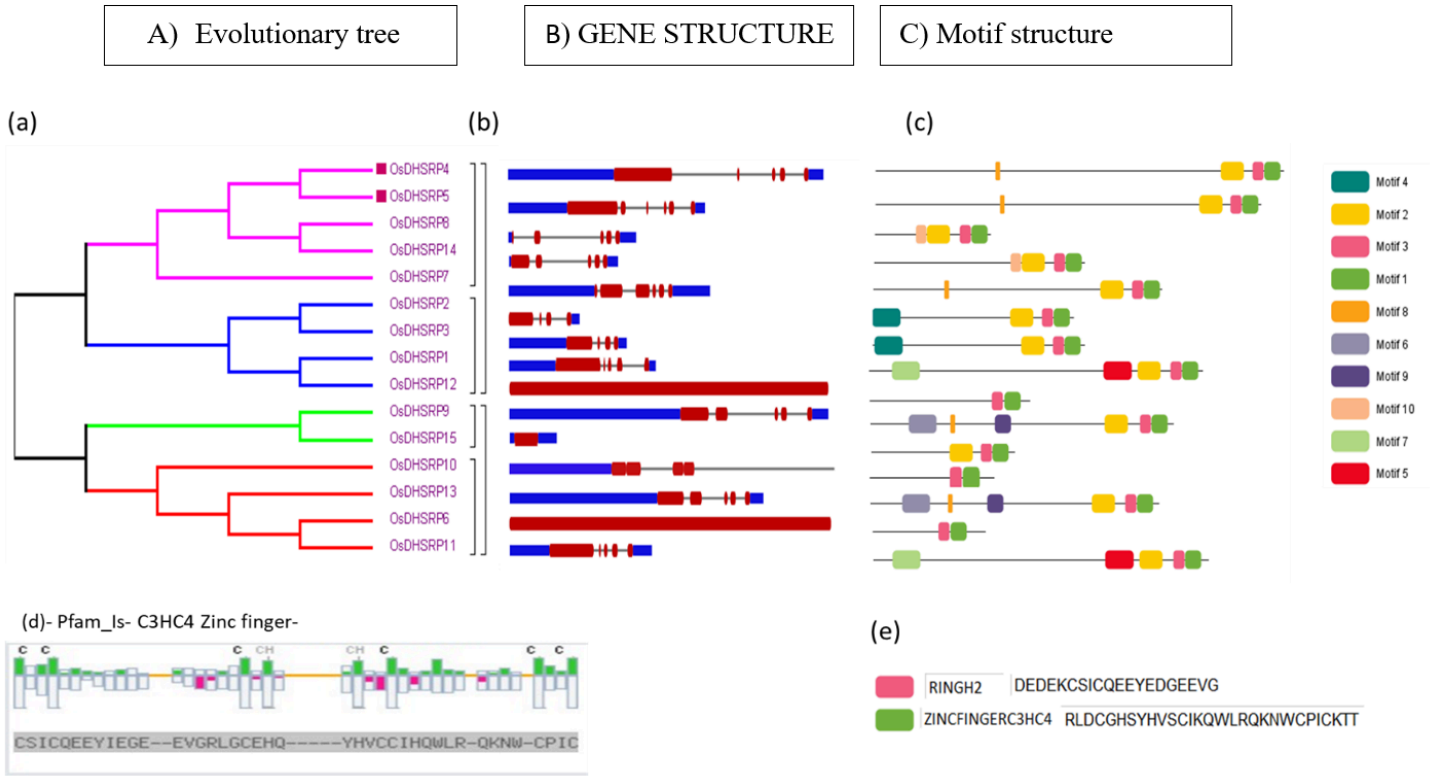


Figure 1

a)The phylogenetic tree was constructed by MEGA v11.0 with the NJ method. **b)**Structures of the 15 putative OsDHSRP genes. **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.

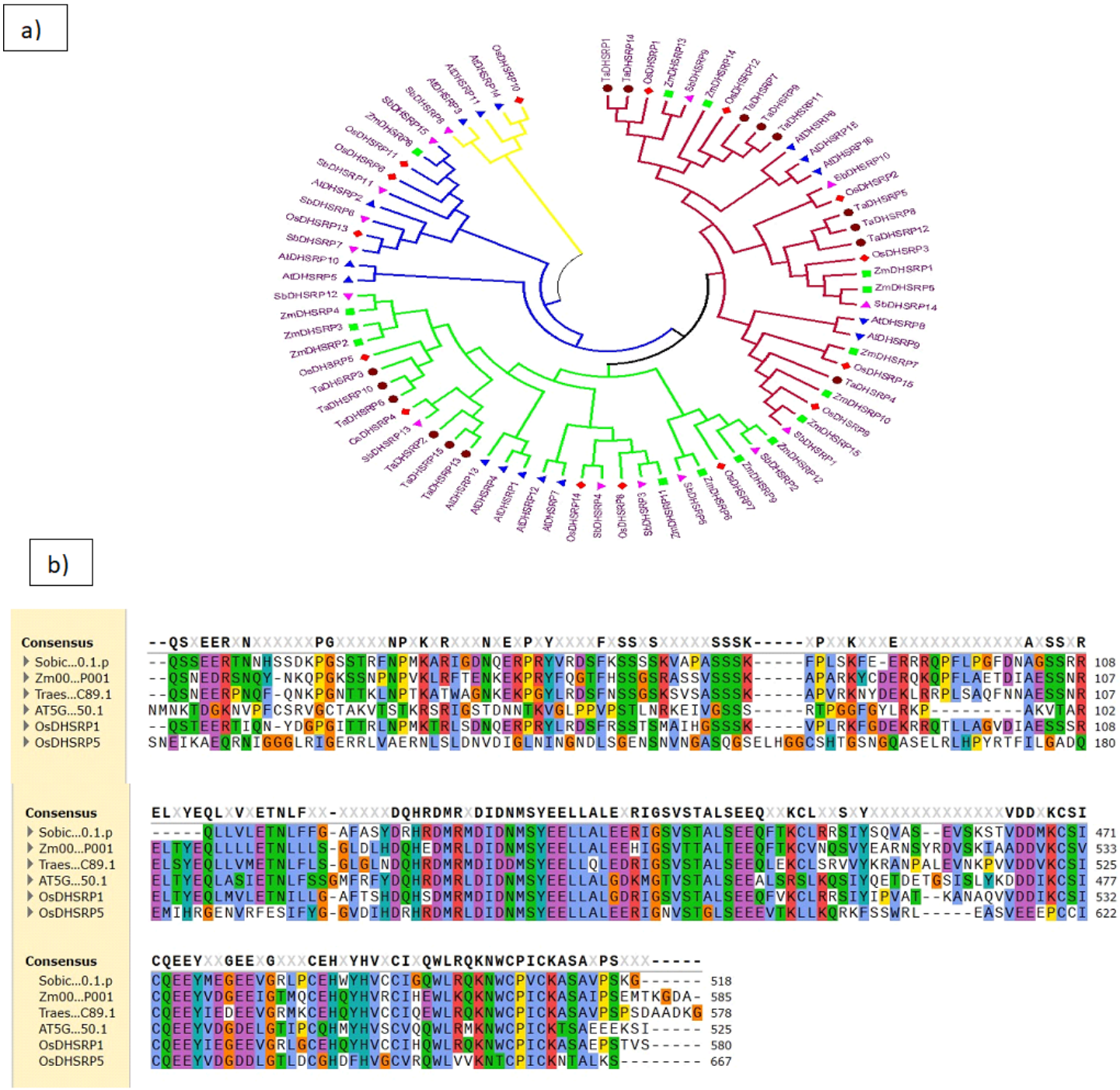


Figure 2

a) The phylogeniuc tree constructed using Mega 11.0 by bootstrap method. The colour of branches indicates four different groups.

b) Multiple sequence allignment of OsDHSRP homologs in five different related species – Sorghum, Maize, Wheat, Arabidopsis and Rice done by mega11.0 and viewed at Snapgene viewer tool. The bold letters indicates conserved sequences among them.

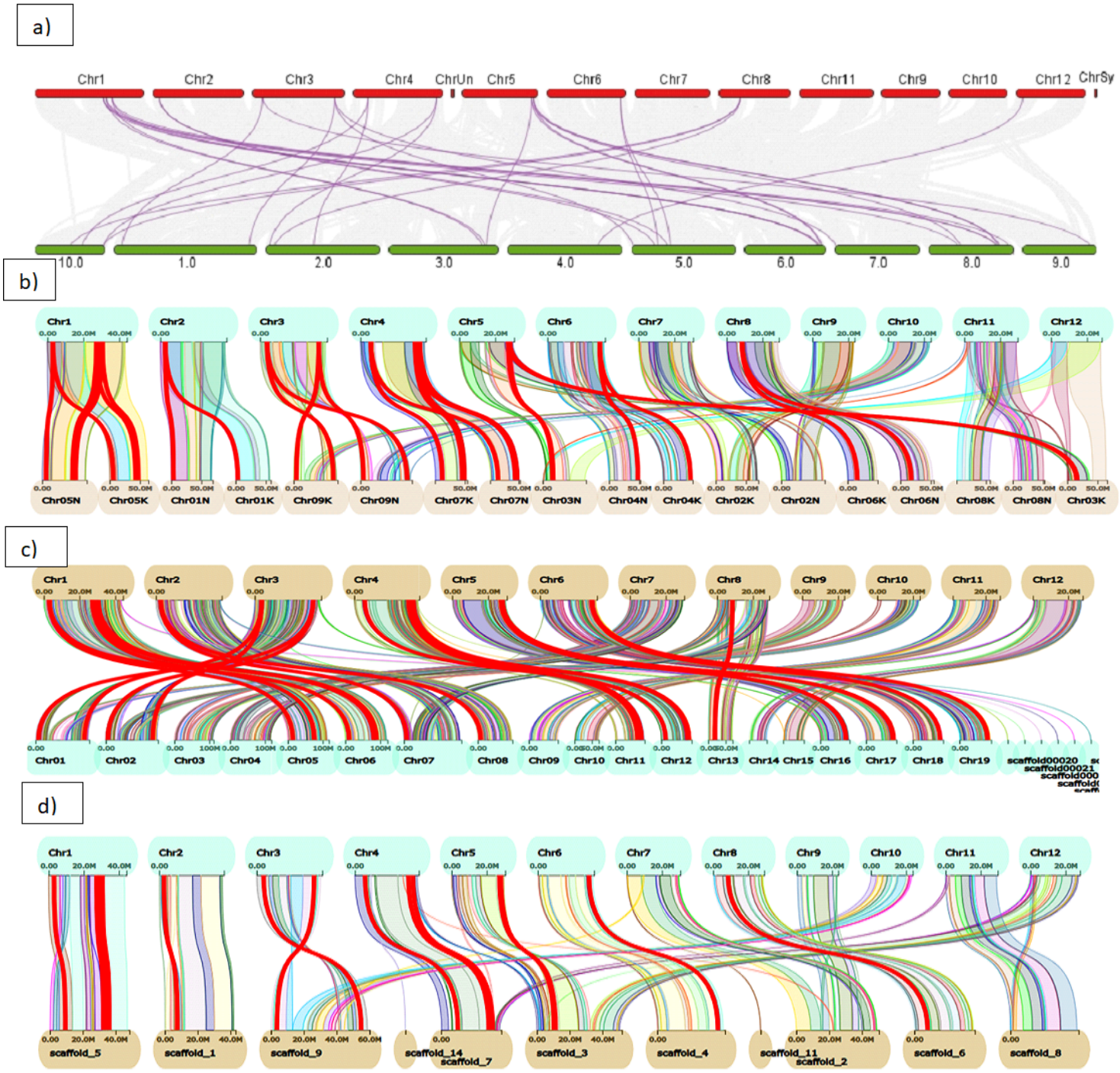


Figure 3

a) It shows collinearity among Maize and Rice species were purpled colored lines indicates collinearity of genes.

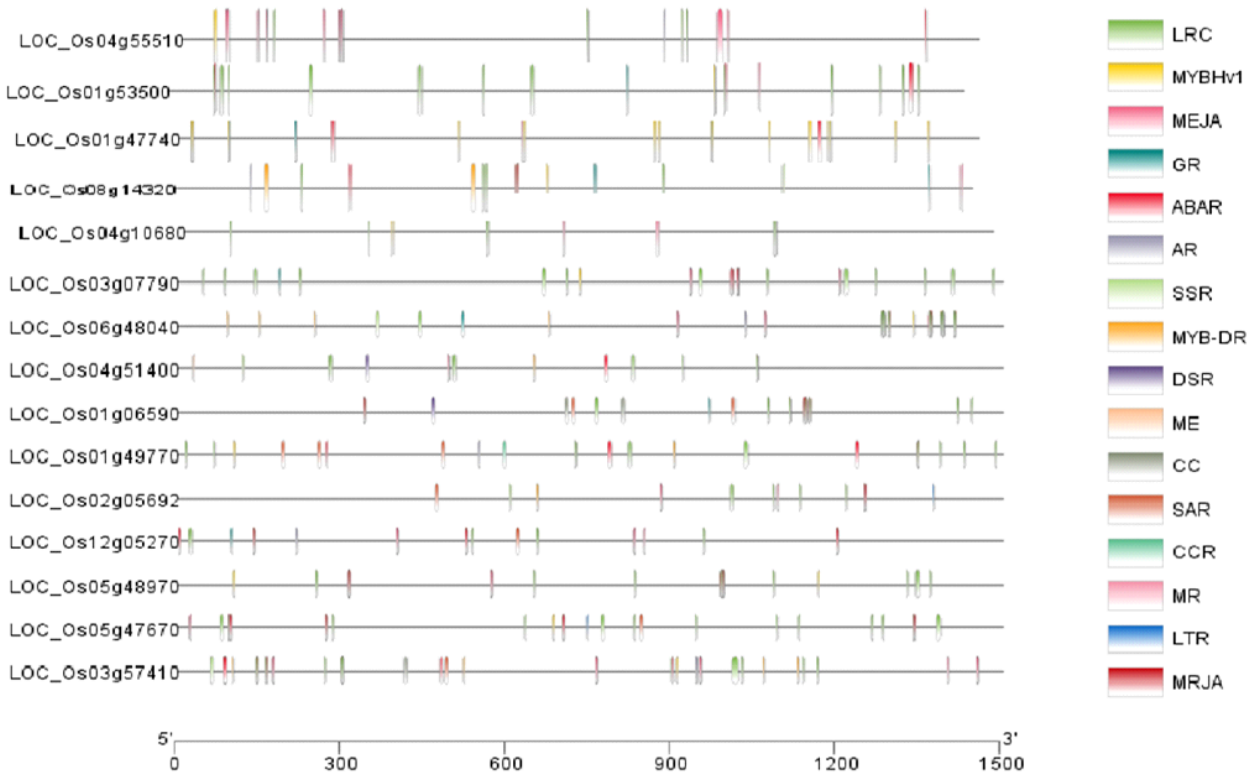
b) Synteny map of Rice and *Panicum virgatam* v5.1 were red lines indicates collinearity among them.

c) Synteny of Rice with *Miscanthus sinesis* were red lines shows collinearity among homologous genes.

d) Collinearity of genes among Rice and *Setaria italica* v2.2.

Red colour lines shows collinearity among homologous genes in two species of b), c), d).

a)



b)

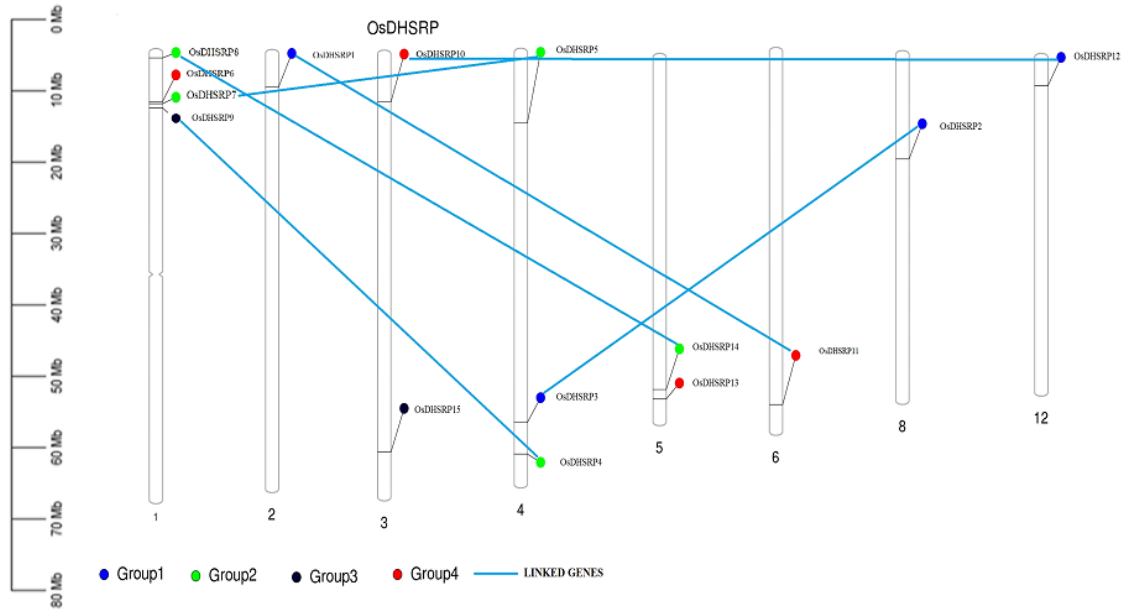


Figure 4

a) Cis regulatory elements present in 15 OsDHSRP gene homologs. Each colour indicates a specific promoter element.

b) Distribution of OsDHSRP genes on the chromosomes of Rice genome.

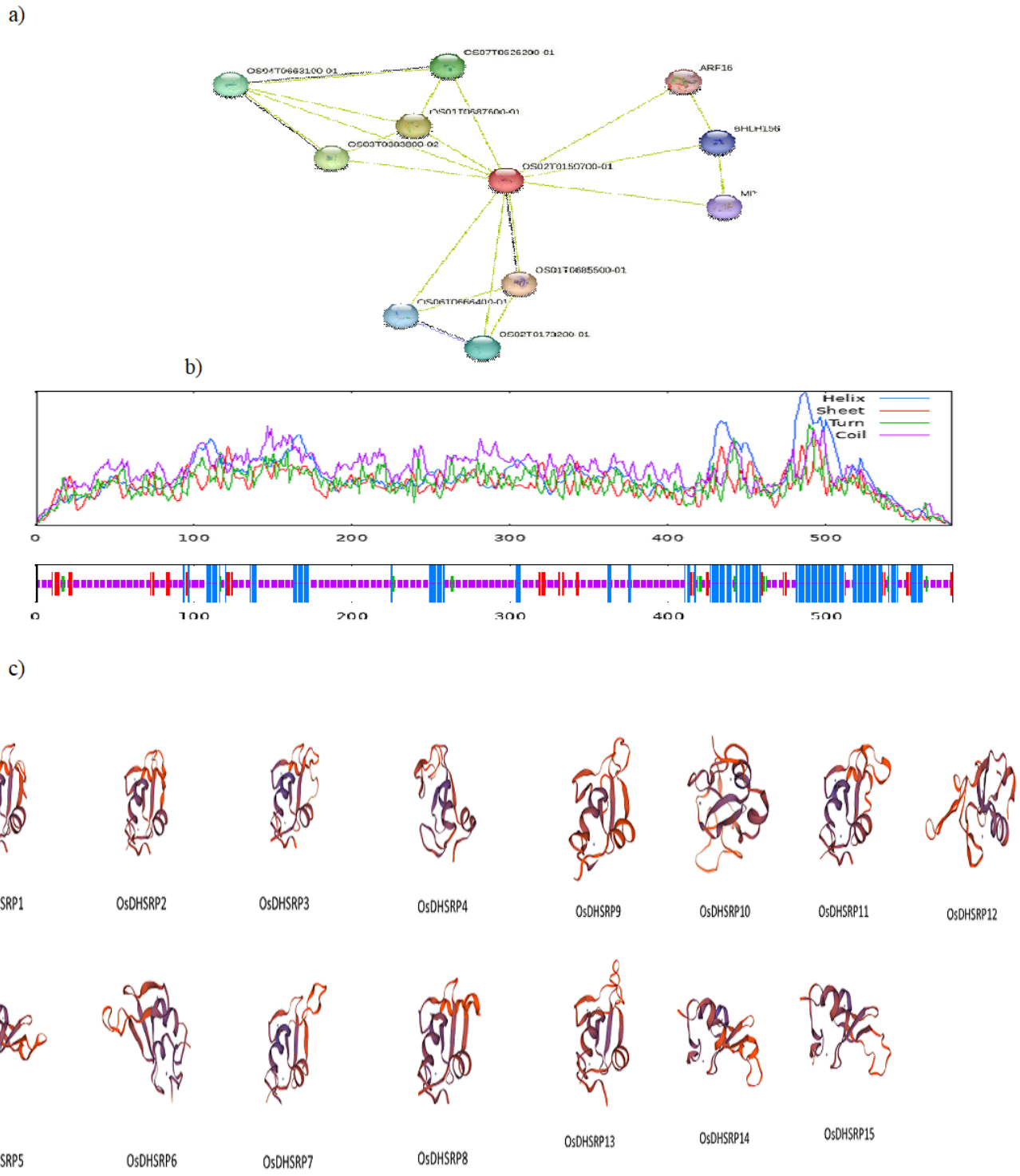


Figure 5

a) Protein -protein interactions of OsDHSRP1 protein from String Database.

b) 2 D structure of OsDHSRP1 protein. The distribution of helix, sheet, turn, coils were indicated with different colours.

c) Tertiary structure of 15 homolog proteins predicted by SWISS MODELLING and validated through Ramachandran plot analysis.

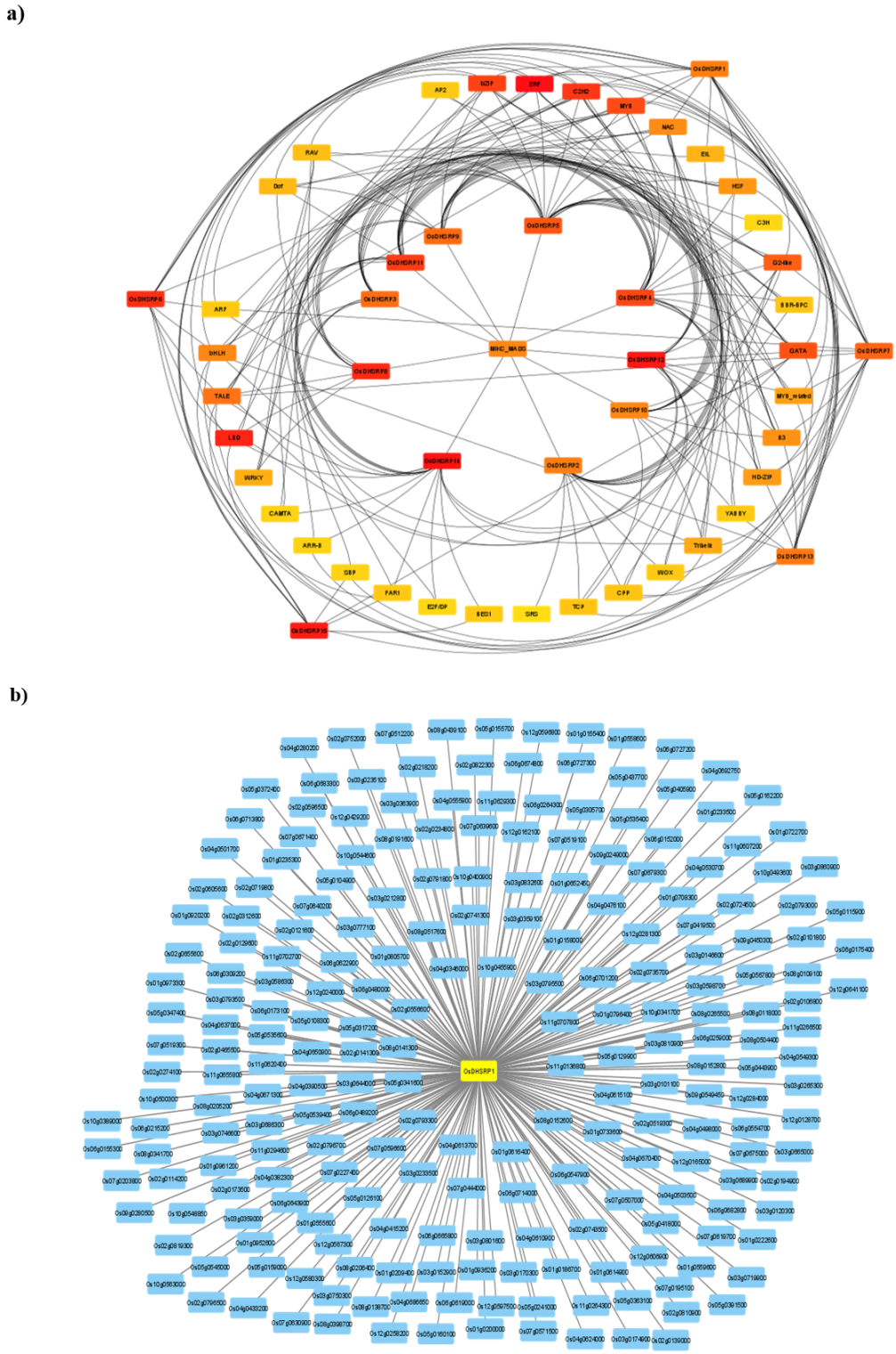


Figure 6

a) Network designed by Cytoscape tool of Predicted Transcriptional factors of OsDHSRP homologs genes. Blue colour indicates genes and others are transcription factors.

b) Network of Coexpression of OsDHSRP1 gene where yellow colour indicates gene and blue are other co- expressed genes

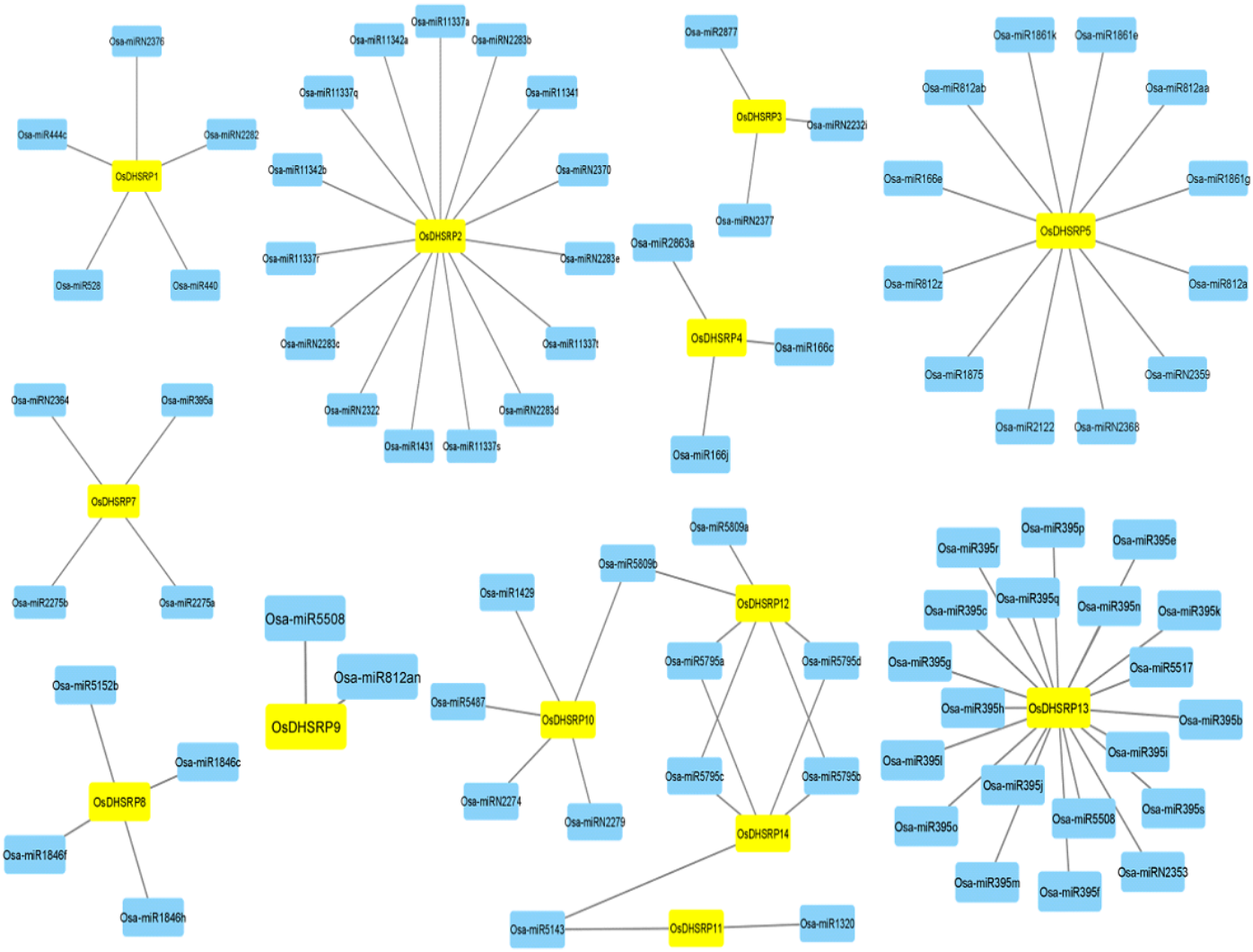


Figure 7

The Network showing predicted MiRNAs of 15 OsDHSRP genes , Here yellow colour indicates genes and blue are predicted MiRNAs of the specific gene.

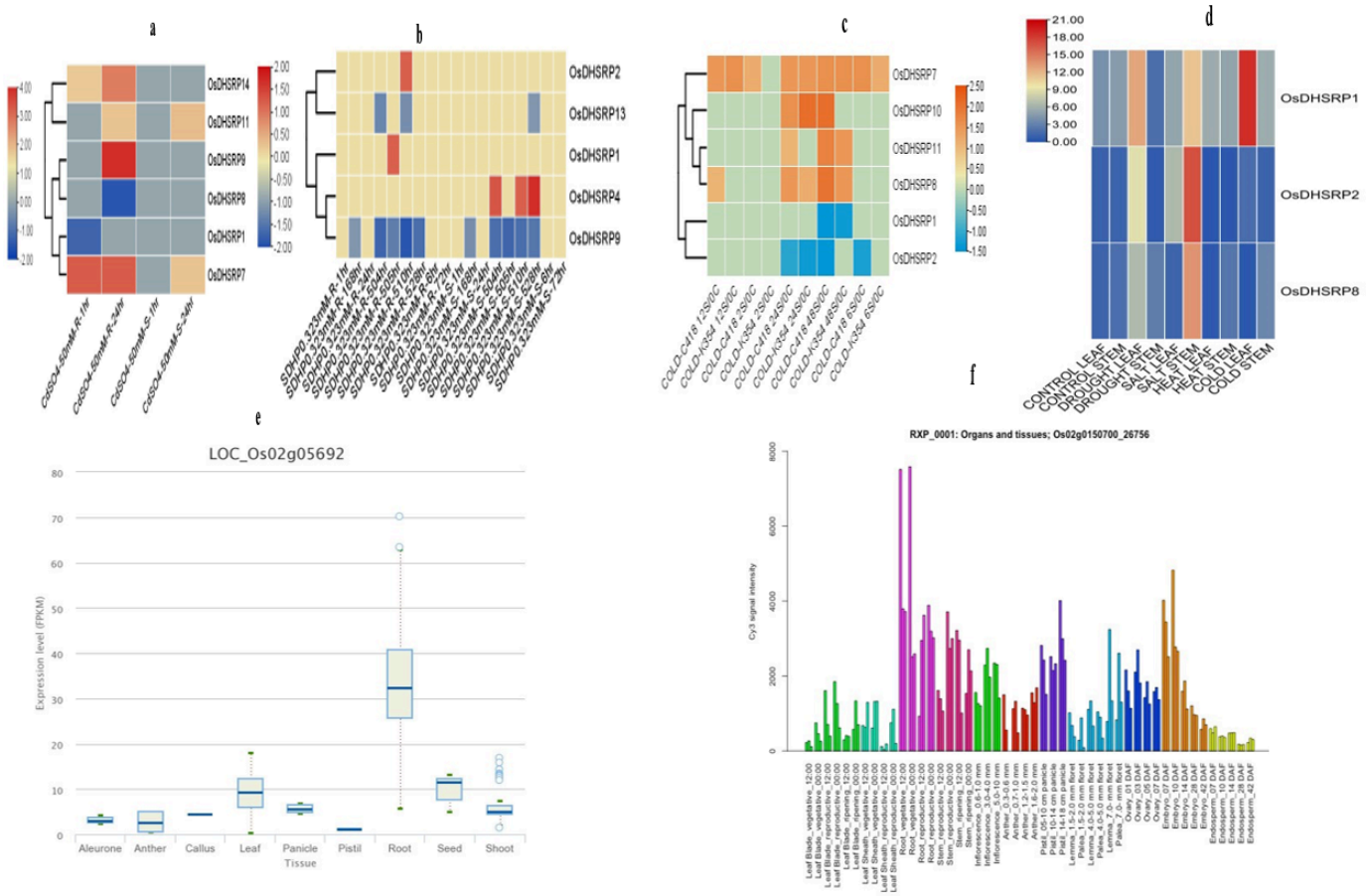


Figure 8

a) Insilco expression heatmap under Cadmium sulphate treatment. b) Heatmap showing expression of homologs under sodium diphosphatase treatment. c) Expression patterns of homolog genes under cold stress.

d) Expression heatmap showing relative foldchange of OsDHSRP homologs done by qRTPCR analysis. e) Basal expression pattern of OsDHSRP1 gene in different tissues and organs of Plant. f) Basal expression patterns of OsDHSRP1 gene.