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Impact of heat stress on expression pattern of nine rice heat shock factor genes and its traits related to tolerance

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Global warming is unusually increasing the earth temperature over the past century at an average rate of 0.07℃ per decade since 1880. The increased temperature exhibit greater impact on grain yield, approximately 5.18 million tons of rice yields due to heat wave. Heat shock factors (HSF) has major role in regulating heat shock proteins which in turn responsible for survival of plants in heat stress by refolding proteins, maintaining functional confirmation, aiding in host defence mechanism. The aim of this research was to analyse phenological, biochemical changes and key genes highly expressed during heat stress at flowering stage in rice. Expression analysis of nine HSF genes had given a differential expression under heat stress as compared to controlled traits. This study suggested OsHSP26.7 as most responsive gene under heat stress and rice line 159, RRF-127, GP-145-103 and Annada with heat tolerant adaptive mechanisms and better performance under high temperatures and was found to be in correlation with the estimated biochemical traits. This can be taken as a base for heat tolerance response of the crop, which may be useful for further validation studies of the candidate genes for heat tolerance in the rice as well as other crop plants.

Keywords: Heat shock factor genes, differential expression, biochemical traits

Introduction

Rice (*Oryza sativa* L.), the edible starchy grain, is the staple food crop of one-half of the world population majorly of East and Southeast Asia. Ninety five percent of world's rice crop is consumed by humans. Although rice can maintain normal growth up to temperature between 27 - 32℃, temperature above 32℃ negatively affects the all stages of rice plant including germination, growth, development, reproduction and yield[1]. High night temperature upto 32℃ increase spikelet sterility in rice which was resulted from decreased pollen germination (36%) of rice. Most critical temperature for rice crop was found to be 33℃ during flowering stage[2]. Losses occur in plants depends on the type of plant and degree and duration of high temperature. Heat stress differentially affects stability of various proteins, membranes, RNA species, cytoskeleton structure and efficiency of enzymatic reactions causes metabolic imbalance, ultimately results in the cellular damage and cell death leads to the catastrophic collapse of cellular organization. A network of interconnected cellular stress response systems is a prerequisite for plant survival and productivity[3]. High temperature might uncouple enzymes and metabolic pathways cause accumulation of unwanted and harmful reactive oxygen species (ROS) through lipid peroxidation and disruption of cell membrane stability. ROS production is seemed to be a primitive event in variety of stress conditions. ROS react with all biomolecules like pigments, proteins, lipids and DNA also oxidize protein and polyunsaturated fatty acids. Lipid peroxidation is one of the possible results in response to thermal stress. Increased leaf temperature reduced antioxidant enzyme activities that increased malondialdehyde (MDA) in leaves of rice plant. MDA is more often used as an indicator of lipid peroxidation resulting from oxidative stress[4]. Tertiary and quaternary structures of membrane proteins get transformed due to heat stress leads to permeability of membranes, which is obvious for electrolyte leakage. Although all plants are susceptible to rise in temperature at all growth and developmental stages, reproductive tissues are the most sensitive one, leads to loss of entire grain crop cycles. Reduced number of pollens retained by stigma obstacle in growth of endosperm, proembryo and unfertilized embryo, reduced ovule viability, impaired pollen germination and pollen tube growth and

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impaired meiosis are some of the consequences of rise in temperature. In general, pollen is more sensitive to heat stress at all stages of its development and performance. At heading stage significantly reduced anther dehiscence and pollen fertility rate causes reduced fertilization, spikelet fertility. Rice is extremely sensitive to short duration heat stress episodes (>35ºC for ≥1 h) coinciding with the reproductive stage, particularly anthesis[5]. At molecular level high temperature alters expression of genes like osmoprotectants, detoxifying enzyme, transporters, regulatory proteins leads to change in biochemical and physiological processes by gene expression. Plants tolerate stresses by modulating multiple genes and by coordinating expression of genes in different pathways. Heat shock proteins (HSPs) play an important role in heat tolerance mechanism by acclimation or ideally by adaptation of plants. For expression of these HSPs require regulatory factors called heat shock factors (HSFs) that occur singly in cytosol and crosses membrane of nucleus by nuclear localization signal domain present on it, then by forming trimer bind themselves on HSP genes, further carry out their expression. HSPs serves functions like protein degradation prevent protein aggregation, protein stabilization and host defence mechanism so called as master players. Accumulation of osmolytes like proline, giberllic acid, trehalose are also the signs of heat injury.

Many rice growing regions already reached upto optimum temperature, therefore, any further increase in mean temperature may be supra optimal for sensitive stages and reduces grain yield. By the end of 21st century it was estimated that rice yield have been reduced by 41%[6]. In rice, heat stress seriously affects the flowering and grain filling stage leads to spikelet sterility and low grain quality. With these considerations, the current study was undertaken to characterize heat tolerance mechanism in rice at phenological, biochemical and molecular levels.

Material and Methods

Plant Material

The experimental material of present investigation consists of sixteen rice genotypes (Table 1). These varieties were planted separately in green house at 28 ± 2℃ in summer 2019-20. Plants were subjected to the same environmental condition as appeared in summer by flooding the trays with water and increasing the green house temperature. Thermal stress is given at flowering stage to 16 rice genotypes. The green house temperature is increased gradually from 30ºC at 6:00 am to 42ºC at 11:00 am. Constant temperature of 42ºC was maintained for 6 hrs continuously. The rice plants were treated with heat stress until 17:00 pm, with gradual adjustments to the greenhouse's temperature down to 28ºC - 30ºC at night (18:00 to 6:00). This stress is given continuously for 6 days from the beginning flowering stage. Leaf samples were collected after stress treatment from control and stress plants for further studies. Leaf samples were collected in liquid nitrogen -80℃ for RNA isolation study.

Membrane Stability Index

Electrolyte leakage from the leaf samples was calculated with the method given by Dinisio-Sese and Tobita (1998)[7] using following formula.

$$\text{Electrolyte leakage (EL \%)} = \frac{EC1}{EC2} \times 100$$

Pollen Fertility

Pollen fertility is calculated by means of staining methods with the help of stains like acetocarmine (2%). The deeply stained / normal looking pollen grains are counted as the viable pollen and the colourless / shrivelled pollen are counted as the non-viable pollen.

The pollen fertility is calculated using the formula.

$$\text{Pollen fertility (\%)} = \frac{\text{No. of viable pollen}}{\text{Total no. of pollen}} \times 100$$

Spikelet Fertility

Spikelet fertility is calculated under both control and stress conditions. The negative effect (% decrease from control) was determined in every genotype. Spikelet fertility is calculated as shown below

$$\text{Spikelet fertility (\%)} = \frac{\text{No. of filled grains}}{\text{Total no. of grains formed (florets)}} \times 100$$

Estimation of MDA Content

MDA content (an indicator of lipid peroxidation) was calculated using the method given by Heath and Packer (1968)[8]. MDA content is expressed as nmoles MDA g^{-1} fresh weight.

Statistical Analysis

The OPSTAT software developed at BHU was applied for statistical analysis.

Table 1 — Detail of rice lines used in experiment

Sr No	ID	Tested lines	Sr. No.	ID	Tested lines
1	R-H-2018-72	72	9	R-H-2018-3	C
2	R-H-2018-73	73	10	R-H-2018-4	D
3	R-H-2018-78	78	11	Nagina-22	N22
4	R-H-2018- 158	158	12	RRF-127	RRF-127
5	R-H-2018-159	159	13	MTU-1010	MTU-1010
6	R-H-2018-164	164	14	CGZR-1	CGZR-1
7	R-H-2018-1	A	15	Annada	Annada
8	R-H-2018-2	B	16	GP-145-103	GP-145-103

RNA Extraction

Total RNA was isolated using TRIzol (Invitrogen, USA) using a manufacturer's protocol. Complementary DNA (cDNA) was synthesized using BIORAD iScript TM cDNA synthesis kit as per manufacturer's instructions.

Semi-Quantitative RT-PCR and Gel Electrophoresis Analysis

Semi-quantitative RT-PCR reactions were carried out with 20 µl of the reaction (APS *Taq* polymerase) solutions using gene specific primers (Table 1) and actin gene primers as internal control. The resultant PCR product was then resolved on 1.5% agarose gel followed by digitalization of fluorescence data to numerical values using GelQuant NET analyzer.

Database search and primer designing for heat shock factor genes in rice

Heat shock factor genes was searched by using iTAK- plant transcription factor and protein kinase identifier and classifier (http://itak.feilab.net/cgi-bin/itak/index.cgi) database. The nucleotide and protein sequences enlisted from above search were retrieved from iTAK. The genes were downloaded by using specific gene ID's enlisted at the database in FASTA format. Primer designing was carried out by using batch primer 3.0 (probes.pw.usda.gov/batchprimer3/) web based tool. Expression primers for 26 HSF genes were designed based on coding sequences (CDS) provided and were used for expression analysis study. These primers were synthesized in 10 *nM* scale with HPSF purification from Eurofins Genomics India Pvt. Ltd. Sequence and other details of primers used in the study are presented in Table 2.

Result and Discussion

Identification and Sequence Analysis of Heat Shock Transcription Factor Genes

In order to identify the heat shock transcription factor genes, the keywords - HSFs was used to the NCBI (National Centre for Biotechnology Information) (https://www.ncbi.nlm.nih.gov/) search engine. Plants having HSFs are shown in Table 3.

Effect of High Temperature on Membrane Stability Index (MSI)

Un-interrupted function of cellular membrane is quite important. Wahid *et al*[9], studied physiological responses to high temperature in order to understand the mechanism of heat tolerance in plants. Hence, to determine the cell membrane stability in high temperature is important as it plays critical role in elucidating the heat stress. Electrolyte leakage is the measure of electrical conductivity of the tissues. Earlier studies presented that heat tolerant genotypes show low levels of electrolyte leakage, indicating less injury to the membrane[10-13] performed a study to know about cell membrane stability of 8 genotypes of bread wheat. The varieties Hindi 62 and NIA W 34 were good general and specific combiners in the tolerant group showing maximum membrane stability index, while HD 2687 and WH 147 were good specific combiners in the heat sensitive group having least membrane stability index.

Similar, kind of experiment about cellular membrane stability due to heat stress in 12 bread wheat genotypes HD 2864, HD 2967, PBW 373, HS 365, Raj 4037, JAUW 584, PBW 175, RSP 561, HW 2045, HD 2385, HD 2687 and HDR 77 (Sharma *et al* 2017). The maximum level of conductivity was found in HDR 77 i.e. less membrane stability index (93%) and minimum conductivity observed in HD 2687 i.e. having maximum membrane stability. Hence HDR 77 was found to be the highly susceptible one and HDR 2687 was the more resistant type to heat stress.

In this study wide variation in the increase of membrane stability among the tested sixteen rice genotypes was recorded which ranged from 1.61% increase in 159 to 39.85% in MTU-1010. The lowest levels of electrolyte leakage was observed in 159 with

Table 2 — Details of primers used in expression analysis

Sr No.	HSF gene	Forward sequence (5'-3')	Reverse sequence (5'-3')	Tm (^{o}c)
1	Osind01g24510.1a	CGTGCAGCAGGTTCTACTCA	TGGAGTGGTCTCCACTTTCC	58
2	Osind01g28640.1	GAGGGATTTGACGTGTAGGC	CACCATCTGGTACGTCTTCG	59
3	Osind01g36950.1	TGACCAACTCACCAGCTCAG	AACACGGGGATCACTTTCAC	58
4	Osind02g10900.1	AGAAGGGTGATGACGACGAC	ACGAAGCTGGAGAAGTTGGA	58
5	Osind02g21410.1	AACAGCGGGTAAATTGATGG	TGCAGATCTCCATCATCCAG	56
6	Osind02g19620.1	CATGCTCGATGGATGTTGAC	TCACTGGCTACTCGTGCATC	58
7	Osind03g09500.1a	ATCCAACTGGTGGAGTCCTG	ACTGCAGGACTTGGAACTGG	59
8	Osind03g09500.2	AACTGGTGGAGTCCTGATGG	ACTGCAGGACTTGGAACTGG	59
9	Osind03g04940.1a	TCGACATTGCTGTTTGATCC	TTCAGAAGCTCCTCCCAGAA	56
10	Osind03g02790.1	TTCCTGACGAAGACGTACCA	TGCGTTGATAGGTGTTGAGC	57
11	Osind03g37700.1	AGACGTACGAGGTCGTGGAC	TTCGCGAACTCCCATCTATC	59
12	Osind03g20380.1	AGTTCGCCAACGAGTTCTTC	GAAATGGTAGGCTGCTGCTG	58
13	Osind03g41450.1	TGATTCGCTGCATCTTGTTC	GCAGCTCAGAACCTCACCTC	58
14	Osind03g41450.2	TGATTCGCTGCATCTTGTTC	GCAGCTCAGAACCTCACCTC	58
15	Osind04g25010.1	AGACCATCTCGTGGAACGAC	GCTTCTCCCCTCTCCTGAAG	60
16	Osind06g21240.1	GGACTACTCGACGGTGAAGC	ACACCACGAAGCTGTTCCTC	60
17	Osind06g20630.1	TCGTGTGGAAGACGTACAGG	GCGGAAACCATAGGTGTTGA	58
18	Osind05g26990.1a	CAGGTTTTGTGCAGAGTCCA	ACCGTGGAGTCTCTGTGAGG	59
19	Osind10g09120.1	GGTGAGTTTGGATTCGAGGA	CTGGAAGAACTCTGGGTTGC	58
20	Osind09g17700.1	ATATACATCGCCGGAAGGTG	ACCCAGAGTTTGACGACAGC	58
21	Osind09g13260.1	AGCTCAGGAAGGACAACCAA	AGCTTCACGCACTTCTCCTC	59
22	Osind09g13180.1	GACGTATCAGCTGGTGGATG	AACCCGTAGGTGTTGAGCTG	59
23	Osind08g26440.1	GGAGCAGGTCATATCCTCCA	AGGCGTACTTGGACATGAGG	59
24	Osind08g20940.1	CCGTTCCTGACCAAGACGTA	CGAAGGAGGAGAAGTTGCTG	59
25	Osind07g24230.1	AGACACTGCAGCCTTTCTCC	CAGCTGGTACGTCTTCGTCA	59
26	Osind07g04910.1	CAAGAACATCAAGCGTCGAA	CCTATCCTCCATGGCTTTCA	58

Table 3 — Details of known heat shock factor genes across different plant species

Species	Total no. of HSF	References
Arabidopsis thaliana	21	Scharf *et al*., 2012
Tomato	24	Scharf *et al*., 2012
Castor bean	19	Scharf *et al*., 2012
Pepper	25	Guo *et al*., 2015
Apple	25	Giorno *et al*., 2012
Tea	16	Liu *et al*., 2016
Soybean	52	Scharf *et al*., 2012
Cotton	40	Wang *et al*., 2014
Chinese cabbage	30	Huang *et al*., 2015
Poplar	27	Scharf *et al*., 2012
Carrot	35	Huang *et al*.,2015
Strawberry	17	Hu *et al*., 2015
Willow	27	Zhang *et al*., 2015
Chinese white pear	29	Qiao *et al*., 2015
Chinese plum	17	Qiao *et al*., 2015
Peach	17	Qiao *et al*., 2015
European pear	33	Qiao *et al*., 2015
Maize	30	Scharf *et al*., 2012
Rice	26	Scharf *et al*., 2012
Wheat	56	Xue *et al*., 2014
Millet	24	Scharf *et al*., 2012
Brachypodium	24	Scharf *et al*., 2012

1.61% increase under stress when compared with control and reported as heat tolerant genotype followed by N22 (1.65), RRF-127 (3.46), D (4.87), Annada (5.82) as shown in Figure 2.

Effect of High Temperature on Pollen Fertility

Heat stress effects pollen development by decreasing the starch concentration before anthesis, which leads to decrease in sugar concentration in mature pollens, contributing to sterile pollen[14]. Many studies reported that pollen fertility decreases under stress conditions and the genotypes showing lowest decrease in pollen fertility are considered as the tolerant ones for heat stress[15-16]. Similar kind of results were shown by with the experiment of heat stress on pollen viability, with 11 rice genotypes during two kharif seasons. In 2010 number of sterile pollen varied from 344.5 in IET21404 to 652.5 in KRH-2 at high temperature stress whereas in control maximum 46.5 was observed in PHB-71. In 2011 the number of sterile pollen was minimum 419.5 in PA6444 and maximum 706.5 in IET21582. Morphological observations revealed that pollen sterility in plants exposed to high temperature was higher than that in control plants.

Pham *et al*[17] reported that long term heat stress of 35℃ increased the deform flowers and damaged 50% pollen viability in tomato. HT7 mutant which was showing highly expressed heat shock factor (SlHsfA1b) and heat shock protein (SlHsp101) produced more viable pollens than wild type in heat stress.

In this study lowest decrease in the pollen fertility, under stress conditions was observed in 159 (10%) followed by Annada (10%), 158 (21%), B (23%), RRF-127 (27%), GP-145-103 (31%) hence appeared as heat tolerant genotypes and highest decrease in pollen fertility was observed in MTU-1010 (88%) (Fig. 3).

Effect of High Temperature on Spikelet Fertility

Wu *et al,* found that high temperature inhibited spikelet formation is associated with the synthesis and decomposition of cytokines. Additionally, high temperature leads to peroxide accumulation in the spikelets, which destroys cellular construction and reduces spikelet number. Previous study showed that in rice genotypes, percent reduction of filled grains under heat stress as compared to control varied from 16 - 66% in 2010 and 11 - 64% in 2011 (Kumar *et al*., 2015). Number of filled grains was higher in heat treatment than in control for IET 21404 and IET 21577 in 2010 and IET 21404 in 2011.

Fig. 1 — Morphological change in rice genotypes under control and stress condition.

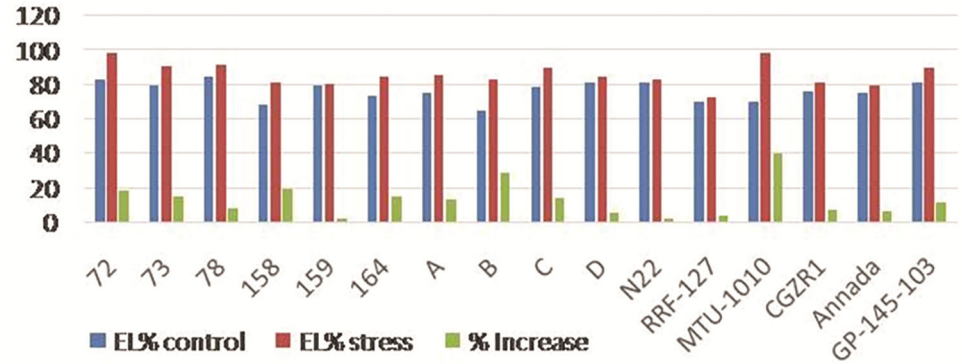

Fig. 2 — Membrane stability index (MSI) of sixteen rice genotypes under control and stress conditions

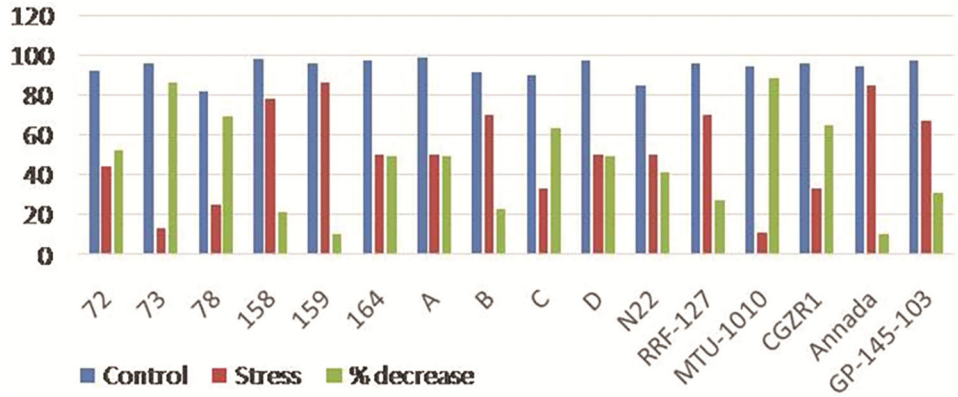

Fig. 3 — Pollen fertility of sixteen rice genotypes under control and stress conditions

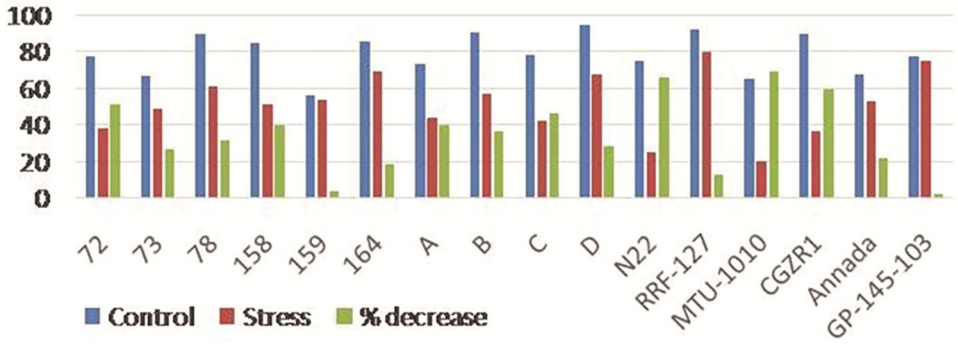

Fig. 4 — Spikelet fertility of sixteen rice genotypes under control and stress conditions

Earlier studies also reported the decreased spikelet fertility under heat stress conditions and the genotype having the lowest change in spikelet fertility under stress conditions when compared to control is considered as the tolerant genotype[18-19]. In the present study, wide variation is observed in the spikelet fertility of the tested genotypes under stress. The decrease in spikelet fertility ranged from 3% in GP-145-103 to 69% in MTU-1010. Lowest decrease in spikelet fertility under stress conditions was observed in GP-145-103 (3%) followed by 159 (4%), RRF-127 (13%), 164 (19%), Annada (22%) (Fig. 4).

Effect of High Temperature on Malondialdehyde (MDA) Content

Heat stress causes the lipid peroxidation of cell membrane which is estimated in terms of MDA. Lipid peroxidation in the cell membranes is the destructive effect of oxidative damage caused due to thermal stress. MDA content has been widely used as a criteria for assessing abiotic stress in various plants[20]. Han *et al*. [21] in studied heat stress on MDA content of four leaf lettuce seedlings. The study showed that, with increasing temperature, the MDA content increased. The MDA content of S24 and S39 was increased by 50.70% and 97.49%, the MDA content of J20 and J2 increased by 55.04% and 77.49%. The increase of the MDA content of heat resistant varieties than the non heat resistant varieties is small. Mansoor and Naqvi[22] in their experiment with four mung bean genotypes NM 19-19, NM 20-21, NM121-123 and NCM 89 showed the effect of heat stress on lipid peroxidation. The result showed that low MDA content and increased antioxidant content under heat stress in NM 19 - 19 indicating that it was most tolerant, while high MDA content and decreased antioxidant content in NM 20 - 21 which said to be as least thermo tolerant.

The mean MDA content in current study under control conditions was recorded as 6.76 n mol/g fresh weight and it ranged from 4.24 nmol/g in GP-145-103 to 10.69 nmol/g in 72, while the mean MDA content under stress conditions increased to 12.64 nmol/g fresh weight and ranged from 7.04 nmol/g in RRF-127 to 24.83 nmol/g in 72 (Table 4). Studies reported that MDA content is relatively more in heat susceptible varieties under stress conditions due to increased lipid peroxidation[23-24]. Thus the heat tolerant genotypes recorded relatively low MDA content under stress than susceptible ones. In the present study lowest amount MDA content was recorded in rice variety 159 with 1.09 fold increase followed by RRF-127 (1.20 fold), Annada (1.36 fold), CGZR-1 (1.64 fold) and 78 (1.64 fold) (Fig. 5) and highest amount of MDA content was recorded in MTU-1010 (2.55 fold).

Table 4 — CRD analysis along with mean and range for MDA enzyme under heat stress conditions

Rice lines	MDA control (nmols / g f.wt) Mean ± S.E.	MDA stress (nmols / g f.wt) Mean ± S.E.
72	10.685 ± 0.679	24.826 ± 0.78
73	7.195 ± 0.879	18.00 ± 0.577
78	7.758 ± 0.754	12.755 ± 0.513
158	4.349 ± 0.726	10.706 ± 0.706
159	8.059 ± 0.685	8.75 ± 0.346
164	6.263 ± 0.373	11.00 ± 1.155
A	5.656 ± 0.603	12.488 ± 0.829
B	6.83 ± 0.768	12.462 ± 0.754
C	5.886 ± 0.979	15.007 ± 0.785
D	7.155 ± 1.192	13.462 ± 0.867
N22	6.643 ± 0.835	11.582 ± 0.696
RRF-127	5.847 ± 1.203	7.037 ± 0.243
MTU-1010	6.62 ± 0.987	13.72 ± 0.9
CGZR-1	6.565 ± 0.885	10.784 ± 0.572
Annada	8.348 ± 0.387	11.357 ± 1.476
GP-145-103	4.243 ± 0.631	8.315 ± 0.656
C.D. (p=0.05)	2.369	2.296
SE(m)	0.819	0.793
SE(d)	1.158	1.122
C.V.	20.852	10.907

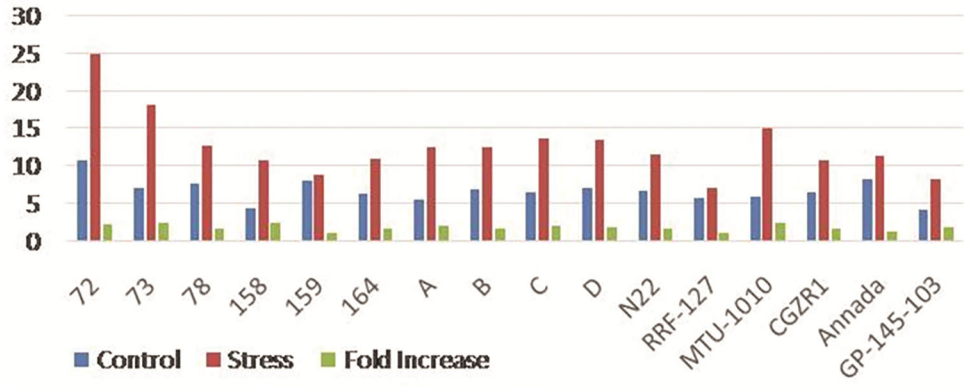

Fig. 5 — MDA content of sixteen rice genotypes under control and stress conditions at flowering stage.

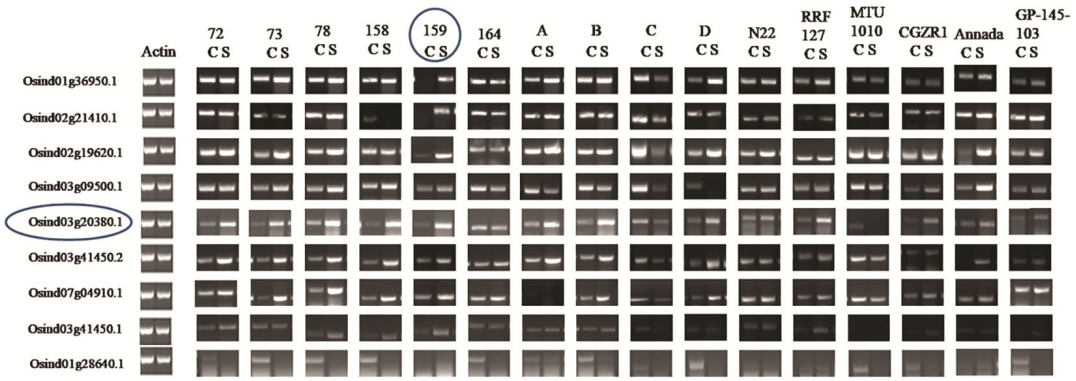

Fig. 6 — Semi quantitative RT-PCR analysis of heat stress responsive genes in different rice genotypes under high temperature.

Semi-Quantitative Expression Analysis of Heat Stress Responsive Genes in Rice Under Heat Stress

Semi quantitative RT-PCR was performed to analyse the expression pattern of nine differentially expressed transcripts in rice under heat stress versus control condition. The differential expression of nine heat stress responsive genes (Osind01g28640.1, Osind02g19620.1, Osind03g09500.1, Osind03g20380.1, Osind07g04910.1, Osind03g41450.1, Osind01g36950.1, Osind02g21410.1, Osind03g41450.2) in all the sixteen rice genotypes under both control and stress conditions was indicated in the Figure 6.

In the present experiment, gene Osind01g36950.1 was investigated as up-regulated in ten rice genotypes out of sixteen studied. Rice varieties 159, D, RRF-127, A, Annada, GP-145-103, 73, 72, 158, N22 was shown up-regulation of 2.79 fold, 1.48 fold, 0.85 fold, 0.38 fold, 0.30 fold, 0.27 fold, 0.15 fold, 0.07 fold, 0.04 fold and 0.02 fold, respectively under heat stress. Rice vartiety, MTU-1010 was shown to be down-regulated by -0.32 fold. Osind02g21410.1 gene was studied by semi quantitative RT-PCR. The MTU-1010 was down regulated by -0.36 fold. Maximum up-regulation were shown by 159 (3.70 fold), RRF-127 (0.59 fold), GP-145-103 (0.24 fold), Annada (0.17 fold), A (0.16 fold), N22 (0.12 fold), 78 (0.04 fold) and B (0.02 fold).

Semi quantitative RT-PCR of Osind02g19620.1 gene, in the present investigation has shown up-regulation in almost all rice genotypes (Fig.7). The rice genotype Annada showed the highest up-regulation of 6.47 fold increase followed by 73 (1.33 fold), D (0.66 fold), N22 (0.48 fold), 159 (7.83 fold), 78 (0.35 fold), GP-145-103 (0.33 fold), CGZR-1 (0.27 fold), A (0.24 fold), 72 (0.10 fold), B (0.08 fold), 158 (0.02 fold), RRF-127 (0.01 fold) and MTU-1010 here also down regulated by -0.04 fold.

In the current study Osind03g09500.1 gene showed up-regulation in rice genotype Annada (2.18 fold), CGZR-1 (1.09 fold), 159 (0.55 fold), 78 (0.53 fold), 73 (0.50 fold), 158 (0.34 fold), GP-145-103 (0.20 fold), RRF-127 (0.08 fold), N22 (0.08 fold) and 72 (0.01 fold) and MTU-1010 shown down-regulation by -0.20 fold.

Osind03g20380.1 gene expression was also studied in the present investigation using semi quantitative RT-PCR and it showed up-regulation in almost all genotypes of rice under heat stress. The rice genotype 158 has shown highest up-regulation of 6 fold increase followed by 73 (5.64 fold), CGZR-1 (4.22 fold), 78

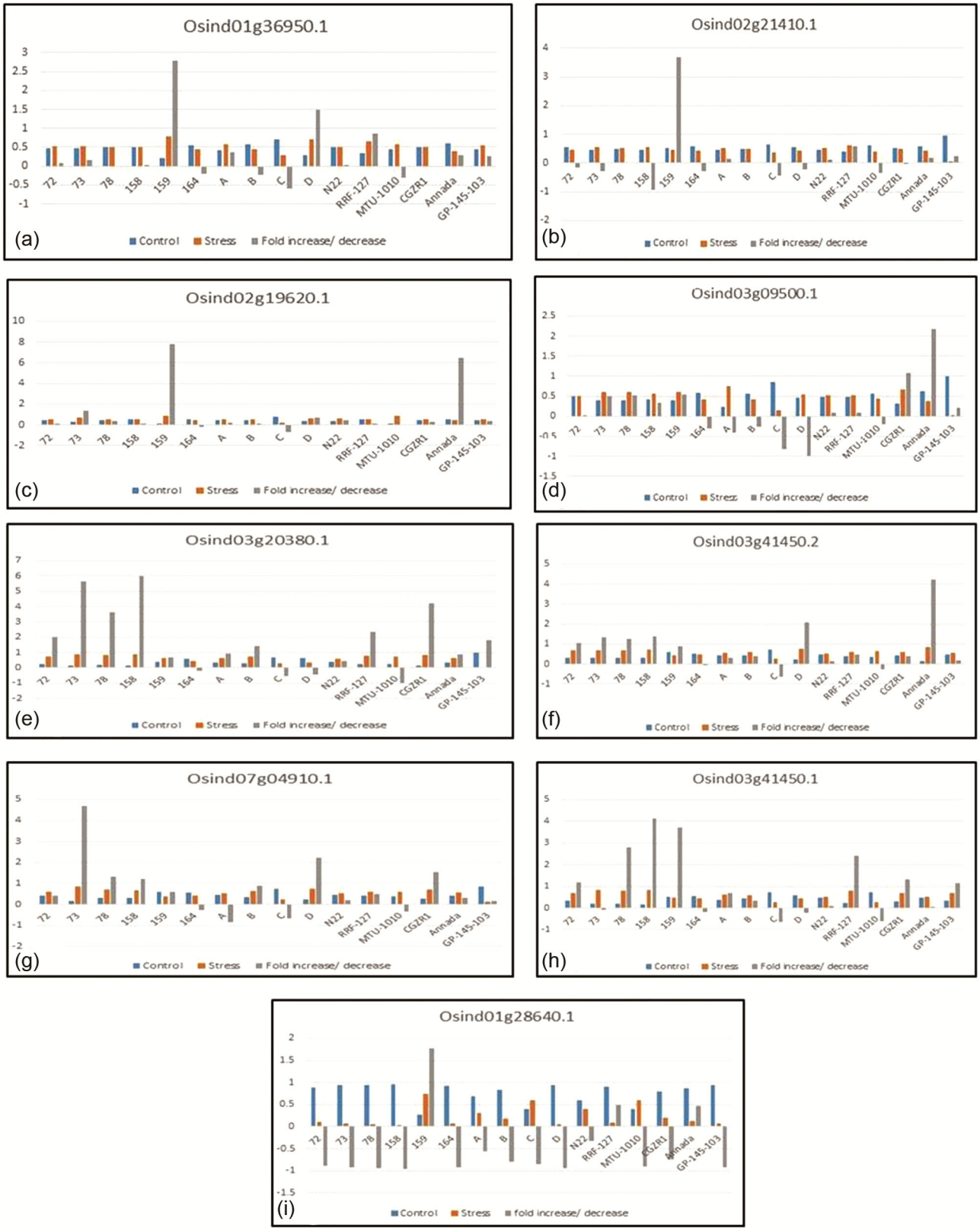

Fig. 7 (a-i) — Gene transcript increase and decrease among sixteen genotypes of rice (72, 73, 78, 158, 159, 164, A, B, C, D, N22, RRF-127, MTU-1010, CGZR1, Annada and GP-145-103) under control and stress condition.

(3.63 fold), RRF-127 (2.36 fold), 72 (2.02 fold), GP-145-103 (1.81 fold), B (1.44 fold), A (0.93 fold), Annada (0.86 fold), 159 (0.70 fold), N22 (0.46 fold) and MTU-1010 shown down-regulation by -0.98 fold.

Also the semi quantitative RT-PCR of gene Osind03g41450.2 was carried out to observe the expression pattern under heat stress on sixteen rice genotypes. Annada, D, 158, 73, 78, 72, 159, RRF-127, B, CGZR-1, A, GP-145-103 and N22 was shown up-regulation, which was up-regulated by 4.22 fold, 2.10 fold, 1.37 fold, 1.34 fold 1.28 fold, 1.06 fold, 0.90 fold, 0.47 fold, 0.38 fold, 0.38 fold, 0.31 fold, 0.17 fold and 0.13 fold, respectively. Down-regulated genotype was MTU-1010 (-0.28).

In the present investigation, semi quantitative RT-PCR of Osind07g04910.1 gene was shown up-regulation in almost all the genotypes under heat stress and down-regulation in some rice genotypes. The rice genotype 73 has shown highest up-regulation of 4.68 fold increase followed by D (2.21 fold), CGZR-1 (1.54 fold), 78 (1.34 fold), 158 (1.22 fold), B (0.89 fold), 159 (0.60 fold), RRF-127 (0.49 fold), 72 (0.42 fold), Annada (0.30 fold), N22 (0.20 fold), GP-145-103 (0.18 fold). Down-regulation was shown by MTU-1010 by -0.35 fold.

Gene Osind03g41450.1 was studied in this experiment through semi quantitative RT-PCR. Rice lines 158, 159, 78, RRF-127, CGZR-1, 72, GP-145-103, A, B, N22, Annada were showing up-regulation. 158 showing fold increase of 4.15 fold, followed by 159 which showed 3.70 fold, 3.79 fold increase was shown by 78, 2.41 fold increase was shown by RRF-127 and 1.30 fold by CGZR-1, 1.17 fold was shown by 72, 1.16 fold by GP-145-103, 0.69 fold by A, 0.32 fold by B, 0.10 fold by N22 and 0.05 by Annada. MTU-1010 (-0.62 fold) shown to be down-regulated.

Semi quantitative RT-PCR of Osind01g28640.1 gene has shown wide variation in all rice genotypes. The maximum up-regulation was shown by 159 (1.77 fold), followed by RRF-127 (0.49 fold), Annada (0.47 fold). MTU-1010 showed down regulation by -0.90 fold. Out of 9 transcripts under control and stress condition for sixteen genotypes of rice a significant level of up-regulation of all genes was observed among 159, RRF-127, GP-145-103 and Annada. HSFs are the primary compounds of the signal transduction pathway to activate the expression of HSP genes. Similar to many other transcription factors, HSF has a conserved modular structure that contains several highly conserved domains[25]. Despite considerable diversification, almost all the HSFs have conserved structure elements of the DNA binding domain (DBD), the oligomerization domain[26]. Some HSFs also have the C- terminal activation domain (CTAD) and nuclear export signal (NES). A nuclear localization signal (NLS) is responsible for the permanent nuclear localization of HSF proteins[27]. The balance between NLS and NES determines the actual nucleocytoplasmic distribution of the protein, which is crucial for many signaling pathways involving transcription factors[28].

Out of 9 transcripts under control and stress condition for sixteen genotypes of rice, a significant level of up-regulation was observed among the following:

Rice genotype 159 showed a higher level of up-regulation for the genes Osind01g36950.1, Osind02g21410.1, Osind02g19620.1, Osind03g09500.1, Osind03g20380.1, Osind03g41450.2, Osind07g04910.1, Osind03g41450.1, Osind01g28640.1 by 2.79, 3.70, 7.83, 0.55, 0.70, 0.90, 0.60, 3.70, 1.77 fold, respectively.

Genotype RRF 127 showed a significant up-regulation for the genes Osind01g36950.1, Osind02g21410.1, Osind02g19620.1, Osind03g09500.1, Osind03g20380.1, Osind03g41450.2, Osind07g04910.1, Osind03g41450.1, Osind01g28640.1 by 0.85, 0.59, 0.01, 0.08, 2.36, 0.47, 0.49, 2.41, 0.49 fold, respectively.

Genotype Annada showed an up-regulation in the transcript level by 0.30, 0.17, 6.47, 2.18, 0.86, 4.22, 0.30, 0.05, 0.47 fold for the genes Osind01g36950.1, Osind02g21410.1, Osind02g19620.1, Osind03g09500.1, Osind03g20380.1, Osind03g41450.2, Osind07g04910.1, Osind03g41450.1, Osind01g28640.1, respectively.

Genotype GP-145-103 showed a greater level of up-regulation for the gene Osind01g36950.1, Osind02g21410.1, Osind02g19620.1, Osind03g09500.1, Osind03g20380.1, Osind03g41450.2, Osind07g04910.1, Osind03g41450.1 by 0.27, 0.24, 0.33, 0.21, 1.81, 0.17, 0.18, 0.16, fold, respectively.

Osind02g21410.1 is a member of A subfamily of unspecified group OsHsfA of HSFs. It has shown its high expression levels in the embryo sac, anther early and mature developing seeds, germinating embryos and leaf[29]. This gene in rice also reported to encodes a transcription factor that functions as a high temperature respective and responsive factor[30].

Osind02g19620.1 also belongs to subfamily A of group OsHsfA4. The over-expression of this gene was observed among all sample tissues while its over-expression was not significantly identified among multiple abiotic stresses.

Osind03g09500.1 is a member of subfamily A1 of HSFs. It is showing high level of expression in the leaf and palea/lemma than other tissues. This gene was shown to be up-regulated by early heat 1 and cold. The functional redundancy was observed in gene Osind03g09500.1 and gene Osind02g21410.1 in terms of development and abiotic stress response. It showed binding to 4P and 3P type HSEs but not with the G and S-type HSEs. These protein also play function as an activator in yeast cells and were found to be induced by both heat stress and oxidative stress of H202[31].

Osind03g41450.2 belongs to subfamily A and group OsHsfA2, commonly described as OsHsfA2e/OsHsf12, this subfamily commonly induced in response to heat stress. It is reported that this gene was shown constitutive predominant expression pattern throughout all the tissues it might play major roles in this subfamily and hence expected to be housekeeping gene. This was also reported to be up-regulated by drought stress as well as salt stress. OsHsfA2e in *Arabidopsis* caused enhanced thermotolerance phenotypes. This gene has shown functional association with a CAMK involved in Ca2+/CaM-dependent signalling pathway while the association with Hsp70 and Hsp90 played roles in thermotolerance response. In rice, the trans-activation activity has previously been reported for OsHsfA2e and its over-expression in *Arabidopsis* led to enhanced thermo and salt tolerance in transgenic plants.

Osind07g04910.1 placed under subfamily A and group OsHsfA2. This showed a higher expression level in the callus, shoot apical meristem, developing panicles and embryo during heat stress. This gene also appeared to be up-regulated by salt stress as well as oxidative stress (H_2O_2).

Osind01g28640.1 belongs to subfamily C but the expression pattern of this gene is very different from that of the other members in the family. It is reported to show expression in germinating seedling and might play role in developmental functions. It also showed trans-activation activity.

The biological functions of the rice HSF genes are still unclear. Functional redundancy appeared among them might be the reason for rare functional identification of the HSF genes.

Conclusion

Current study helps us to identify the key genes expressed in response to heat stress in the tested rice genotypes. Induction of rice transcripts Osind03g20380.1 suggests that these genes may impart heat stress avoidance capacity to the tolerant genotypes. Genes who were up-regulated suggests their function in positive regulation in adaptation of the heat stress, this can be taken as a base for heat tolerance response of the crop, which may be useful for further validation studies of the candidate genes for heat tolerance in the rice as well as other crop plants.The rice genotypes 159, RRF-127, GP-145-103 and Annada (popular rice variety) were identified as heat tolerant rice varieties based on the component traits analysis.

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References

1 Aghamolki M T K, Heat stress effects on yield parameters of selected rice cultivars at reproductive growth stages, *J Food Agric Environ*, 12 (2014) 741-746.
2 Jagadish S V K, Craufurd P Q & Wheeler T R, High temperature stress and spikelet fertility in rice (*Oryza sativa* L.), *J Exp Bot*, 58 (2007) 1627-1635.
3 Scharf K, Berberich T & Nover L, The plant heat stress transcription factor (HSF) family: Structure, function and evolution, *Biochim Biophys Acta*, 1819 (2012) 104-19.
4 Malenčić D, Popović M, Miladinović J, Stress tolerance parameters in different genotypes of rice, *Biol Plant*, 46 (2004) 141-143.
5 Prasad P V V, Boote K J, Allen Jr L H, Sheehy J E & Thomas J M G, Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress, *Field Crop Res*, 95 (2006) 398-411.
6 Ceccarelli S, Grando S, Maatougui M, Michael M, Slash M *et al*, Plant breeding and climate changes, *J Agr Sci*, 148 (2010) 627-637.
7 Dinisio-sese M L & Tobita S, Antioxidant responses of rice seedlings to salinity stress, *Elsevier*, 135 (1998) 1-9.
8 Heath R L & Packer L, Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation, *Arch Biochem Biophys*, 125 (1968) 189-198.
9 Wahid A, Gelani S, Ashraf M & Foolad M R, Heat tolerance in plants: An overview, *Environ Exp Bot*, 61 (2007) 99-223.
10 Sarsu F, Ghanim A M, Das P, Bahuguna R N, Kusolwa P M *et al*, Pre-field screening protocols for heat-tolerant mutants in rice, *Springer*, (2018).
11 Sharma R, Sharma R, Sudan J, Salgotra R K & Singh R, Alterations in cellular membrane stability due to heat stress in different genotypes of bread wheat, *Electron J Plant Breed*, 8 (2017) 1022-1027.
12 Hemantaranjan A, Bhanu A N, Singh M N, Yadav D K, Patel P K *et al*, Heat stress responses and thermotolerance, *Adv Plants Agric Res*, 1 (2014) 62-70.

13 Dhanda S & Munjal R, Inheritance of cellular thermotolerance in bread wheat, *Plant Breed*, 125 (2006) 557-564.

14 Pressman E, Peet M M & Pharr D M, The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers, *Ann Bot*, 90 (2002) 631-636.

15 Jagadish S V K, Murty M V R & Quick W P, Rice responses to rising temperatures-challenges, perspectives and future directions, *Plant Cell Environ*, 38 (2015) 1686-1698.

16 Kumar N, Kumar N, Shukla A, Shankhdhar S C & Shankhdhar D, Impact of terminal heat stress on pollen viability and yield attributes of rice (*Oryza sativa* L.), *Cereal Res. Commun*, 43 (2015) 616-626.

17 Pham D, Hoshikawa K, Fujita S, Fukumoto S, Hirai T *et al*, A tomato heat-tolerant mutant shows improved pollen fertility and fruit-setting under long-term ambient high temperature, *Environ Exp Bot*, 178 (2020) 104150.

18 Fu G, Feng B, Zhang C, Yang Y, Yang X *et al*, Heat stress is more damaging to superior spikelets than inferiors of rice (*Oryza sativa* L.) due to their different organ temperatures, *Frontiers in Plant Science*, 7 (2016) 1637.

19 Zhang C, Li G, Chen T, Feng B, Fu W *et al*, Heat stress induces spikelet sterility in rice at anthesis through inhibition of pollen tube elongation interfering with auxin homeostasis in pollinated pistils, *Rice*, 11 (2018) 1-12.

20 Jain M G, Mathur S K & Sarin N B, Ameliorative effects of proline on salt stress induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea L.*), *Plant cell Rep*, 204 (2001) 63-68.

21 Han Y, Fan S, Zhang Q & Wang Y, Effect of heat stress on the MDA, proline and soluble sugar content in leaf lettuce seedlings, *Agric Sci*, 4 (2013) 112-115.

22 Mansoor S & Naqvi F, Effect of heat stress on lipid peroxidation and antioxidant enzyme in mung bean (*Vigna radiata* L.) seedlings, *Academic Journals*, 12 (2013) 3196-3202.

23 Kazim A M, Abid A & Saddia G, Response of rice (*Oryza sativa* L.) under elevated temperature at early growth stage: Physiological markers, *Russ J Agric Soc Econ Sci*, 20 (2013) 11-19.

24 Zafar S A, Hameed A, Nawaz M A, Wei M A, Noor M A *et al*, Mechanisms and molecular approaches for heat tolerance in rice (*Oryza sativa* L.) under climate change scenario, *J Integr Agric*, 17 (2018) 726-738.

25 Doring P, Treuter E, Kistner C, Lyck R, Chen A *et al*, The role of AHA motifs in the activator function of tomato heat stress transcription factors HsfA1 and HsfA2, *Plant Cell*, 12 (2000) 265-78.

26 Clos J, Westwood J T, Becker P B, Wilson S, Lambert K *et al*, Molecular cloning and expression of a hexameric *Drosophila* heat shock factor subject to negative regulation, *Cell*, 63 (1990) 1085-97.

27 Nover L, Lyck R, Harmening U, Treuter E & Scharf K, Intracellular distribution and identification of the nuclear localization signals of two plant heat-stress transcription factors, *Planta*, 202 (1997) 117-125.

28 Heerklot H, Membrane stress and permeabilization induced by asymmetric incorporation of compounds, *Biophys J*, 81 (2001) 184-195.

29 Jin G, Gho H & Jung K, A systematic view of rice heat shock transcription factor family using phylogenomic analysis, *J Plant Physiol*, 170 (2013) 321-329.

30 Liu G T, Chai F, Wang Y, Jiang J, Duan W *et al*, Genome-wide identification and classification of HSF family in grape, and their transcriptional analysis under heat acclimation and heat stress, *Hortic Plant J*, 4 (2018) 133-143.

31 Singh A, Mittal D, Lavania D, Agarwal M, Mishra R *et al*, OsHsfA2c and OsHsfB4b are involved in the transcriptional regulation of cytoplasmic OsClpB (Hsp100) gene in rice (*Oryza sativa* L.), *Cell Stress Chaperones*, 17 (2011) 243-254.

32 Guo M, Lu P J, Zhai F Y , Chai G W, Gong H Z *et al*, Genome-wide analysis, expression profile of heat shock factor gene family (CaHsfs) and characterisation of CaHsfA2 in pepper (*Capsicum annuum* L.), *BMC Plant Biol*, 15 (2015) 1-20.

33 Giorno F, Guerriero G, Baric S & Mariani C, Heat shock transcriptional factors in *Malus domestica*: Identification, classification and expression analysis, *BMC Genomics*, 13 (2012) 1-13.

34 Li H, Huang W, Liu W Z, Wang X Y & Zhuang J, Transcriptome-based analysis of dof family transcription factors and their responses to abiotic stress in tea plant (*Camellia sinensis*), *Int J Genomics*, 2016 (2016) 1-15.

35 Wang J, Sun N, Deng T, Zhang L & Zuo K, Genome-wide cloning, identification, classification and functional analysis of cotton heat shock transcription factors in cotton (*Gossypium hirsutum*), *BMC Genomics*, 15 (2014) 1-19.

36 Huang X Y, Tao P, Li B Y, Wang W H, Yue Z C *et al*, Genome-wide identification, classification, and analysis of heat shock transcription factor family in Chinese cabbage (*Brassica rapa pekinensis*), *Genet Mol Biol*, 14 (2015) 2189-2204.

37 Hu Y, Han Y T, Wei W, Li Y J, Zhang K *et al*, Identification, isolation and expression analysis of heat shock transcription factors in the diploid woodland strawberry *Fragaria vesca*, *Plant Sci*, 6 (2015) 1-16.

38 Zhang J, Li Y, Jia H X, Li J B, Huang J *et al*, The heat shock factor gene family in *Salix suchowensis*: A genome-wide survey and expression profiling during development and abiotic stresses, *Front Plant Sci*, 6 (2015) 1-14.

39 Qiao X, Li M, Li L, Yin H, Wu J *et al*, Genome-wide identification and comparative analysis of the heat shock transcription factor family in Chinese white pear (*Pyrus bretschneideri*) and five other Rosaceae species, *BMC Plant Biology*, 15 (2015) 1-15

40 Xue G P, Drenth J & McIntyre L, TaHsfA6f is a transcriptional activator that regulates a suite of heat stress protection genes in wheat (*Triticum aestivum* L.) including previously unknown Hsf targets, *J Exp Bot*, 66 (2015) 1025-1039.