Exploring Response of Rice towards Heat Stress from Germination to Maturity

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Abstract
Among the abiotic stresses, heat stress is continuously posing great challenges before the scientific community around the world by adversely affecting the growth and development of the crop plants. Productivity of rice, one of the world's most essential cereals, is threatened by high temperature stress deepened by climate change. It is imperative to develop heat stress tolerant verities in rice to counter the high temperature stress and to maintain and enhancement in the productivity of rice to feed the expected 9 billion populations by 2050. At early growth and developmental stages of plants thescreening based on physiological parameters plays a pivotal role in identifying heat tolerant varieties. With the advent of new molecular biology techniques, the crop improvement program for maximum yield enhancement to meet the challenges of food security around the world, despite numerous environmental barriers, has been speeded up.This work is focused on early seedling growth and development in rice under temperature stress. Screening at early seedling stage for heat tolerance is critical for crop establishment specifically under direct seeded condition.

I.   Introduction:

Rice (*Oryza sativa* L.) is one of the staple food crops for approximately half of the world population. India is the second largest producer of rice and also a key cereal of half of the Indians specially the South Indian people .Keeping in view of growing human population, decreasing arable land and environmental pressure, rice production must increase by 0.6-0.9% annually to meet with the food demand. Among all abiotic stresses, extreme temperature severely affects the quality and yield of rice. Furthermore, predictions of future climate change especially higher probability of an increase in mean temperature of the atmosphere during rice growing seasons will certainly intensified this issue many folds. The fluctuations in temperature during day time inrice producing areas are optimal. Though ,rice at 27^0c to 32^0c, can maintain normal growth and development without substantial reduction in yield(Agha molki et.al.2014)while 32^0c or more adversely affect at different stages of growth and development of rice. The most critical temperature was found to be 33^0c at flowering stage was found to be critical. High temperature is injurious to most physiological process including stomatal conductance, photosynthesis, growth and grain yield. High temperature due to global warming leads to sterility and instability in rice yield even in temperate regions. To negate the threat of global warming and subsequent high temperature stress on rice production, the creation of genetically thermo-tolerance rice varieties is urgently required. In rice the genetic basis of thermo tolerance is not well established.

To investigate the genetic flexibility to heat stress ,investigation of heat acclimation can be explored whichshows a positive correlation to thermo tolerance. Therefore, in this study eight local rice varieties were evaluated to explore their thermotolerance on the basis of growth, yield, physiological and biochemical responses under high temperature stress. Specifically, expression and accumulation of 70kDa heat shock protein (HSP70) and antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) were investigated during stress and recovery treatments.

II.   Material and Methods:

This research study is designed to explore response of Rice (*Oryza sativa* L.) towards heat stress (42±2°C) at seedling, flowering and grain growth stages. This research was conducted consecutively for three years i.e., mid May to November 2016, 2017 and 2018. During the first year, response of eight rice cultivars were determined in terms of growth characteristics, cell membrane thermo-stability (CMT), peroxidation of lipid (MDA), oxidative stress (H_2O_2), osmolyte accumulation (Proline), protein quantification at seedling stage. During second year in addition of previous work, proliferation of total soluble protein through electrophoresis (SDS-PAGE), identification of molecular chaperon (HSP70) via western blotting and quantitative and qualitative (Native-PAGE) analysis of antioxidant, catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POD), enzymes ascertained at 20 days old seedlings. At later, growth, flowering and grain filling, growth characteristics i.e. plant height (PH), shoot fresh weight (SFW), shoot dry

weight (SDW), number of tillers per plant (TPP), panicle length (PL), number of grains per panicle (GPP), along with measurement of electrolyte leakage (RMP), hydrogen peroxide (H_2O_2), lipid peroxidation (MDA), accumulation of osmo protectents (Proline), extraction and quantification of total soluble protein, extraction and quantification of reactive oxygen scavengers were carried out. During the last year in view of the past results and findings, changes in the expression and accumulation of heat induced chaperonin (HSP70)via immune blotting and substrate specific gel based activity of antioxidant enzymes (isoforms) was studied at flowering and grain filling growth stages of eight rice cultivars. All the experiments were carried out in the controlled growth chambers, and in the labs in Hyderabad India.

Selection of Rice Cultivars
Rice cultivars used in this investigation kindly provided by Rice Research Institute,??? (Table 1). Some of the selected rice cultivars although are cultivatedin other areas of India but are dominantly growing in Telangana.

Table 1: Name and some characteristics of rice cultivars used in this study

S. No.	Genotype	100 grains weight (g)	Seed Size
1	IR-6	1.40	Small
2	IR-8	1.45	Large
3	DR-82	1.08	Medium
4	DR-83	0.97	Small
5	DR-92	1.20	Small
6	Kawal-95	1.12	Large
7	Sona Masoori	1.23	Medium
8	Shahkar	1.18	Medium

Growth Conditions:
Temperature preview of rice field
Rice were grown in the month of May and harvested during October and November for three years, 2016-2018. Three years data of mean temperature of seven months i.e., May, June, July, August, September, October and November of Pudur Manneguda Vikharabad are presented in Fig. 1.
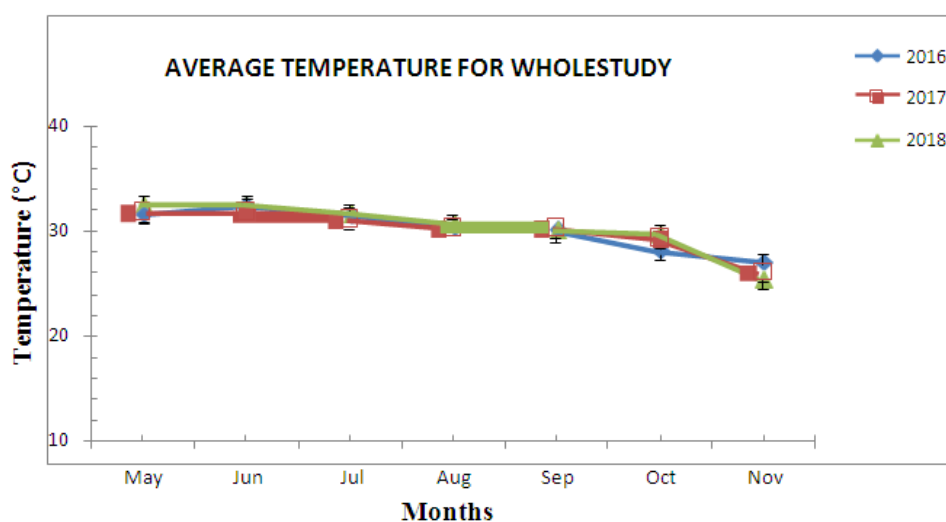

Figure 1: Three year's, 2016-2018, mean temperature of Pudur Manneguda Vikharabad from the month of May-Nov.

Water and soil used in the study:
Soil and water used in this experiment were assessed according to the standard protocol, to minimize any other possible effects on rice growth and development. Brief methodology of water and soil analysis is given below.

Water analysis
Fresh water used to irrigate rice field from seedling to maturity. Water provided with enough requirements for rice field. During each year, water and soil properties were assessed for its purity as shown in Table 2.

Soil   properties
Some biochemical studies of soil are presented in the Table 2. The textural type of soil was analyzed with the reference of International Textural Triangle (Moodie et al., 1959). For mechanical analysis, the percentage of sand clay and silt were determined using hygrometer, with 1% sodium hexametaphosphate as the dispersing chemical. Chemical analysis was carried out using method of US Salinity Laboratory Staff (Richards, 1954). Saturated soil past extract were used to determine pH using pH meter while electrical conductivity of the soil determined with the help of EC meter. Water soluble potassium (K^+), calcium (Ca^{++}) and sodium (Na^+) in the extract were assessed with flame photometer and nitrogen was estimated according to Bremner (1965). Soil available phophate were quantified using method of Watenbe and Olsen (1954). Total soluble carbonate and bicarbonate in the soil were determined via titration with sulfuric acid as standard having phenolphthalein and methyl orange as indicator and chloride was calculated using Cl^- analyzer. Ratio of sodium adsorption was calculated using the formula:

$$SAR = N^+ / [\sqrt{(Ca^{2+} + Mg^{2+}/2)}]$$

Table 2: Some physio-chemical characteristics of soil and water used in the experiment

Physiochemical properties	2016	2017	2018
Water properties			
Electrical Conductivity (dS/m)	0.80	0.81	0.83
pH	7.07	7.12	7.00
Sodium adsorption ratio	3.64	3.68	3.66
RSC	1.89	1.81	1.94
Soil Characteristics			
Saturation percentage	25	26	24
Nitrogen percentage	0.58	0.55	0.57
Moister percentage	19	17	18
Available P (mg/ml)	5.9	6.4	6.1
K^+ mg/Kg	148	142	151
Ca^{2+} mg/Kg	10.5	12.04	11.4
Soil texture	Sandy loam	Sandy loam	Sandy loam
Sand (%)	21	20	19
Silt (%)	14	12	16
Clay (%)	64	65	63
pH	8.3	8.2	8.1
EC (dS/m)	2.9	2.7	2.4

Screening for germination potential under heat stress
Seeds were first washed with tap and distilled water for 3-5 times and then dipped in 70% ethanol for 20 seconds. 10% Sodium Hypochlorite (commercial bleach) was used for sterilization for 30 minutes. Seeds were rinsed five times with autoclaved distilled water and then primed in water for overnight. Sterilized seeds were divided into two groups. One group was allowed to germinate in petri-dishes of 25 mm diameter with filter

paper under normal temperature (28±2°C) considered as control (C) and the other group were treated with high temperature (42±2°C) stress for 24 h (T24), 48 h (T48) and 72 h (T72) and then allowed to germinate in same type of petri-dishes of 25 mm layered with filter paper. Heat treated seeds after 24, 48 and 72 h of heat stress transferred to normal (28±2°C) temperature and allowed to germinate and seedling establishment. Germination was considered when the radical emerged through seed coat. Data were documented for two weeks from the day of beginning. Number of germinated seeds counted after every two days till 10 days and then promptness index (P.I), germination stress index (GSI) were calculated for determination of germination potential using following formula according to Bouslama and Schapaugh(1984).

P.I.= nd_2 (1.0) + nd_4 (0.8) + nd_6 (0.6) + nd_8 (0.4) + nd_{10}(0.2)

Where "n" is number of seeds germinated at day d. In which nd2, nd4, nd6, nd8, and nd10 represent the germinated seeds after 2, 4, 6, 8 and 10 days, respectively.

G.S.I(%) =(P.I of stressed seeds/P.I. of Control seeds) x100.

For seedling growth, rice seeds are soaked overnight in water. Seeds sowed in line at proper distance and allowed to germinate and establish seedling in plastic trays lined with sandy loam soil. For nursery, seedlings were grown in natural sunlight as well as in controlled growth chambers with daily mean temperature 28±2 °C and humidity 40-45%. 20 days old seedlings used to subject heat stress treatments.

Flowering and maturity Stage:

Seedlings after 20 days transplanted in pots filled with 60-70% sandy loam soil. Each pot had approximately 10 seedlings and later thinned to 3-5 plants per pot. After 10-15 days of transplantation, fertilizer DAP nearly 3-5 g/ pot, were used. Plants were also nourished with full and half strength Hoagland nutrient solution (Hoagland and Arnon, 1950) after every second week up to maturity. Plants were watered every day at two times, early in the morning and evening and maintained in standing condition approximately 2-4 mm. Flowering started nearly after 3 month while grain filling took 4 -5 month from the date of sowing. Average time for grain filling were 20 days after flowering starts. Cultivars showed differences in terms of flowering onset. DR-82, DR-83 showed early, IR-6, IR-8, DR-92, Sona Masoori showed mid while K-95 showed late flowering. Similar pattern also observed at grain filling stage.

Stress treatment

Heat stress was applied at seedling stage in controlled growth chambers while at later growth stages heat shock room was used. Plants maintained at 28±2 °C and plants collected 24 hours before heat stress and it was considered as control (C). Then plants exposed to heat stress with gradually increasing temperature and maintained at 42 °C. Temperature was raised with the help of electric heaters installed as well as white lights. Light were supplemented with white fluorescent tube lights and mercury lamps fixed to the walls on four sides. In the stress room at leaf surface area of rice plants photosynthetically active radiation (PAR) were maintained in the range of 700-900μmole m^{-2} s^{-}. The minimum requirement of PAR for a C3 plant is 500-600 μmole $m^{-2}s^{-1}$. Heat stress was given at 42±2 °C during day for 16 h while at 38±2 °C during night. Humidity was maintained in the range 55-60% with the help of water vapors, allowed to evaporate in water tub. Light intensity, humidity and temperature were recorded carefully during day and night on daily basis. For data recording and watering during stress protective measures were taken. Rice plants were collected after 24, 48 and 72 h of heat stress and named the treatments as T24, T48 and T72, respectively. After 72 h of heat stress, stressed plants were again transferred to field for recovery treatments and again collected plants after 24, 48 and 72 h of recovery and named as R24, R48 and R72, respectively. Plant height, fresh weight, electrolyte leakage and concentration of hydrogen peroxide (H_2O_2) were determined on the day of treatment using fresh leaves while remaining leaf tissue stored at -80 °C for biochemical and proteomic analysis.

III.   Results and Discussions

Comparative response for thermo-tolerance in eight rice cultivars, IR-6, IR-8, DR-82, DR- 83, DR-92, K-95, Sona Masoori, and Shahkar was studied at seedling, flowering and grain filling stages of rice growth. Determinations were made in terms of changes in growth and yield characteristics along with cell membrane thermo-stability, H_2O_2 concentration, lipid peroxidation content (MDA), osmolyte accumulation (Proline), expression of molecular chaperon (HSP70) and antioxidant enzymes (SOD, CAT, APX and POD), under control (28±2 °C), heat stress (42±2 °C/38±2 °C, day time / night time) for 24h (T24), 48h (T48), 72h (T72) and during recovery (28±2 °C) for 24h (R24), 48h (R48), 72h (R72), at above mentioned rice growth stages. At germination stage, promptness index (P.I.) and germination stress index (G.S.I.) were evaluated. Findings of the above study are asunder:

Germination Stage Analysis

At germination stage, screening of eight rice cultivars for thermotolerance was carried out on the basis of speediness to germination and germination percentage, under high temperaturestress.Promptness index (P.I.) is the ratio of speediness to germination between non-stressed and heat stressed seeds. These stressed and non-stressed seeds then allowed to germinationinlaboratoryconditionfor10days.Afterevery2ndday,numberof germinated seeds counted and determined promptness index (P.I.), according to the given formula. Under control condition, rice seeds showed more rapid germination and within less time as compared to heat shocked seeds, which showed late germination. Statistical analysis showed significant ($p < 0.05$) differences among cultivars (C), treatments (T) and their interaction (CxT) for this attribute (Fig. 2).Under control condition, cultivar "IR-8" had maximum P.I. followed by "DR-82" while "DR-83" showed minimum P.I. as compared to others. Heat stress treatment for 24h (T24), showed highest P.I. in "IR-8" and "DR-82" while after 48h (T48), "K-95" and "SonaMasoori" had maximum P.I. among the group. At T72, cultivar "K-95" exhibited maximum P.I. as compared to others while lowest P.I. showed by cultivar "DR-82" and "Shahkar".
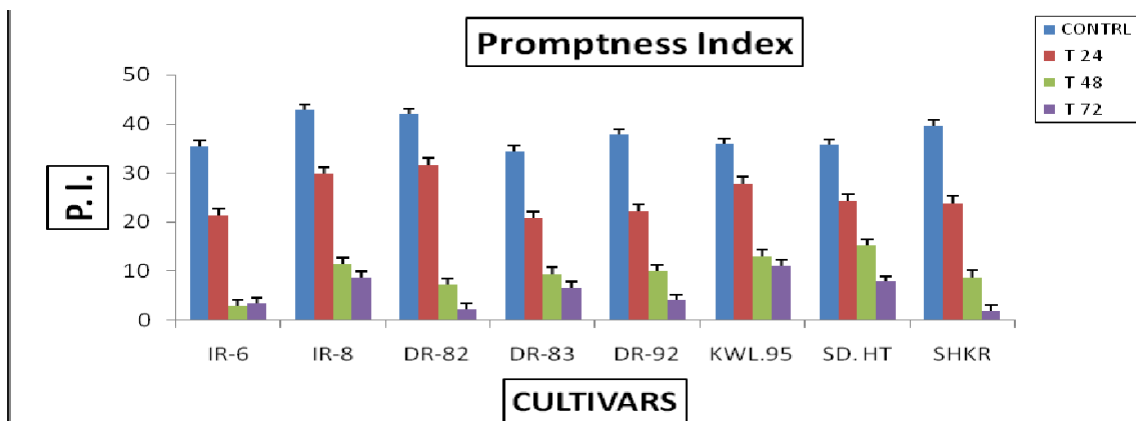

Figure 2: Effect of heat stress (42 ±2 °C) on promptness index (P.I.) of eight rice cultivars during germination.

Germination Stress Index (G.S.I.)

Number of germinated seeds from total seeds were also compared between heat stressed and non-stressed seeds for germination stress index (G.S.I.). The results showed that cultivar (C), treatments (T) and interaction (C x T) had significantly ($p < 0.05$) differencesfor G.S.I. Temperature treatments exhibited that only "Sona Masoori" and "K-95" had highest germination percentage, at T24 and T72 respectively. Varietal differences were observed for this parameter at different temperature regimes and lowest germination was exhibited by "DR-83" and "DR-92" at T24, "IR-6" and "DR-82" at T48 while Shahkar" and "DR-82" at T72 (Fig. 3).
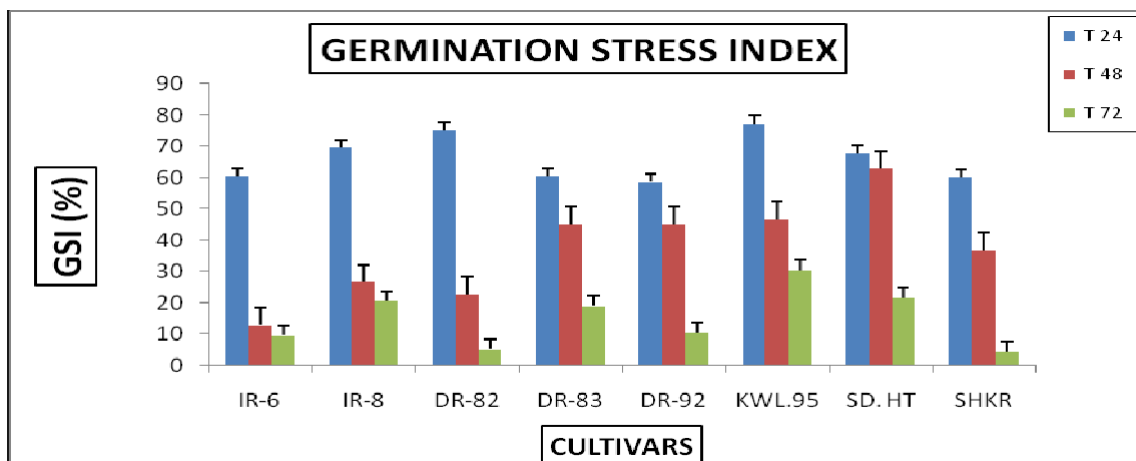

Figure 3: Effect of heat stress (42 ±2 °C) on Germination Stress Index (G.S.I.) of eight rice cultivars at germination stage**.**

Success has been achieved to overcome current challenges faced with high temperature induced yield losses but examining the complex issues surrounding grain quality losses is still not well established. Keeping in view of the future global warming, the adoption of new water saving technologies and the rice varieties with

increased tolerance of high temperature coupled with drought stresstolerance at floral meristem stage will be crucial to explain the progress attained in overcoming the heat stress induced damage at sensitive developmental stages such as flowering. The major factor driving climate change is increased CO_2 and it is projected to continue to increase gradually, but research indicates a lack of amelioration of heat stress induced yield loss under elevated CO_2 conditions. However, progress achieved through breeding efforts to safeguard sensitive reproductive processes from heat stress would allow better use of the additional biomass accumulated from gradually increasing CO_2. Indeed, this is an intriguing hypothesis that could partially address the persisting challenge to increase rice productivity in spite of a projected war.

The ability of the plant to cope with or adjust to the heat stress varies with the species and developmental stages, in particular anthesis and grain filling are more susceptible .Apart from them, pollen viability, embryo development, patterns of assimilate partitioning, growth and development of seed/grain,structural changes in tissues and cell organelles, disorganization of cell membranes, disturbance of leaf water relations, impedence of photosynthesis and lipidPeroxidation ismajor concerns in this elevated temperature

Plants manifest numerous adaptive changes. Signal transduction mechanism need to befocusedfrom heat stress point of view where Ca+2 role is very important. The heat shock proteins and molecular chaperons which ensured three dimensional structure of membrane proteins helps in understanding the sustained cellular functions and survival strategies under heat stress, a clear cut idea on proteomics is tne need of the hour,applications of exogenous osmoprotectants play a major role in heat tolerance. Execution of traditional breeding practice should take place keeping phenotypic flexibility in mind

Identification of temperature signal perceiving regions, Understanding the signal transduction pathway cross talk, Temperature stress regulators, Understanding post transcriptional and translational changes, Identification of genes with specific function, Understanding the nature of cross protection and Identifying targets of injury.

Furthermore, applications of genomics, proteomics and transcriptomics approaches to a better understanding of the molecular basis of plant response to heat stress as well as rice plant heat tolerance are of essence.

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