www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(8): 985-989

www.thepharmajournal.com
Received: 27-06-2022
Accepted: 30-07-2022

Nawale Archana Bhausaheb
Department of Plant Biotechnology, Center for Plant Molecular Biology and Biotechnology, Tamil Nadu, India

Soundarrajan Vadivel
Department of Plant Biotechnology, Center for Plant Molecular Biology and Biotechnology, Tamil Nadu, India

Geethalakshmi Vellingiri
Agro-Climate Research Centre, Directorate of Crop Management, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Gnanam Ramasamy
Department of Plant Molecular Biology and Bioinformatics, Center for Plant Molecular Biology and Biotechnology, Tamil Nadu, India

Manikanda Boopathi Narayanan
Department of Plant Biotechnology, Center for Plant Molecular Biology and Biotechnology, Tamil Nadu, India

Saranya Nallusamy
Department of Plant Molecular Biology and Bioinformatics, Center for Plant Molecular Biology and Biotechnology, Tamil Nadu, India

Thangaraj Kandasamy
Agricultural Research Station, Paramakudi, Tamil Nadu, India

Yogameenakshi Parameswaran
Krishi Vigyan Kendra, Tirur, Kerala, India

Meenakshisundaram Palaniappan
Department of Plant Biotechnology, Center for Plant Molecular Biology and Biotechnology, Tamil Nadu, India

Corresponding Author
Meenakshisundaram Palaniappan
Department of Plant Biotechnology, Center for Plant Molecular Biology and Biotechnology, Tamil Nadu, India

Diurnal fluctuation as well as effect of heat stress on catalase activity in select rice genotypes

Nawale Archana Bhausaheb, Soundarrajan Vadivel, Geethalakshmi Vellingiri, Gnanam Ramasamy, Manikanda Boopathi Narayanan, Saranya Nallusamy, Thangaraj Kandasamy, Yogameenakshi Parameswaran and Meenakshisundaram Palaniappan

Abstract
In the present study, seven different rice varieties, *viz.,* N22, CO51, TKM13, ASD16, Kuliyadichan, Nootripathu and Sivappu Chithiraikar were studied for assessing diurnal fluctuations (under controlled conditions) as well as the effects of heat stress on catalase activity. Comparisons of mean catalase activity in control samples during different time periods *viz.,* morning (6 a.m.), afternoon (1 p.m.) and evening (6 p.m.) revealed the existence of diurnal fluctuations. Catalase activity was found to increase from morning through afternoon and then dropped back to levels observed in the morning, but at different rates. This reveals that rice varieties exhibit diurnal fluctuations at varying rates. Based on the pairwise comparisons of mean catalase activity under different time and temperature conditions, it was found that catalase activity was affected by exposure to heat stress and rice varieties were having differential response. All varieties, but for N22, had elevated catalase activities upon heat stress showing that they are highly susceptible to ROS damage. Among the six varieties, CO51 exhibited significant differences in catalase activity. Based on the present investigation, molecular evidence is presented to support that N22 is a globally accepted heat tolerant variety and CO51 could be declared as heat susceptible rice variety. All other varieties have different degrees of heat tolerance and heat tolerance mechanisms are complex.

Keywords: Rice, catalase, diurnal fluctuations, heat stress effects

Introduction
Global warming is a major challenge faced now-a-days by the farming community across the globe. Average global temperature on earth has risen by about 0.8 °C since 1880, according to recent research by scientific communities including NASA's GISS. Two-thirds of the warming has occurred since 1975, at a rate of about 0.15 to 0.20 ℃ per decade (Lorenz *et al.,* 2019) [3]. Every 1 ℃ rise in the global mean temperature is predicted to reduce global yields of wheat by 6 *per cent*, rice by 3.2 *per cent*, maize and soybean by 3.1 *per cent* (Zhao *et al.,* 2017) [11]. Rice is the staple food crop for half of the world's population. It is predicted that, by 2030, 16 *per cent* of rice harvesting regions will be exposed to at least two days of temperature above the critical point which shall rise to 27 *per cent* by 2050 (Gourdji *et al.,* 2013) [1]. Heat stress has detrimental impacts on crop physiology which includes photosynthesis, respiration, plant growth and development (Prasad *et al.,* 2017) [6]. High temperature deteriorates metabolic processes and promotes membrane damage, which impacts agronomic qualities (Mohammed and Tarpley 2009) [5]. When stress occurs, the stability of the plasma membrane is affected which plays a key role in heat tolerance in plants (Rehman *et al.,* 2016) [7]. Heat stress triggers an excessive production of reactive oxygen species (ROS), which can harm cells by causing programmed cell death (Xu *et al.,* 2006) [8]. Plants produce anti-oxidant enzymes to counteract the negative effects of increased ROS levels. One of the most commonly found enzymes is catalase. It is pivotal in preventing cells from oxidative damage by ROS. In plants, catalase eliminates H_2O_2 produced during photorespiration under both normal and stressful conditions, as well as during mitochondrial electron transport and fatty acid oxidation. In this present study, we evaluated the diurnal fluctuations of catalase production as well as the effects of heat stress on catalase activity at different time intervals in various rice genotypes.

Materials and Methods

Plant Material and Growth Conditions

Rice seeds of seven (7) varieties *viz.*, N22, CO51, TKM13, ASD16, Kuliyadichan, Nootripathu and Sivappu Chithiraikar were obtained from Plant Breeding Station, TNAU, Coimbatore; Agricultural Research Station, Paramakudi as well as from Krishi Vigyan Kendra, Tirur. The seeds were sown on moistened germination paper placed in a petri dish and incubated at room temperature in the dark until germination. After seven days, the germinated seeds were transferred to pots, filled with clay soil and nursed in a greenhouse, at the Department of Plant Biotechnology, CPMB & B, TNAU, Coimbatore. Twenty-eight (28) days after sowing (DAS), the plants were transferred to two environmental growth chambers (M/s. Genesis Technologies, Thane, Maharashtra, India) which were used for assessing the diurnal fluctuation of catalase production as well as the effect of heat stress on catalase activity. For the purpose of experimentation, plants (for all varieties) were maintained as two biological replicates for each treatment condition *viz.*, control / unstressed (25 °C) and treatment / heat stressed (37 °C). Hoagland's solution was used to water the plants once a week. In the growth chambers, the following environmental parameters were maintained: 35 Klux of photosynthetically active radiation (PAR) at canopy level, 8 hours day / 16 hours night cycle and 25 °C / 20 °C day / night temperatures.

Heat Stress Experiment

Heat stress experiment was carried out in plant growth chambers on the plants whose age was 30 DAS. The first growth chamber served as a control chamber (where temperature was maintained at 25 °C throughout the experimental period) and the second growth chamber served as heat stress chamber (where temperature was raised to 37 °C and brought back to 25 °C during the experimental period) [Note: On the day of experimentation, fluctuations in voltage was observed between 4 p.m. and 7 p.m. which will be evident in Figure 1]. In the heat stress chamber, temperature was increased by 2 °C every hour from 6 a.m. to 12 p.m. The highest temperature was maintained at 37 °C between 12 p.m. to 1 p.m. After 1 p.m. temperature was reduced at the rate of 2 °C per hour to reach back to 25 °C at 6 p.m. The temperatures were recorded using the data logger Hobo Pro v2 U23-001 (M/s. Onset, Bourne, MA 02532, USA). The temperature profile of the chambers used as control and heat stress chambers is given in Figure 1.

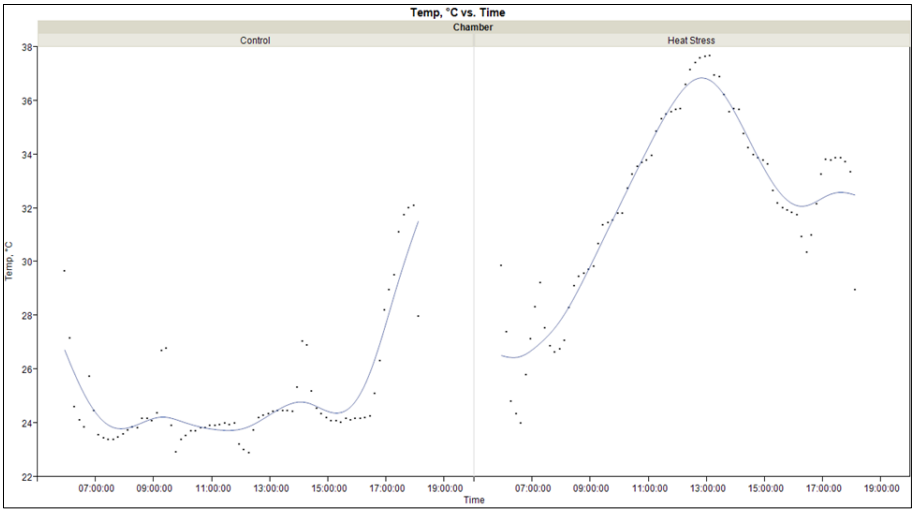

Fig 1: Temperature profile of growth chambers used in the experiment

Collection of Leaf samples and Assaying Catalase Activity

Leaf samples were collected thrice on the day of experimentation from both control and heat stressed plant samples, for carrying out the catalase enzymatic assay. Leaf samples were collected at 6 a.m. (before initiating temperature increase in the heat stress chamber); 1 p.m. (after subjecting plants in the heat stress chamber to 37 °C for 1 hr) and at 6 p.m., (after relieving the plants from heat stress and bringing them back to the control condition of 25 °C), to ascertain the temporal variation in catalase activity as well as the effect of heat stress on catalase activity. Always, the second leaf from the top was collected for enzyme assay. During sample collection from the second leaf, first ⅓ portion of the leaf was collected at 6 a.m., second ⅓ portion of the leaf was collected at 1 p.m. and the last ⅓ portion was collected at 6 p.m., to observe the temporal effects as well as the effect of heat stress on catalase activity from the same leaf.

Immediately after detachment from the plants, the leaf samples were kept on ice until it was transferred to -80 °C deep freezer. Catalase assay was performed on the leaf samples (two biological and two technical replicates) using the method developed by H. Luck (1974). In brief, the samples were ground with a phosphate buffer, centrifuged at 12000 r.p.m. for 20 min at 4 °C. After centrifugation, supernatant was collected and used for enzyme assay. Before adding the enzyme extract, the OD value of the substrate, *i.e.*, phosphate buffered hydrogen peroxide (H_2O_2) solution was adjusted to an O.D of 0.5 at a wavelength of 240 nm. This was followed by the addition of 0.04 ml of enzyme extract and then the time taken for the OD value of phosphate buffered H_2O_2 to decrease from 0.450 to 0.40 was noted. This change in time (in seconds) was used for calculating the catalytic activity (reported in units / ml). The results of catalase activity are expressed as "Units / ml". One Unit of enzyme activity is the amount of enzyme that can liberate half the peroxide oxygen from a hydrogen peroxide solution of any concentration in 100 sec at 25 °C"

Statistical Analysis

Mean catalase activity in seven varieties of rice was calculated for control (25 °C) and treatment (37 °C) using two biological and two technical replicates collected at 6 a.m, 1

p.m. and 6 p.m. Then, the statistical analysis was done to ascertain the diurnal fluctuations as well as effects of heat stress on each rice variety. Firstly, for assessing the diurnal fluctuations, mean catalase activity was compared in control samples (25 ℃) as follows: (1) Mean catalase activity (6 a.m. Vs 1 p.m.) (2) Mean catalase activity (1 p.m. Vs. 6 p. m.) and (3) Mean catalase activity (6 a.m. Vs. 6 p. m.). Secondly, for assessing the effects of heat stress, mean catalase activity between control (25 ℃) and heat stressed samples (37 ℃) were compared as follows: (1) Mean catalase activity (25 ℃ Vs. 37 ℃) at 1 p. m. (2) Mean catalase activity (37 ℃ Vs. 37 ℃) at 1 p.m. and 6 p.m. In order to compare the means of catalase activity under different conditions, an independent t-test assuming equal variance was conducted. The test was carried out using the software SAS JMP Statistical Discovery V 10.0.

Results and Discussion

Assessing Diurnal Fluctuations in Catalase Activity

For assessing the diurnal fluctuations of catalase production (based on catalase activity) in control samples (grown at 25 ℃), all pairwise comparisons of 'mean catalase activity' estimated at 6 a.m., 1 p.m., and 6 p.m. were made. The results of comparison of enzyme activity are given in Table 1 and Figure 2. An inspection of Figure 2 gives us an idea of the diurnal fluctuations in mean catalase activity (catalase production) in different rice varieties (under study).

Table 1: Fluctuation in Catalase Activity in Samples Not Subjected to Heat Stress

Variety	Mean catalase activity (Units / ml) at 25° C			t ratio			Probability (Alpha level = 0.05)		
	6:00 AM	1:00 PM	6:00 PM	6 AM / 1 PM	1 PM / 6 PM	6 AM / 6 PM	6 AM / 1 PM	1 PM / 6 PM	6 AM / 6 PM
N22	70.8300	106.2500	63.7500	1.7320	-2.0370	-1.7320	0.1340	0.0800	0.1340
CO51	43.8500	51.9400	70.8300	2.4150	6.9220	13.8600	0.0522	0.0004*	<0.0001*
TKM13	60.2100	47.2200	54.3070	-3.6690	3.0000	-1.3860	0.0105*	0.024*	0.2148
ASD16	82.6350	94.4400	63.7500	1.7320	-7.5080	-2.3760	0.1340	0.0003*	0.0550
Kuliyadichan	47.2200	51.9450	47.2200	1.7320	-1.7320	-	0.1340	0.1340	-
Nootripathu	56.6700	56.6700	51.9450	-	-1.7320	-1.7320	-	0.1340	0.1340
Sivappu Chitiraikar	37.9500	43.8500	43.8500	2.4250	0.0000	2.4250	0.0515	1.0000	0.0515

Based on Table 1, is observed that the varieties N22 and ASD16 exhibit an increased mean catalytic activity during the mid-noon period (but statistically not significant) whereas, in the variety TKM13, there is a statistically significant drop-in mean catalase activity during the mid-noon period. Other varieties exhibited no significant change in terms of mean catalase activity. Also, it is observed that the varieties CO51, TKM13 and ASD16 exhibit statistically significant enhancement of mean catalytic activity (in the mid-noon period) even when maintained at 25 ℃. This could be a potential sign indicating that these varieties are highly susceptible to Reactive Oxygen Species (ROS) damage under normal conditions. On the other hand, the varieties, N22, Kuliadichan, Nootripathu and Sivappu Chithiraikar did not exhibit significant change in mean catalytic activity suggesting that damage from ROS is less in these varieties under normal growth conditions. Further, it is observed that during the day time, only CO51 exhibits statistically significant change in mean catalase activity even when maintained at 25 ℃. On the other hand, all other rice varieties exhibited no significant change in mean catalase activity when compared between 6 a.m. and 6 p.m. This suggests that the rice varieties exhibit differential response to catalase production and hence their susceptibility to ROS damage. Among the rice varieties, N22 is less susceptible to ROS damage which supports the fact that it is a globally accepted heat tolerant variety. On the other hand, CO51 could be declared as a heat susceptible variety.

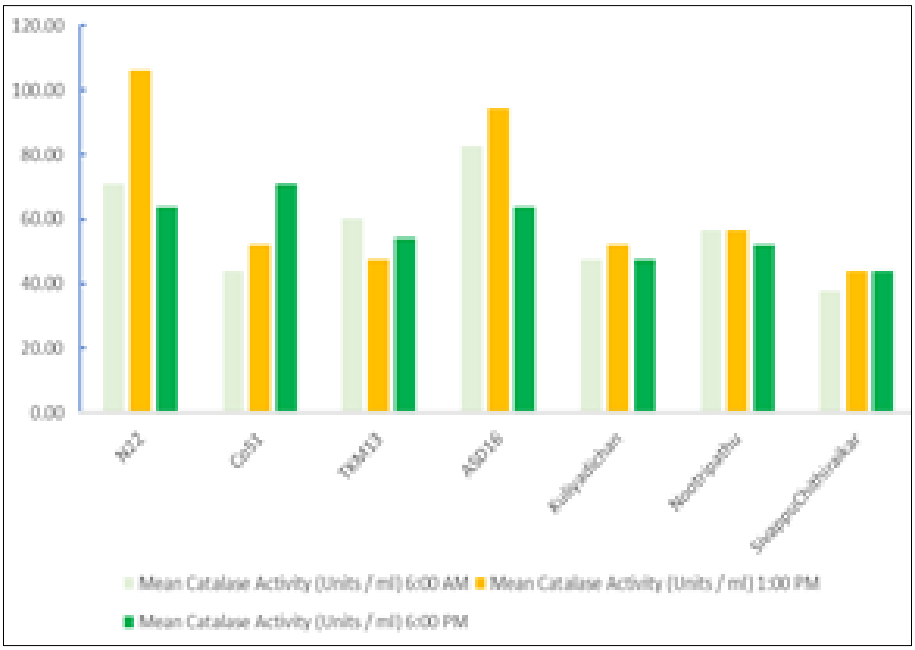

Fig 2: Diurnal Fluctuations in Catalase Production

Assessed by Catalase Activity

Assessing Effect of Heat Stress on Catalase Activity

For assessing the effect of heat stress on catalase activity, pairwise comparisons of 'mean catalase activity' among control and heat-treated samples were taken up (for all rice varieties under study). Mean catalase activity of varietal samples collected at 1 p.m. and 6 p.m. were used for the analysis. The results of comparisons of mean catalase activity (25 ℃ Vs. 37 ℃) at 1 p.m. and 6 p.m., are given in Table 2.

Table 2: Effect of Heat Stress Treatment on Catalase Activity

Variety	Mean catalase activity (Units / ml) at 25 °C and 37 °C at 1 PM		t ratio	Probability (Alpha level = 0.05)
	25 °C	37 °C	25 °C / 37 °C	25 °C / 37 °C
N22	106.2500	212.5000	2.3238	0.0591
CO51	51.9450	82.6350	4.1800	0.0058*
TKM13	47.2200	82.6350	5.1960	0.0020*
ASD16	94.4400	177.0850	4.0410	0.0068*
Kuliyadichan	51.9450	94.4400	15.5770	<0.0010*
Nootripathu	56.6700	82.6300	3.8090	0.0089*
Sivappu Chitraikar	43.8500	63.7500	4.3950	0.0046*

Based on Table 2, it is concluded that all varieties, except N22, show differential response to heat stress by exhibiting statistically significant increase in the mean catalase activity immediately after heat stress exposure. This reveals that all the varieties are affected by ROS activity and hence to scavenge the ROS, catalase activity was enhanced in the heat stressed samples. N22 remains unaltered in terms of catalase activity showing that it is tolerant to heat stress. Similar observations were made by Ying *et al.*, (2008) [9] and Zafar *et al.*, (2020) [10]. Both the studies report that all heat susceptible varieties / mutants exhibited enhanced ROS clearance via catalase activity and vice versa.

Assessing the temporal response of catalase activity after heat stress exposure

To understand the temporal response of catalase activity after heat stress exposure, comparison of mean catalase activity was evaluated in samples subjected to heat stress. Mean catalase activity of samples collected at 1 p.m. (immediately after heat stress at 37 ℃) and 6 p.m. (temperature brought from 37 ℃ to 25 ℃) were used for statistical analysis. The results are presented in Table 3.

Table 3: Temporal response of catalase activity after removal of heat exposure

Variety	Mean catalase activity of heat stressed samples (exposed to 37 ℃) (Units / ml)		t ratio	Probability (Alpha level = 0.05)
	1:00 PM	6:00 PM		
N22	212.5000	118.0550	-2.1900	0.0700
CO51	82.6350	70.8300	1.7320	0.1340
TKM13	82.6350	82.6350	0.0000	1.0000
ASD16	177.0850	94.4400	-4.0410	0.0068*
Kuliyadichan	94.4400	63.7500	-7.5080	0.0001*
Nootriathu	82.6350	56.6700	-3.8090	0.0089*
Sivappu Chitiraikar	63.5000	56.6700	-1.7320	0.1340

Based on Table 3, it can be inferred that the heat stress memory response in rice varieties differs by exhibiting differential catalase activity after relief from heat stress. Among the seven varieties, ASD16, Kuliyadichan and Nootripathu exhibit significant differences in mean catalase activity. There is a drastic reduction in mean catalase activity at 6 p.m. when compared to 1 p.m. On the other hand, other varieties namely, N22, CO51, TKM13 and Sivappu Chithiraikar exhibit no significant difference in mean catalase activity at 1 p.m. and 6 p.m. indicating that there exists heat stress memory in these varieties.

Conclusions

In the present study, rice varieties subjected to catalase activity analyses under both control and heat stress conditions revealed that rice plants exhibit diurnal fluctuations in terms of catalase activity. Also, catalase activity is affected by heat stress. Comparisons of mean catalase activities among rice varieties revealed that heat stress tolerant rice varieties do not show any statistically significant change in catalytic activity. On the other hand, varieties that are susceptible to heat stress show significant enhancement of catalase activity upon heat stress. The present study provides molecular evidence that N22 is a heat tolerant variety and CO51 is a heat susceptible variety.

Acknowledgements

Dr. PMS wishes to acknowledge The MRDP grant from the Department of Science and Technology, Government of India, New Delhi under the SPLICE-Climate change program (2018 - 19); Dr. PMS also wishes to acknowledge Dr. M. Raveendran, Department of Plant Biotechnology, CPMB & B, TNAU, Coimbatore, who provided us with the seed materials used in this study. Dr. PMS also wishes to thank Dr. Santosh Patil Ganapathi, Assistant Professor (Statistics), Department of Physical Sciences and Information Technology, AEC & RI, Coimbatore who assisted in Statistical Analyses.

References

1. Gourdji SM, Sibley AM, Lobell DB. Global crop exposure to critical high temperatures in the reproductive period: historical trends and future projections. Environmental Research Letters. 2013;8(2):024041.
2. Kraus TE, Mc Kersie BD, Fletcher RA. Paclobutrazol-induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. Journal of Plant Physiology. 1995;145(4):570-576.
3. Lorenz R, Stalhandske Z, Fischer EM. Detection of a

climate change signal in extreme heat, heat stress, and cold in Europe from observations. Geophysical Research Letters. 2019;46(14):8363-8374.
4. Luck H. Methods in enzymatic analysis II (ed.). Bergmeyer (publication). 1974;885-894.
5. Mohammed AR, Tarpley L. Impact of high night time temperature on respiration, membrane stability, antioxidant capacity, and yield of rice plants. Crop Science. 2009;49(1):313-322.
6. Prasad PV, Bheemanahalli R, Jagadish SK. Field crops and the fear of heat stress-opportunities, challenges and future directions. Field Crops Research. 2017;200:114-121.
7. Rehman SU, Bilal M, Rana RM, Tahir MN, Shah MKN, Ayalew H, *et al*. Cell membrane stability and chlorophyll content variation in wheat (*Triticum aestivum*) genotypes under conditions of heat and drought. Crop and Pasture Science. 2016;67(7):712-718.
8. Xu S, Li J, Zhang X, Wei H, Cui L. Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. Environmental and Experimental Botany. 2006;56(3):274-285.
9. Yun-Ying CAO, DUAN Hua, YANG Li-Nian, WANG Zhi-Qing, ZHOU Shao-Chuan, YANG, *et al*. Effect of heat stress during meiosis on grain yield of rice cultivars differing in heat tolerance and its physiological mechanism. Acta Agronomica Sinica. 2008;34(12):2134-2142.
10. Zafar SA, Hameed A, Ashraf M, Khan AS, Li X, Siddique KH. Agronomic, physiological and molecular characterisation of rice mutants revealed the key role of reactive oxygen species and catalase in high-temperature stress tolerance. Functional Plant Biology. 2020;47(5):440-453.
11. Zhao Chuang, Bing Liu, Shilong Piao, Xuhui Wang, David B. Lobell, Yao Huang, Mengtian Huang *et al*. Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences. 2017;114(35):9326-9331.