

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.611.129>

Impact of High Temperature on Antioxidant Enzymes during Reproductive Phase in Rice Cultivars

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ABSTRACT

Keywords

Antioxidant enzymes, Rice, Ascorbate peroxidase, Catalase, Heat stress, Hydrogen peroxide.

Article Info

Accepted:
10 September 2017
Available Online:
10 November 2017

The inductive response of H₂O₂ scavenging enzymes was studied in leaves of twenty rice cultivars when the plants were exposed to elevated temperature 55 days after transplanting (DAT). High temperature stress preferentially enhance the activities of ascorbate peroxidase (APX) and non-specific oxidase (POX), catalase (CAT) activity decreased with continuous exposure to heat stress although it was higher than control upto 15 days of stress (DAS) treatment. Thereafter, plants under normal temperature showed increased catalase activity as they experienced the stressful conditions generated by shift towards reproductive stage, hence our results suggest that (a) peroxidase enzymes detoxify H₂O₂ under high temperature (b) catalase enzyme scavenges H₂O₂, when the plant shifts from vegetative to reproductive stage.

Introduction

Rice is the most diverse cereal crop in the world, it is poorly adapted to high temperature and flowering (anthesis) is the most heat sensitive stage (Farrel *et al.*, 2006). Exposure of rice crop to heat stress at least during a part of its growth stage may drastically reduce the yield. High temperature at reproductive stage can decrease rice yield due to reduced pollen viability and increased spikelet sterility. At the cellular level, it has been observed in many crops that the level of active oxygen species (AOS) like superoxide radicle, hydrogen peroxide, single oxygen and hydroxyl radicle and antioxidant enzymes like superoxide dismutase, catalase and peroxidase increase when plants are exposed to high

temperature (Bowler *et al.*, 1992) and also when the plants undergo the shift from vegetative to reproductive stage (Gielis *et al.*, 1999). The present study was undertaken to assess the contribution of different H₂O₂ scavenging enzymes in detoxification under two different stressful environment experienced by the same plants.

Materials and Methods

Seeds of 20 rice cultivars were sown in pots with 2 kg of autoclaved soil. Fertilizers @ 60 mg urea, 30 mg superphosphate and 60 mg potash per plot were applied at the time of sowing. The plants were transplanted 30 DAS

and three plants are maintained per plot. All plants were maintained at 28/25⁰C at 75± 5% relative humidity. At 55 DAT (Primordia initiation stage) one set of plot was transferred to a growth chamber under condition of elevated temperature 35/28⁰C (high temperature exposure) and other was kept in growth chamber maintained at 26/22⁰C (normal for rice cultivar). First sampling was done after 10 days of treatment followed by 5 days interval in the second samples. Fully expanded leaf was taken for extraction of the enzyme from stressed and control plants.

Enzyme assay

Leaves were cut into small pieces and ground in potassium phosphate buffer (1:25 w/v, pH 7.0) containing 0.2 mmol L⁻¹ ascorbate and 1% PVPP. The ground tissue was spun at 13,000 g at 9⁰C for 10 min. the supernatant was collected and used for enzyme assay. All operations were carried out at 4⁰C. Total soluble protein was measured using BGA as a standard (Lowry *et al.*, 1951). The peroxidase (POX) activity was estimated by recording the decrease in ascorbate ($\epsilon = 2.8 \text{ m M}^{-1} \text{ cm}^{-1}$) content at 290 nm, as ascorbate was oxidized (Nakano *et al.*, 1981). Catalase (CAT) was assessed by measuring the disappearance of H₂O₂ ($\epsilon = 39.4 \text{ m M}^{-1} \text{ cm}^{-1}$) (Aebi *et al.*, 1984). SOD activity was assessed by following method of (Dhindsa *et al.*, 1981). SOD was measured by spectrophotometer at 560 nm wavelength.

Results and Discussion

The data pertaining to the effect of high temperature on CAT, SOD, POX activity in rice cultivars is presented in Table 1 and Figure 1.

Catalase (CAT) was found to be significantly different among different temperature treatments. Catalase activity decreased in

plants exposed to long period of heat stress. In the first stage after heat stress, the cultivars IURON 13-11, R 2032-50-6-1-134, OR 2376-1, 1RH-103 and R 1138-668-3-533-1 experienced increased catalase activity to degrade H₂O₂ production which was not maintained subsequently.

During long period of stress, level of catalase has been shown to drop in a wide range of species. Water deficit indicated oxidative stress in rice plants has also been reported to result in decreased level of SOD, APX, POX and CAT with catalase activity being maximally affected (Bio *et al.*, 2012). An increase in catalase activity under normal temperature in IURON 13-11, OR 2376-1, 1RH-103 and R 1138-668-3-533-1 before flowering indicates that stressful condition generated due to the transition of the plant towards the reproductive stage is overcome by the increased antioxidant activity of catalase.

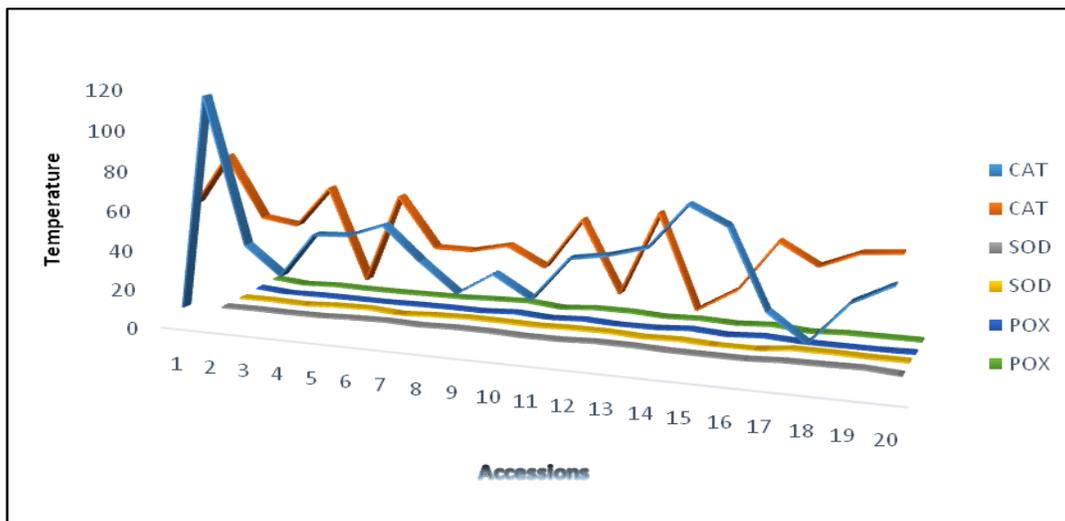
It has been reported that during the transition from vegetative to reproductive phase, the level of active oxygen species and antioxidant enzymes increases suggested that plants undergo stressful conditions during flowering process (Lui *et al.*, 2013). In IURON 13-11, R 2032-50-6-1-134, OR 2376-1 and 1RH-103 flowering initiated 73 days after transplanting under normal conditions. This was followed with the increase in catalase activity which shows that H₂O₂ which may be produced under such conditions scavenged by catalase enzyme.

Peroxidase (POX) activity increased in all genotypes under high temperature. Level of enzyme activity was higher in R-GM-AS-42 and R 2196-1150-1-412-1 at all stages of growth. Thus it conclusively support that both peroxidases compliment towards the breakdown of H₂O₂ in protection against the oxidative damage.

Table.1 Effect of high temperature on CAT, SOD, POX activity in rice cultivars

Accessions	CAT (H ₂ O ₂ ⁻¹ min ⁻¹ g ⁻¹)		SOD (g ⁻¹)		POX (H ₂ O ₂ ⁻¹ min ⁻¹ g ⁻¹)		
	Temperature	26 ⁰ C	35 ⁰ C	26 ⁰ C	35 ⁰ C	26 ⁰ C	35 ⁰ C
IURON 13-5		10.0	60.0	0.46	0.77	1.60	2.35
IURON 13-11		117.5	85.0	0.65	0.81	0.20	0.80
R 1625-1204-1-754-1		45.0	55.0	0.42	0.24	0.55	1.55
R 1661-605-84-1		31.0	52.5	0.45	1.25	0.45	1.20
R 1896-268-1-80-1		53.0	72.5	1.10	1.55	0.25	1.20
R 2032-506-1-134-1		54.0	28.0	1.46	0.26	0.55	1.35
R 1762-780-1-242-1		60.5	71.0	0.91	1.28	0.65	1.50
R 2196-1150-1-472-1		44.5	47.0	1.43	1.78	0.45	2.20
R-GM-AS-42		30.5	47.0	1.32	1.56	1.50	2.70
R1656-430-10-1965-1		41.5	51.0	0.70	1.12	0.30	1.0
R2164-1069-440-1		31.0	42.0	0.67	1.56	1.20	2.40
R 2196-1150-1-472-1		52.0	66.5	1.47	1.57	0.20	2.40
OR2376-1		55.0	32.5	1.32	0.65	0.40	1.40
Gopalbhog		60.0	72.5	0.65	1.11	1.65	2.0
IRH 103		81.5	28.0	0.53	0.11	0.45	1.30
R1138-668-3-533-1		73.0	39.0	0.42	0.10	1.55	2.35
Indira Sona		35.5	63.5	1.36	2.13	0.50	0.80
R RF 75		22.5	53.5	1.51	2.13	0.30	1.70
R1656-3173-1-415-1		43.0	61.0	1.79	1.68	0.25	1.70
R2093-1536-1-660-1		52.50	62.5	0.59	1.68	0.70	1.70
CD at 5%		32.24	14.98	0.22	0.96	2.86	3.29

Fig.1 Effect of high temperature on CAT, SOD, POX activity in rice cultivars



Superoxide dismutase (SOD) activity at control ranged from 0.042 to 1.79. The higher SOD enzyme activity was recorded in accession R 1656-3173-415-1. SOD activity increased in almost all accession in higher temperature except accessions R 1625-1204-

1-754-1, R 2032-506-1-134-1, 1RH-103 and R 1138-668-3-553-1 overexpression of SOD in plants affected a number of physiological phenomenon which included the removal of H₂O₂, oxidation of toxic reductants, biosynthesis and degradation of lignins in cell

walls, auxin catabolism, defensive response to wounding and some respiratory process (Lui *et al.*, 2012).

The interactive effect of high temperature and flowering stress on catalase activity which decreases under these conditions remain unclear. In the heat stressed plants which flower earlier than control condition. The peroxidase scavenging system has an overriding influence over catalase in detoxifying H₂O₂.

The differential response of antioxidant enzymes as a consequence of oxidative stress under high temperature and transition to reproductive phase leads us to propose that (a) peroxidase enzymes detoxify H₂O₂ under heat stress condition (b) catalase enzyme scavenges H₂O₂ during the shift in the vegetative to reproductive stage.

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How to cite this article:

Shabnam Khan, Arti Guhey and Kuruwanshi, V.B. 2017. Impact of High Temperature on Antioxidant Enzymes during Reproductive Phase in Rice Cultivars. *Int.J.Curr.Microbiol.App.Sci.* 6(11): 1099-1102. doi: <https://doi.org/10.20546/ijcmas.2017.611.129>