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SHORT COMMUNICATION

EFFECT OF HIGH TEMPERATURE ON HYDROGEN PEROXIDE SCAVENGING ENZYMES DURING REPRODUCTIVE PHASE IN AROMATIC RICE CULTIVARS

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The inductive response of H_2O_2 scavenging enzymes was studied in leaves of aromatic rice when the plants were exposed to elevated temperature 55 days after transplanting (DAT). High temperature stress preferentially enhanced the activities of ascorbate peroxidase (APX) and non specific peroxidase (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 condition generated by shift towards reproductive stage. Hence our results suggest that (a) peroxidase enzymes detoxify H_2O_2 under high temperature (b) catalase enzyme scavenges H_2O_2 when the plant shifts from vegetative to reproductive stage.

***Key words*: Antioxidant enzymes, ascorbate peroxidase, catalase, guaiacol peroxidase, heat stress, hydrogen peroxide, rice**

Rice appears to be most sensitive to high temperature at flowering and the effect of high temperature on spikelet sterility is widely reported in literature (Yoshida *et al*. 1981). Quality traits of aromatic rice are highly influenced by temperature particularly at the time of flowering, grain filling and maturity. Basmati rice requires relatively cooler temperature during crop maturity for better retention of aroma (Juliano 1972, Mann 1987). Exposure of basmati rice to high temperature would result in a loss of the quality traits. At the cellular level, it has been observed in many crops that level of active oxygen species (AOS) like superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radical and antioxidant enzymes like superoxide dismutase, catalase and peroxidases 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 H_2O_2 scavenging enzymes in detoxification under two different stressful environments experienced by the same plant.

Seeds of aromatic rice (*Oryza sativa* L.) cultivars, Tarawari basmati and Pusa basmati 370 (local selection and photosensitive), Pusa basmati 1 and Pusa Sugandh 2 (improved lines and photoinsensitive) were sown in pots with 2 kg of autoclaved soil in the phytotron of Indian Agricultural Research Institute, New Delhi. Fertiliser @ 60 mg urea, 30 mg superphosphate and 60 mg potash per pot was applied at the time of sowing. The plants were transplanted at 30 DAS and three plants were maintained per pot. All plants were maintained at 28/25^{0}C at 75+5% relative humidity and photosynthetically active radiation (PAR) of 300 µmol $m^{-2}s^{-1}$. At 55 DAT (Primordia initiation stage) one set of pots was transferred to a growth chamber under condition of elevated temperature of 35/28^{0}C (high

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temperature exposure) in dark/light of 12/12 h at 75+5% relative humidity and the other was kept in growth chamber maintained at 25/22^{0}C (normal for aromatic rice). First sampling was done after 10 days of treatment followed by 5 days interval in the subsequent samples. Fully expanded leaf was taken for extraction of the enzyme from stressed and control plants. Samples were collected from four plants but each sample of enzyme extract was assayed twice. The results are thus mean of n=8 observations in all cases.

Enzyme assays : 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 12,000 g at 0^0 C for 30 min. The supernatant was collected and used for enzyme assay. All operations were carried out at 4^{0}C. Total soluble protein was measured using BSA as a standard (Lowry *et al*. 1951). The ascorbate peroxidase (APX) activity was estimated by recording the decrease in ascorbate (ε = 2.8 mM^{-1} cm^{-1}) content at 290 nm, as ascorbate was oxidized (Nakano *et al.* 1981). Catalase (CAT) was assayed by measuring the disappearance of H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}) (Aebi 1984). Total peroxidase (POX) activity was measured by monitoring the formation of tetraguaiacol (ε = 26.6 mM^{-1} cm^{-1}) from guaiacol at 470 nm in presence of H_2O_2 (Rao *et al.* 1996).

Under normal conditions P. Sugandh 2 started flowering at 70 DAT followed by T. basmati and P. basmati 370 at 73 DAT and P. basmati 1 at 80 DAT respectively. Flowering initiated 5-7 days earlier in pots kept under high temperature than those at normal conditions. APX activity increased in all genotypes under high temperature stress and the activity was highest in Pusa Sugandh 2 and Pusa basmati 370 at 10 DAS (Fig. 1). Tarawari basmati and Pusa basmati 1 showed a gradual increase in APX activity till 20 DAS whereas in Pusa Sugandh 2 and Pusa basmati 370 the activity declined after an initial burst followed by a gradual increase subsequently. This indicated that Pusa basmati 370 and Pusa Sugandh 2 had better scavenging capacity as observed by the sharp increase in APX activity at the brief exposure (10 DAS) of high temperature. It has been reported that heat inducible transcriptional activation of cytosolic APX genes corresponds with an increase in APX activity (Foyer *et al.* 1997).

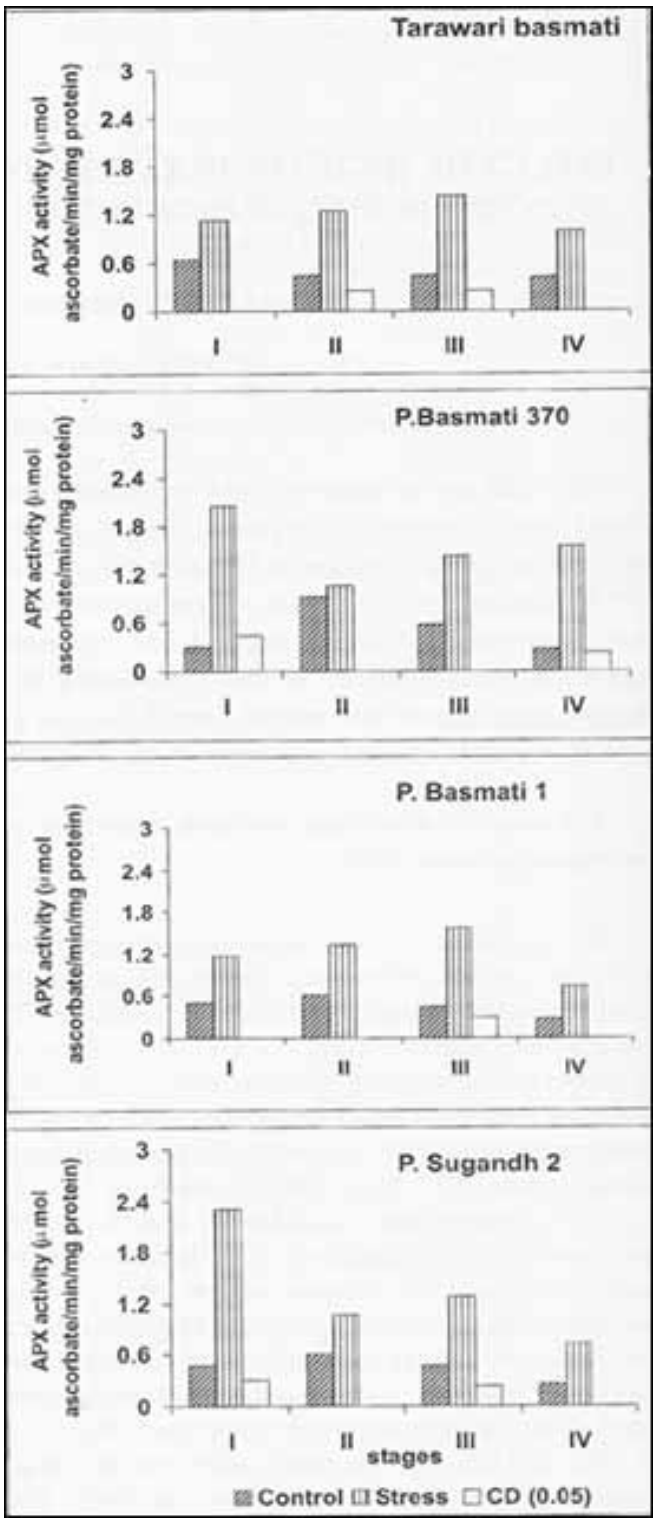

Fig. 1. Effect of high temperature on ascorbate peroxidase activity in rice cultivars. I- 10, II- 15, III- 20, IV- 25 days of stress.

The co-ordinate involvement of other antioxidant enzymes like POX and CAT in breakdown of H_2O_2 is also known (Scandalios 1993). POX activity increased in all genotypes under high temperature with T. basmati and Pusa Sugandh 2 showing maximal induction at 25 DAS. Level of enzyme activity was higher in Pusa Sugandh 2 and Pusa basmati 370 at all stages of growth (Fig. 2). Thus it conclusively supports that both peroxidases complement towards the breakdown of H_2O_2 in protection against the oxidative damage. In Pusa Sugandh 2 and Pusa basmati 370 initial outburst in AOS is controlled by APX whereas in Pusa basmati 1 it is POX activity which is a major contributor. The predominance of APX over POX is evident from the activity pattern observed in the rice cultivars. After 20 days exposure to stress, APX activity reduced and POX activity increased to mitigate the damaging effects of AOS.

Another important H_2O_2 detoxifying enzyme is catalase. Catalase activity decreased in plants exposed to long periods of heat stress. In the first stage after heat stress the plants of local selection (Tarawari basmati and P. basmati 370) experienced increased CAT activity to degrade H_2O_2 production which was not maintained subsequently (Fig. 3). During long periods of stress, levels of catalase have been shown to drop in a wide range of species (Lopez-Delgado *et al.* 1998, Dat *et al.* 1998, Jiang *et al.* 2001). Water deficit induced 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 (Boo *et al*. 1999). An increase in catalase activity under normal temperature in Pusa basmati 1 and Pusa Sugandh 2 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, suggesting that plants undergo stressful conditions during the flowering process (Lokhande *et al*. 2003). Plant growth and transition from vegetative to reproductive development are regulated by interactions between the environment and the endogenous developmental programs (Koornneef *et al*. 1998, Gomez-Mena *et al.* 2001). In Tarawari basmati and Pusa basmati 370, flowering initiated 73 days after transplanting under

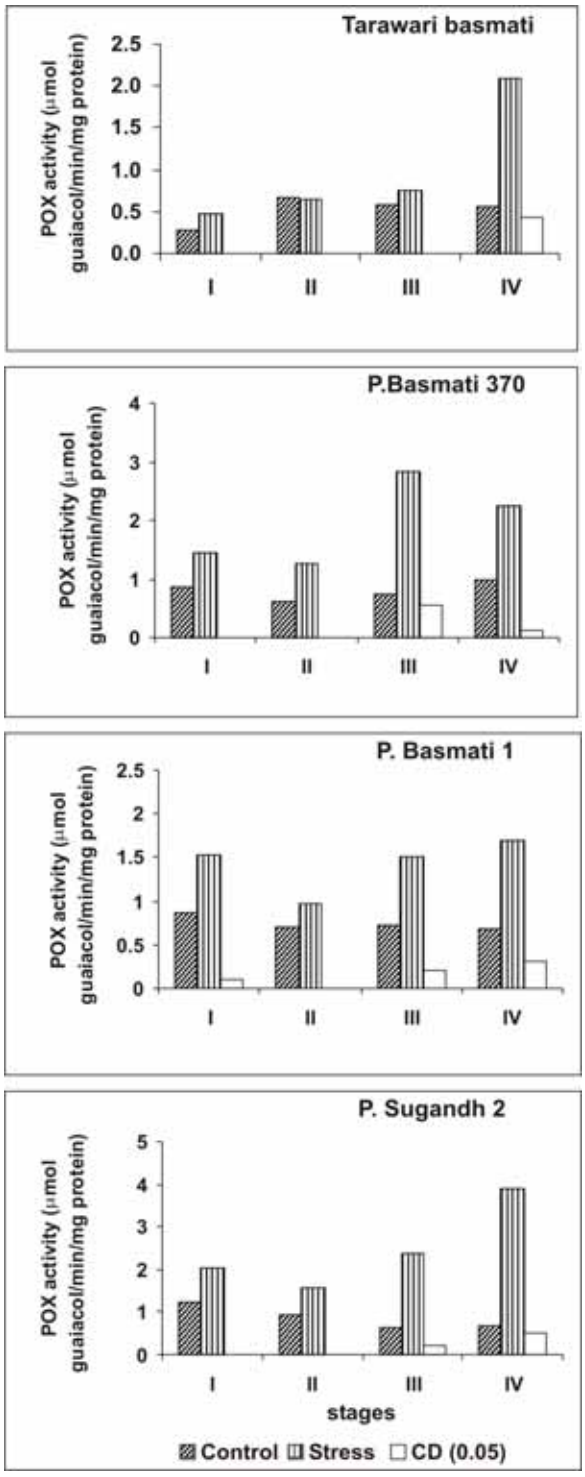

Fig. 2. Effect of high temperature on total peroxidase activity in rice cultivars. I- 10, II- 15, III- 20, IV- 25 days of stress.

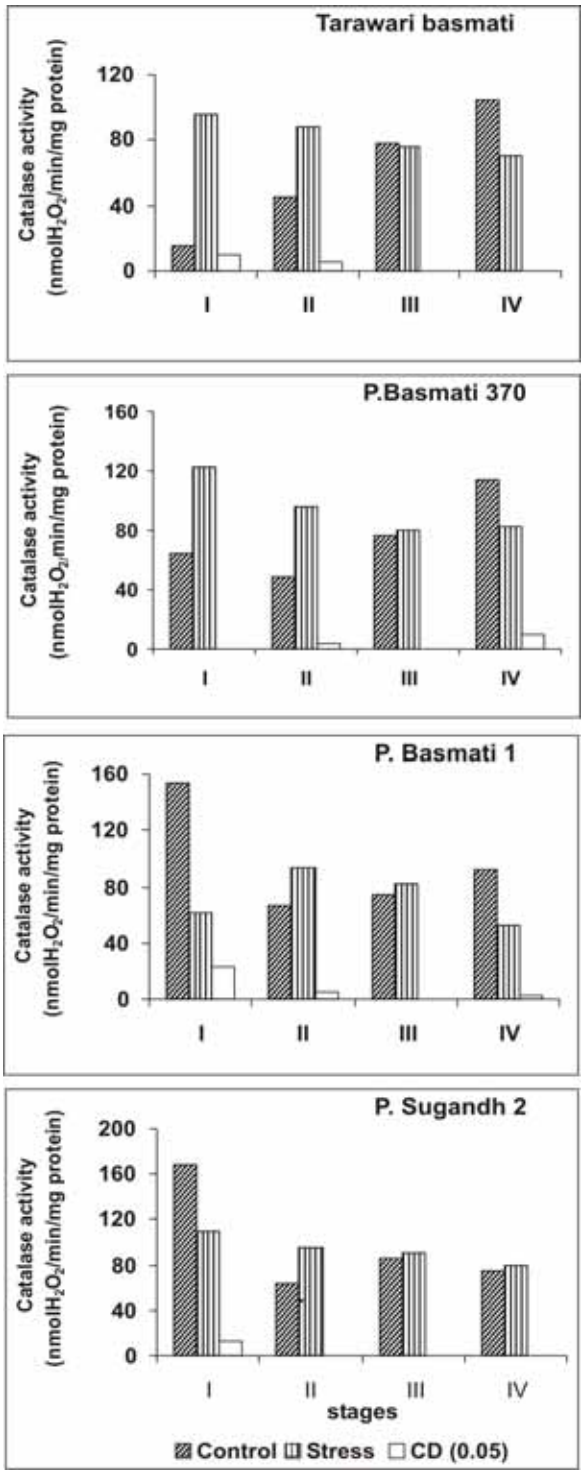

Fig. 3. Effect of high temperature on catalase activity in rice cultivars. I- 10, II- 15, III- 20, IV- 25 days of stress.

normal conditions. This was followed with the increase in catalase activity which shows that H_2O_2 which may be produced under such conditions is scavenged by catalase enzyme. In the heat stressed plants, which flower earlier than control conditions the peroxidase scavenging system has an overriding influence, over catalase in detoxifying H_2O_2. The interactive effect of high temperature and flowering stress on catalase activity, which decreases under these conditions remains unclear. The predominance of APX in scavenging H_2O_2 under these conditions is observed.

Therefore, 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 H_2O_2 under the heat stress conditions (b) catalase enzyme scavenges H_2O_2 during the shift in the vegetative to reproductive stage.

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