**Temperature stress-induced biochemical changes in pearl millet** [***Pennisetum glaucum* (L.) R. Br] genotypes at the seedling** **stage**

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# Abstract

The biochemical and physiological changes due to high temperature were observed in twelve genotypes of pearl millet (*Pennisetum glaucum* (L.) R. Br.). The 20 days old seedlings were exposed at 40°C for 6 hrs, 44°C for 4 hrs and 46°C for 2 hrs in BOD incubator. Data on chlorophyll content, activity of antioxidative enzymes and MSI were recorded after two days of treatment. The genotypes HTP94/54, J-2588 and PPMI 1263 performed better having high activity of antioxidative enzymes. These genotypes can be used for breeding programmes to develop high temperature stress tolerance.

**Key words:** RWC, MSI, chlorophyll, SOD and CAT

**Introduction**

The humans and livestock in rainfed regions of the country mostly rely on pearl millet [*Pennisetum glaucum* (L.) R. Br.] for food, feed and fodder. The heat stress in the semi-arid tropics can result in inadequate seedling establishment leading to reduction in productivity and stability of pearl millet (Peacock *et al.,* 1993).The growth and development of the pearl millet is mostly affected by environmental temperature. In India and Africa, the temperatures commonly exceed 45oCand reaches to as high as 60 oC occasionally. The duration and degree of heat stress invites different types of response of plants to heat stress (Hasanuzzaman *et al.,* 2013). High temperature exerts negative impact due to inhibition of photosynthesis process (De Ridderandsalvucci, 2007). Plants continuously struggle and tolerate heat stress resulting physical changes within the plant body for changing metabolism. Plants alter their metabolism in various ways in response to high temperature, particularly by producing compatible solutes that are able to organize proteins and cellular structures, maintain cell turgor by osmotic adjustment and modify the antioxidant system to re-establish the cellular redox balance and homeostasis. The reactive oxygen species (ROS) are generated during heat stress as a byproduct of aerobic metabolism. The ROS negatively affect cellular metabolism such as peroxidation of lipid membranes and damage to nucleic acids and proteins (Bita & Gerats, 2013). The stability of various membranes, cytoskeleton structures, RNA species and proteins are affected differentially due to high temperature stress which alters the effectiveness of enzymes. This ROS production can be avoided by ROS scavenging enzymes system. The superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and peroxidase (POX) are the main ROS scavenging enzymes. The non-enzymatic systems include glutathione (GSH) and ascorbic acid (ASC) (Suzuki*et et al.,* 2012, Maheshwari, C., *et al.,* 2024). To impart thermo tolerance in plants high levels of these antioxidants are required (Awasthi *et al.,* 2015). Keeping all this in view, the present study on biochemical changes in pearl millet genotypes due to induced high temperature stress at seedling stage was done.

# Materials and methods

The experiment was carried out during *Kharif* 2018 at ICAR-AICRP on Pearl Millet, Project Coordinating Unit, Jodhpur with twelve pearl millet genotypes H77/29-2, HTP94/54, H77/833-2-202, JMSB 20171, J-2588 J-2591, PPMI 1239, PPMI 1263, PIB 143, PIB 921, PIB 686 and 02777 B. The plants were initially raised under normal conditions in soil in small plastic pots (15x16 cm) and 20 days old seedlings were exposed to high temperature (40°C for 4 hrs, 44°C for 4 hrs and 46°C for 2 hrs) in BOD incubator to create the heat stress condition. After 2 days of treatment, second mature fresh leaves were collected for the analysis. The physiological index (membrane stability index) was observed when crop was under heat stress. The key biochemical parameters viz., superoxide dismutase (SOD), catalase (CAT) and chlorophyll content were also measured.

**Chlorophyll extraction**

It was done as per procedure given by Arnon, (1949) in which 100 mg of finely cut fresh leaves were taken and grinded with 10 ml of 80% acetone. It was then centrifuged at 5000 –10000 rpm for 5 mins. The supernatant was transferred. The absorbance of the solution was recorded at 645 nm and 663 nm.

**Membrane stability index (MSI)**

The procedure for calculating MSI was used as described by Premchandra *et al*., (1990) and modified by Sairam, (1994). Leaf samples (0.1 g) were placed in distilled water (10 ml). One set was kept at 40oC for 30 minutes and its conductivity of electrolytic leakage (C1) was recorded using conductivity meter. The second set was kept in boiling water bath (100 oC) for 10 minutes and its conductivity (C2) was recorded after cooling at room temperature.

 The MSI was calculated according to the formulae:

**MSI% = (1-C1 /C2) x 100**

**Estimation of Chlorophyll content**

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation (Awasthi *et al.,* 2015):

Chlorophyll a: 12.7(A663) – 2.69(A645)

Chlorophyll b: 22.9(A645) – 4.68(A663)

Total Chlorophyll: 20.2(A645) + 8.02(A663)

**Antioxidant enzyme assays**

The protocol of Chance and Maehly (1955) was used to assess the catalase (CAT) activities. Samples were prepared by grinding 0.5 g fresh leaves in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 1% polyvinyl polypyrrolidone (PVP). The homogenate was filtered through four layers of cheese cloth and then centrifuged at 4 oC for 20 mins at 15,000 X g. The supernatant was collected and an appropriate aliquot dilution of the crude extract was used for enzyme assays. CAT activity was measured by following the decomposition of H2O2 at 240 nm (e = 39.4 mM-1 cm-1) in a reaction mixture containing 50 mM phosphate buffer (pH 7.0) and 15 mM H2O2. Enzyme activity was expressed as mole of H2O2 decomposed mg-1 (protein) min-1.

The procedure given by Dhindhsa *et a*l. (1981) was used to assess Superoxide dismutase (SOD) activities. A leaf sample (0.5 g) was homogenized in 10 cm3 chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The buffer was filtered through cheese cloth and after centrifugation at 20,000 X g for 20 mins aliquots of the supernatant were used for enzymatic quantify. The 3.0 cm3 reaction mixture contained 13 mM methionine, 25 mM nitroblue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer pH (7.8), 50 mM sodium bicarbonate and 0.1 cm3 enzyme extract. The reaction was started by adding 2 ml riboflavin and placing the tubes below 2 X 15.00 W fluorescent lamp for 15 mins. It was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme develops maximum colour. A non-irradiated complete reaction mixture did not develop colour and served as blank. Absorbance was recorded at 560 nm, and one unit of enzyme activity was taken as that quantity of enzyme, which reduced the absorbance reading to 50 % in comparison with the tubes lacking enzymes.

**Results and discussion**

The key biochemical parameters viz., superoxide dismutase (SOD), catalase (CAT) and chlorophyll content were measured. All these parameters helped in assessing tolerant versus susceptible genotypes under heat stress. The values for stability of cellular membrane in the pearl millet genotypes indicated that there was decline in MSI percent of stressed plant in all genotypes. The MSI values varied from 52.72 to 73.56 percent on fresh weight basis in control at 40oC while under heat stress it varied from 39.33 to 65.90 percent. MSI was high in J-2588 and H77/833-2-202 under control at 40oC, whereas, at 46oC, genotypes J-2588 and H77/833-2-202 followed by H77/29-2, HTP94/54 and PPMI 1263 recorded high MSI (Figure 1).

Figure 1: Effect of high temperature stress on membrane stability index in pearl millet genotypes. Values of ± SE

The low MSI were found in genotypes H77/29-2, HTP94/54 and PPMI 1263 indicating that they are comparatively tolerant at high temperature. Similar kind of results were obtained by Maavimani *et al.,*(2014), Golam *et al*.,(2012), Blum *et al.,*(2001), Wahid & Shabhir, (2005) and Gupta *at el.,*(2013). The total chlorophyll content varied from 2.17 to 2.94 mg g-1 fresh weight under control at 40 oC while under high temperature stress at 46 oC it varied from 1.49 to 2.51 mg g-1 (Figure 2).

Fig. 2: Effect of high temperature stress on Chlorophyll content in pearl millet genotypes. Values of ± SE

Literature revealed that many quaternary ammonium compounds are synthesized in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane thereby maintaining photosynthetic efficiency. It suggested that plants must be protected from heat-induced oxidative stress so that they can survive under high temperature. The tolerance to high temperature in crop plants has been associated with an increase in antioxidative capacity (Almeselmani *et a*l., 2006, Devraj, 2008 and Meena, *et al.*, 2021, Yadav, *et al.,* 2022). The catalase activity was maximum in H77/833-2-202 , J-2591 and PIB 921 at 46oC (Figure 3) whereas maximum percent increase in catalase and superoxide dismutase were recorded in J-2588, H77/833-2-202 and J-2591 (Fig.4) under high temperature stress conditions. The tolerant and susceptible varieties can be differentiated on basis of activities maintained under high temperature. The tolerant varieties could maintain increased activities at high temperature in comparison to the susceptible ones (Chakraborty and Pradhan, 2011, Gupta, N.K. *et.al.,* 2022).

Fig.3: Effect of high temperature stress on antioxidant enzyme catalase in pearl millet genotypes. Values of ±SE

Fig.4: Effect of high temperature stress on activity of antioxidant enzyme Superoxide dismutase in pearl millet genotypes. Values of ± SE

Tolerant plants have a tendency of protection against the damaging effects of ROS with the synthesis of various enzymatic and non-enzymatic ROS scavenging and detoxification systems (Apel, 2004). The activities of these enzymes increases with increasing temperature. Catalase and Superoxide dismutase are the most important enzymes involved in regulation of intracellular level of H2O2. Many quaternary ammonium compounds are synthesized or found abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane thereby maintaining photosynthetic efficiency.

**Conclusion**

Temperature stress is one of the most affecting factors in plant growth and development which is normally beyond the control of man. However, the selection of high temperature tolerant genotypes is in the hands of plant physiologists and breeders. Based on various physiological parameters analyzed in the present study, HTP94/54, J-2588 and PPMI 1263 were identified as heat tolerant. It is suggested that these heat tolerant genotypes can be used in future breeding program to develop heat tolerant varieties of pearl millet.

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**References**

1. Almeselmani, M., Deshmukh, P.S., Sairam, R.K., Kushwaha, S.R., & Singh, T.P., (2006). Protective role of antioxidant enzymes under high temperature stress. *Plant Sci.* 171 (117), 382–388.
2. Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Ann. Rev. Plant Biol.* 55, 373–399
3. Arnon, D.I. (1949). Copper enzymes in isolated chloroplast, polyphenol oxidase in Beta vulgaris. *L. Plant physiology*. 24, 1-15
4. Awasthi, R., Bhandari, K., & Nayyar, H. (2015). Temperature stress and redox homeostasis in agricultural crops. *Front. Environ. Sci*. 3, 11. doi: 10.3389/fenvs. 2015.00011
5. Bita, C.E., & Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science.* 4, 273.
6. Blum, A., Klueva, N., & Nguyen, H.T. (2001). Wheat cellular thermo-tolerance is related to yield under heat stress. *Euphytica*. 117, 117–123.
7. Chakraborty, U., & Pradhan, D. (2011). High temperature-induced oxidative stress in Lens culinaris, role of antioxidants and amelioration of stress by chemical pre-treatments. *J. Plant Interact*. 6, 43–52
8. Chance, B., & Maehly, A.C. (1955). Assay of catalase and peroxidises. Methods Enzymol. 2, 764–775.
9. DeRidder, B.P., & Salvucci, M. E. (2007). Modulation of Rubisco activase gene expression during heat stress in cotton (*Gossypium hirsutum* L.) involves post-transcriptional mechanisms. *Plant Science.* 172, 246–254.
10. Devi, R. S. & Prasad, M. N. V. (1998). Copper toxicity in *Ceratophyllum demeresum* L. (Coontail), a free floating macrophyte: Response of antioxidant enzymes and antioxidants, *Plant Sciences*. 138, 157.
11. Devraj, V. R. (2008). High temperature and salt stress response in French bean (Phaseolus vulgaris). *Aust. J. Crop Sci*. 2, 40–48.
12. Dhindhsa. R. S., Plumb-Dhidsa, P., & Thorne, T. A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid per oxidation, and decreased levels of superoxide dismutase and catalase. *J Expt Bot*. 32, 93–101.
13. Golam, F., Prodhan, Z. H., Nezhadahmadi, A., & Rahman, M. (2012). Heat tolerance in tomato. *Life Science Journal*. 9, 1936–1950.
14. Gupta, N. K., Agrawal, S., Agrawal, V. P., Gupta, S., & Singh, G. (2013). Effect of short -term heat stress on growth, physyiology and antiozxidative defence system in wheat seedling. *Acta Physiol plant*. DOI10.1007/s11738-013-1122-1.
15. Gupta, N. K., Gupta, S., Singh, J., Garg, N. K., Saha, D., Singhal, R. K., ... & Siddiqui, M. H. (2022). On-farm hydro and nutri-priming increases yield of rainfed pearl millet through physio-biochemical adjustments and anti-oxidative defense mechanism. *Plos one*, *17*(6), e0265325.
16. Hasanuzzaman, M., Nahar, K., Alam, M. M. et al. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences.*14, 9643–9684.
17. Maavimani, M., Jebaraj, S., Raveendran, M. et al. (2014). Cellular membrane thermostability is related to rice (*Oryza sativa* L.) yield under heat stress. *International Journal of Tropical Agriculture.* 32, 201–208.
18. Maheshwari, C., Garg, N. K., Singh, A., & Tyagi, A. (2024). Ameliorative Effects of Paclobutrazol via Physio-Biochemical and Molecular Manifestation in Rice under Water Deficit Stress. *Rice Science*, *31*(5), 603-616.
19. Meena, R. C., Ambawat, S., Satyavathi, C.T., & Meena, R. (2021). High temperature induced biochemical changes in pearl millet genotypes at seedling stage, *International Journal of Chemical Studies*. 9(1), 764-768.
20. Peacock, J. M., Soman, P., Jayachandran, R., Rani, A.U., Howarth, C.J., Thomas, A. (1993). Effects of high soil surface temperature on seedling survival in pearl millet. *Exp Agric*. 29, 215–225.
21. Premchandra, G. S., Saneoka, H., & Ogata, S. (1990). Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. *Journal Agric. Sci. Camb.* 115, 63-66.
22. Sairam, R. K. (1994). Effect of moisture-stress on physiological activities of two contrasting wheat genotypes. *Indian J. Experimental Biology*. 32, 594-597.
23. Suzuki, N., Koussevitzky, S., Mittler, R., & Miller, G. (2012). ROS and redox signalling in the response of plants to abiotic stress. *Plant Cell Environ*. 35, 259–270. doi: 10.1111/j.1365-3040.2011.02336.x
24. Wahid, A., & Shabbir, A. (2005). Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. *Plant Growth Regulation.* 46, 133–141.
25. Yadav, M. R., Choudhary, M., Singh, J., Lal, M. K., Jha, P. K., Udawat, P., ... & Prasad, P. V. (2022). Impacts, tolerance, adaptation, and mitigation of heat stress on wheat under changing climates. *International Journal of Molecular Sciences*, *23*(5), 2838.