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RESEARCH ARTICLE

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-day-old seedlings were exposed to temperatures of 40 °C for 6 hrs, 44 °C for 4 hrs, and 46 °C for 2 hrs in a 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 in breeding programs to develop high-temperature stress tolerance.

Keywords: *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. Heat stress in the semi-arid tropics can result in inadequate seedling establishment, leading to a reduction in the productivity and stability of pearl millet (Peacock *et al.,.,* 1993). The growth and development of the pearl millet are mostly affected by environmental temperature. In India and Africa, the temperatures commonly exceed 45 °C and reach as high as 60 °C occasionally. The duration and degree of heat stress *et al.,*elicit different types of responses in plants to heat stress (Hasanuzzaman *et al*.,., 2013). High temperatures exert a negative impact due to the inhibition of the photosynthesis process (De Ridder and Salvucci, 2007 and Gupta *et al.,* 2013). Plants continuously struggle and tolerate heat stress, resulting in physical changes within the plant body that alter metabolism. Plants alter their metabolism in various ways in response to high temperatures, particularly by producing compatible solutes that can organize proteins and cellular structures, maintain cell turgor through osmotic adjustment, and modify the antioxidant system to re-establish 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 the peroxidation of lipid membranes and damage to nucleic acids and proteins (Bita and 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. The ROS scavenging enzymes system can avoid this ROS production. 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 al.,.,* 2012 and

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Yadav *et al*., 2022). To impart thermotolerance in plants, high levels of these antioxidants are required (Awasthi *et al.,.,* 2015). Keeping all this in mind, the present study on biochemical changes in pearl millet genotypes due to induced high-temperature stress at the seedling stage was conducted.

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). Twenty-day-old seedlings were then exposed to high temperatures (40 °C for 4 hours, 44 °C for 4 hours, and 46 °C for 2 hours) in a BOD incubator to create a heat stress condition. After 2 days of treatment, the second mature fresh leaves were collected for the analysis. The physiological index (membrane stability index) was observed when the 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 the procedure given by Arnon (1949), in which 100 mg of finely cut fresh leaves were ground with 10 ml of 80% acetone. It was then centrifuged at 5000–10000 rpm for 5 minutes. 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). Leaf samples (0.1 g) were placed in distilled water (10 ml). One set was kept at 40 °C for 30 minutes, and its conductivity of electrolytic leakage (C1) was recorded using a conductivity meter. The second set was kept in a boiling water bath (100 °C) 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: $12.7(A_{663}) - 2.69(A_{645})$

Chlorophyll b: $22.9(A_{645}) - 4.68(A_{663})$

Total Chlorophyll: $20.2(A_{645}) + 8.02(A_{663})$

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 of fresh leaves in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetraacetic acid (EDTA) and 1% polyvinyl polypyrrolidone (PVP). The homogenate was filtered through four layers of cheesecloth and then centrifuged at 4 °C for 20 minutes 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 H_2O_2 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 H_2O_2. Enzyme activity was expressed as moles of H_2O_2 decomposed mg^{-1} (protein) min^{-1}.

The procedure given by Dhindhsa *et al.,*. (1981) was used to assess Superoxide dismutase (SOD) activities. A leaf sample (0.5 g) was homogenized in 10 cm^3 chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The buffer was filtered through cheesecloth, and after centrifugation at 20,000 X g for 20 minutes, aliquots of the supernatant were used for enzymatic quantification. The 3.0 cm^3 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 cm^3 enzyme extract. The reaction was initiated by adding 2 mL of riboflavin and placing the tubes below a 2 × 15.00 W fluorescent lamp for 15 minutes. It was stopped by switching off the light and covering the tubes with a black cloth. Tubes without an enzyme develop maximum colour. A non-irradiated complete reaction mixture did not develop colour and served as a blank. Absorbance was recorded at 560 nm, and one unit of enzyme activity was defined as the quantity of enzyme that reduced the absorbance reading to 50% compared to the tubes lacking enzyme.

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 the stability of the cellular membrane in the pearl millet genotypes indicated a decline in MSI percent in all stressed plants. The MSI values varied from 52.72 to 73.56 percent on a fresh weight basis in the control at 40 °C, while under heat stress, they varied from 39.33 to 65.90 percent. MSI was high in J-2588 and H77/833-2-202 under control at 40 °C, whereas, at 46 °C, genotypes J-2588 and H77/833-2-202 followed by H77/29-2, HTP94/54 and PPMI 1263 recorded high MSI (Fig. 1). 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 results were obtained byBlum *et al.,.,* (2001), Wahid and 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 °C while under high temperature stress at 46 °C it varied from 1.49 to 2.51 mg g^{-1}(Fig. 2). 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 is suggested that plants must be protected from heat-induced oxidative stress so that they can survive under high temperatures.

The tolerance to high temperatures in crop plants has been associated with an increase in antioxidative capacity (Almeselmani *et al.,.,* 2006and Meena *et al.,.,* 2021). The catalase activity was maximum in H77/833-2-202, J-2591, and PIB 921 at 46 °C (Fig. 3)

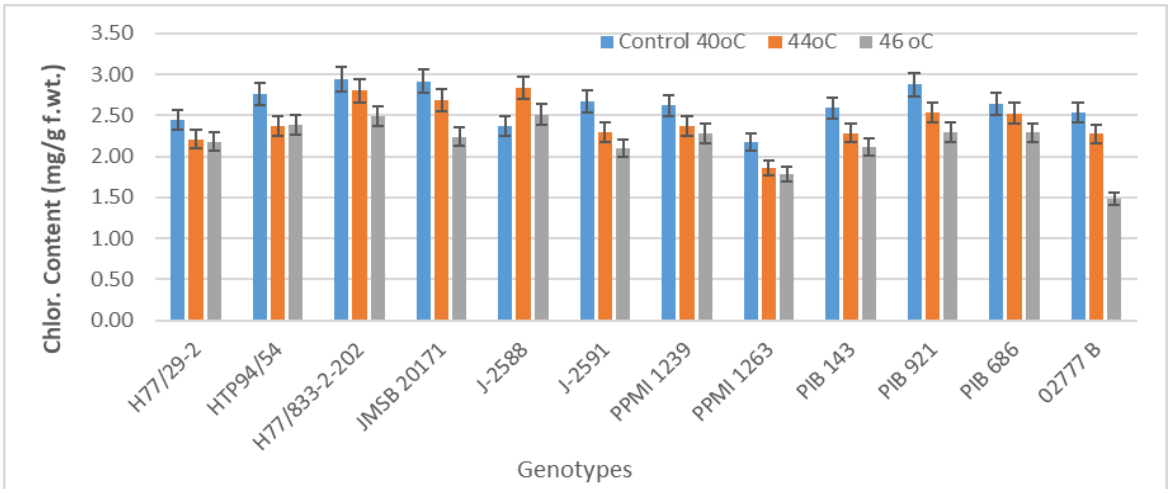

Fig. 2: Effect of high temperature stress on Chlorophyll content in pearl millet genotypes. Values of ± SE

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

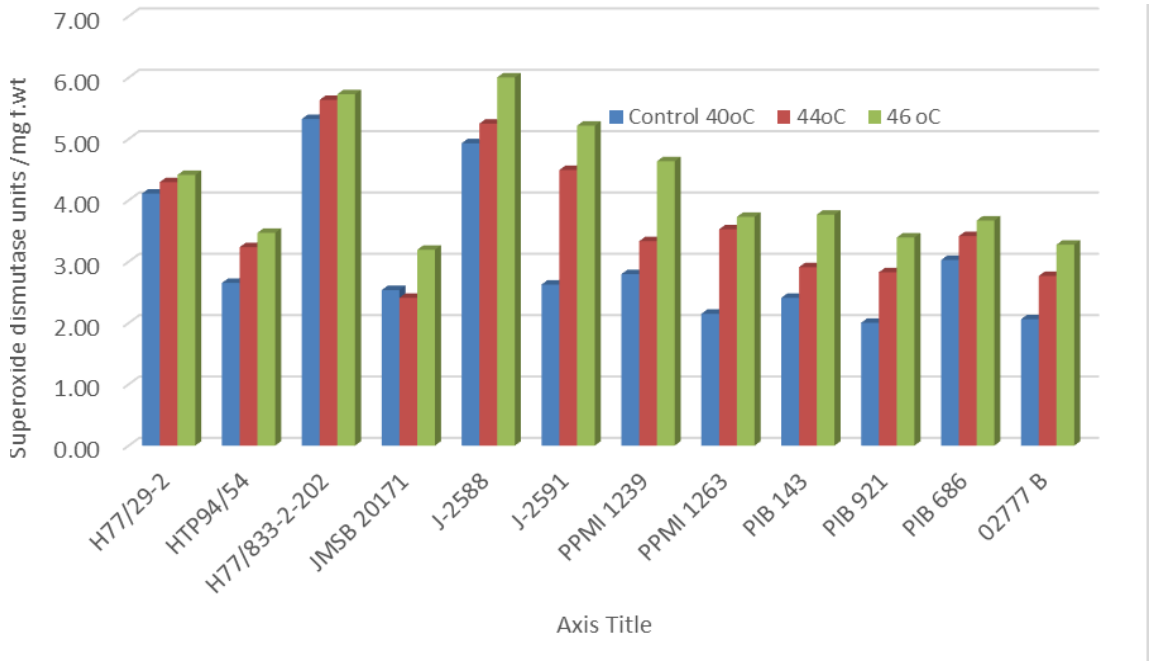

Fig.4: Effect of high temperature stress on activity of antioxidant enzyme Superoxide dismutase in pearl millet genotypes. Values of ± SE

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 based on the activities they maintain under high temperatures. The tolerant varieties can maintain increased activity at high temperatures compared to the susceptible ones (Chakraborty and Pradhan, 2011). Tolerant plants have a tendency to protect themselves against the damaging effects of ROS through the synthesis of various enzymatic and non-enzymatic ROS scavenging and detoxification systems (Apel, 2004). The activities of these enzymes increase with increasing temperature. Catalase and Superoxide dismutase are the most important enzymes involved in the regulation of the intracellular level of H_2O_2. Many quaternary ammonium compounds are synthesized or found abundantly mainly in chloroplasts, where they play a vital role in the adjustment and protection of the thylakoid membrane, thereby maintaining photosynthetic efficiency.

CONCLUSION

Temperature stress is one of the most significant factors affecting plant growth and development, which is normally beyond human control. 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 programs to develop heat-tolerant varieties of pearl millet.

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