Research Article

Impacts of High Temperature Stress on Enzyme Activities of Mulberry Varieties/Genotypes

S. Ranjith kumar[1]*, G. Swathiga[1], R. Ramamoorthy[1] and D. Vijayalakshmi[2]

[1]Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Coimbatore, Tamil Nadu – 641 301

[2]Department of Crop physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu – 641 003

Abstract

High temperature stress is a detrimental factor for many crops in relation to crop growth and productivity. It severely affects the metabolic process in plants leads, to loss of plant life. Nitrate reductase activity is important enzyme activity which plays important role in nitrogen metabolism and regulation. In this study, there was no such great variation in proline accumulation and enzyme activities in mulberry varieties analysed before imposing high temperature stress. Then after stress, proline accumulation was noticed in high temperature stressed mulberry plants. The varierty V1 recorded maximum of NRase activity (14.26 µg NO_2 g^{-1} h^{-1}) compared to other varieties. The highest catalase activity was observed in variety V1 followed by S36, MR2 and least activity was recorded in G2 and G4. In the present study, the varieties V1, MR2 and S36 showed higher level of CAT and POD enzyme activity compared to other to varieties.

Keywords: Proline, NRase, Catalase, Peroxidase, High temperature stress

***Correspondence**

Author: Ranjith kumar

Email: ranjithsiva1294@gmail.com

Introduction

Mulberry is an important crop plant in sericulture sector and its leaf is the primary food of domesticated silkworm *Bombyx mori*. L which produces nature silk used in textile industries. Mulberry is cultivated mostly under semi-irrigated conditions and often gets exposed to high temperature upto 42° C during summer in Tamil Nadu.

High temperature stress is a detrimental factor for many crops in relation to crop growth and productivity. It severely affects the metabolic process in plants leads, to loss of plant life [1]. High temperature is a serious threat that causes decline in yield and total dry matter accumulation in many crop plants [2].

Nitrate reductase activity is important enzyme activity which plays important role in nitrogen metabolism and regulation. Under high temperature stress, nitrate reductase activity is declined [3]. Due to high temperature stress, as a mechanism to tolerate the stress many plant species accumulates various osmolytes such as proline, polyols, sugars and tertiary and quartenary ammonium compounds [4]. Accumulation of Reactive oxygen species (ROS) caused due to high temperature stress causes major loss to crops worldwide [5]. High temperature also causes oxidative stress that causes cell membrane damage, lipid peroxidation [6]. To limit the damages caused by oxidative stress plants have developed a series of detoxification systems that breaks the toxicity levels of ROS [7]. [8] stated that antioxidants such as catalase, peroxidase and superoxide dismutase are highly involved in scavenging the ROS produced during stress conditions.

Plant tolerance to stress is the major objective to mulberry plants in many regions of the world. A very little information is known about the antioxidant defense mechanism in mulberry plants against environmental stresses. The present study is undertaken to assess the physiological basis of tolerance to HTS in mulberry varieties by analyzing i) Effect of high temperature stress on proline accumulation and NRase activity, ii) Changes in ROS scavenging enzymes like CAT and POX under high temperature stress, iii) Identify the variety that can better tolerate HTS.

Materials and Methods

Five promising mulberry varieties *viz*., V1, G2, G4, MR2 and S36 were grown in pot culture through mulberry cuttings. 120 days old saplings were used for the study, in which three sets of 20 pots one control and one for high temperature stress for 7 days and the third set for high temperatures stress upto 14th day were maintained for each variety. Each variety was replicated four times.

The high temperature experiment was conducted at temperature controlled open top chambers (OTCs) available at Department of Crop Physiology, TNAU, Coimbatore, is used for the study. Inside the OTCs, required levels of

temperature and relative humidity can be maintained by giving commands through control panel. In high temperature chamber, temperature was maintained at 40º C starting from 9.00 hrs and maintained upto 13.00 hrs respectively. Crop management practices were taken as per recommendation.

Plants were grown normally until 120 days. After 120 days the plants were pushed into OTC. Plants were exposed to high temperature at 40º C for period of two weeks. The observations taken during the experiment *viz*., before imposing stress, 7th day after stress and 14th day after stress.

Proline content of the leaf was estimated by [9] method and expressed as µg g^{-1} of fresh weight. Nitrate reductase enzyme activity was estimated in fully expanded leaves from control and stress imposed plants [10] method. The enzyme activity was expressed as µmol NO_2 g^{-1} h^{-1}.

Catalase activity was assayed from the rate of H_2O_2 decomposition extinction coefficient of 39.4 mmol as measured by the decrease in the absorbance at 240 nm, following the procedure of [11]. The reaction mixture contains 50 mmol potassium phosphate buffer (pH 7.0) and the appropriate volume of extract. The reaction was initiated by adding 10 mmol of H_2O_2. One unit of catalase is defined as the amount of enzyme that liberated half of the peroxide oxygen from 10 mmol H_2O_2 solutions in 100 sec at 25 °C.

Peroxidase activity (change in OD value at 430 nm g^{-1} min^{-1}) was determined by [12] and [13] method. One gram of leaf was extracted using 0.1M phosphate buffer (pH 7.0) and a known volume of the extract was added to a cuvette containing 3 ml phosphate buffer and 3 ml pyrogallol was added and the increase in absorbance at 430 nm was recorded. The change in absorbance in minutes was used to calculate the enzyme activity.

Completely Randomized Design was used for data analysis of mulberry varieties before imposing stress (120th Day after planting) and Factorial Completely Randomized Design (FCRD) was used for data analysis of mulberry varieties maintained at high temperature stress (127th and 134th day after planting) maintained under OTCs. The experiment is carried out on various parameters as per the procedure suggested by [14].

Result and Discussion

The study clearly demonstrated that the level of metabolites like proline, catalase and peroxidase enzyme activities were increased as the defense mechanism to reduce the ROS formed during high temperature stress. Increase in proline content due to stress is known to be one of the earlier response of crops to high temperature stress [15]. Increase in proline content was noticed in mulberry leaves exposed to high temperature stress [16]. There was no such great variation in proline accumulation and enzyme activities in mulberry varieties analysed before imposing high temperature stress (**Table 1**). Proline accumulation was noticed in high temperature stressed mulberry plants (**Figure 1**). The variety V1 recorded greater accumulation of about (9.84), following this variety MR2 recorded (9.27) and S36 recorded (9.12) and least values were recorded in G2 and G4 (8.99 and 8.79) respectively. Proline interaction with enzymes to preserve protein structure and activities reduces enzymes denaturation caused due to abiotic stress and acts as energy reservoir during recovery after stress.

Table 1 Alteration in enzyme activities of mulberry varieties at 120th day after planting (Before imposing stress)

S. No	Variety	Proline (µg g^{-1})	Nitrate reductase (µg NO_2 g^{-1} h^{-1})	Catalase (µg H_2O_2 min^{-1} g^{-1})	Peroxidase (Δ 430 nm g^{-1} min^{-1})
1.	V1	3.51±0.04	13.96±1.10	2.61±0.13	1.96±0.01
2.	G2	2.94±0.02	11.39±0.98	2.38±0.15	1.71±0.03
3.	G4	2.84±0.02	8.39±0.92	2.21±0.10	1.60±0.00
4.	MR2	3.46±0.03	12.19±1.05	2.70±0.07	1.88±0.04
5.	S36	3.15±0.03	11.69±1.00	2.50±0.09	1.90±0.03
S.Ed		0.228	0.627	0.023	0.032
CD *p<0.05		0.487	1.33	0.049	0.069

Nitrate reductase activity of the leaf showed decreasing under high temperature stress (**Figure 2**). The varierty V1 recorded maximum of NRase activity (14.26) compared to other varieties. Following this, MR2 recorded (12.72), S36 (12.16) and least activity was recorded in G2 and G4 (11.34 and 8.37) respectively. Nitrate reductase activity is the key mechanism for overall nitrogen assimilation process and important for crop life [17]. A decline in nitrate reductase activity during stress period was reported by [18].

Catalase and Peroxidase associated with the scavenging of ROS and increase in their activity related to increase in stress tolerance. Higher CAT and POD activities were reported in wheat genotypes tolerant to abiotic stress [19]. It is seen that high temperature and water stress increases the levels of these enzymes in different mulberry varieties [20]. In this study, catalase activity of the leaf showed an increasing trend from the time of exposure to high temperature stress upto two weeks (**Table 2**). The highest catalase activity was observed in variety V1 (5.69), followed by S36

(5.31), MR2 (5.26) and least activity was recorded in G2 and G4 (3.56 and 3.30) respectively. Peroxidase activity of the leaf showed an icreasing trend in mulberry varieties exposed to high temperature stress (**Table 3**). The variety V1 has highest peroxidase activity (4.51), followed by MR2, S36, G2 and G4 (4.28, 4.26, 3.57 and 2.61) respectively. In the present study, the varieties V1, MR2 and S36 showed higher level of CAT and POD enzyme activity compared to other to varieties. These three varieties showed high oxidative metabolism. Therefore, it is concluded that mulberry variety V1 has highest tolerance in terms of antioxidant response to prolonged high temperature stress closely followed by MR2, S36 and less tolerance was noted in varieties G2 and G4 respectively.

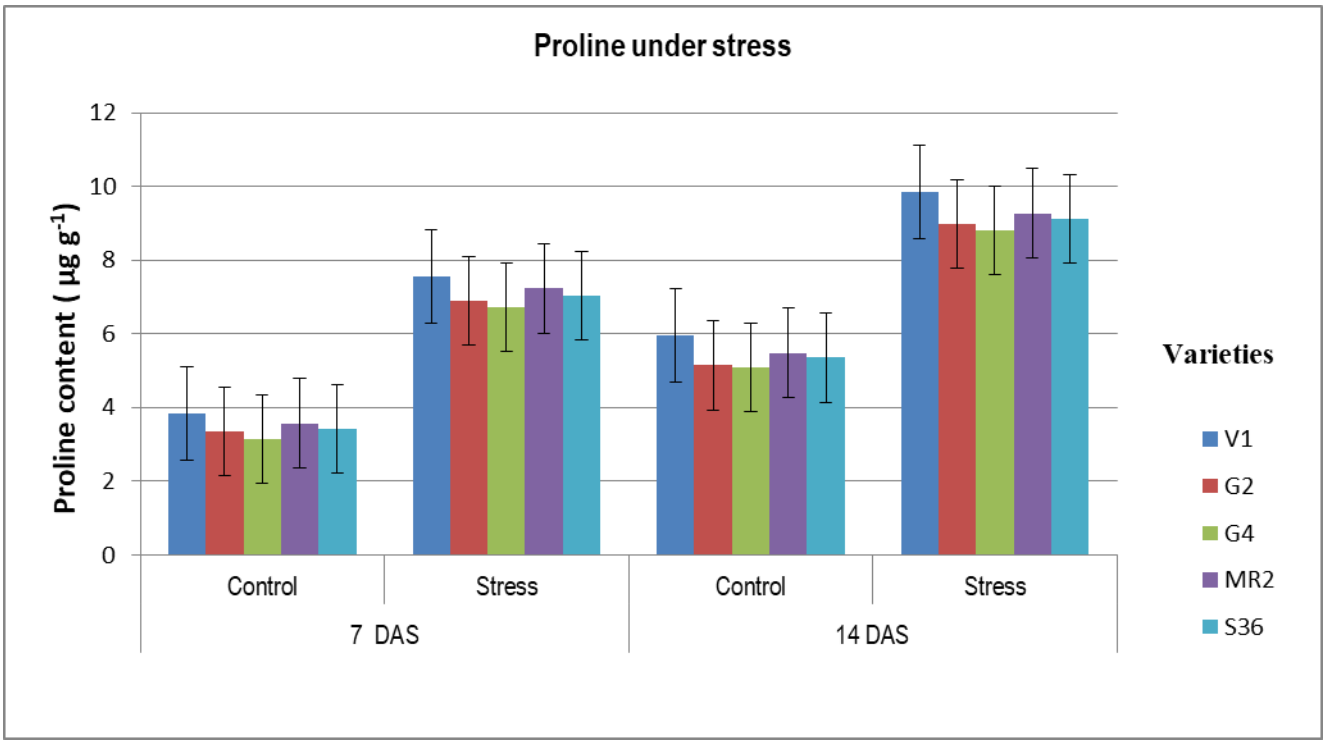

Figure 1 Proline content (µg g^{-1}) in mulberry varieties exposed to high temperature stress for two weeks interval

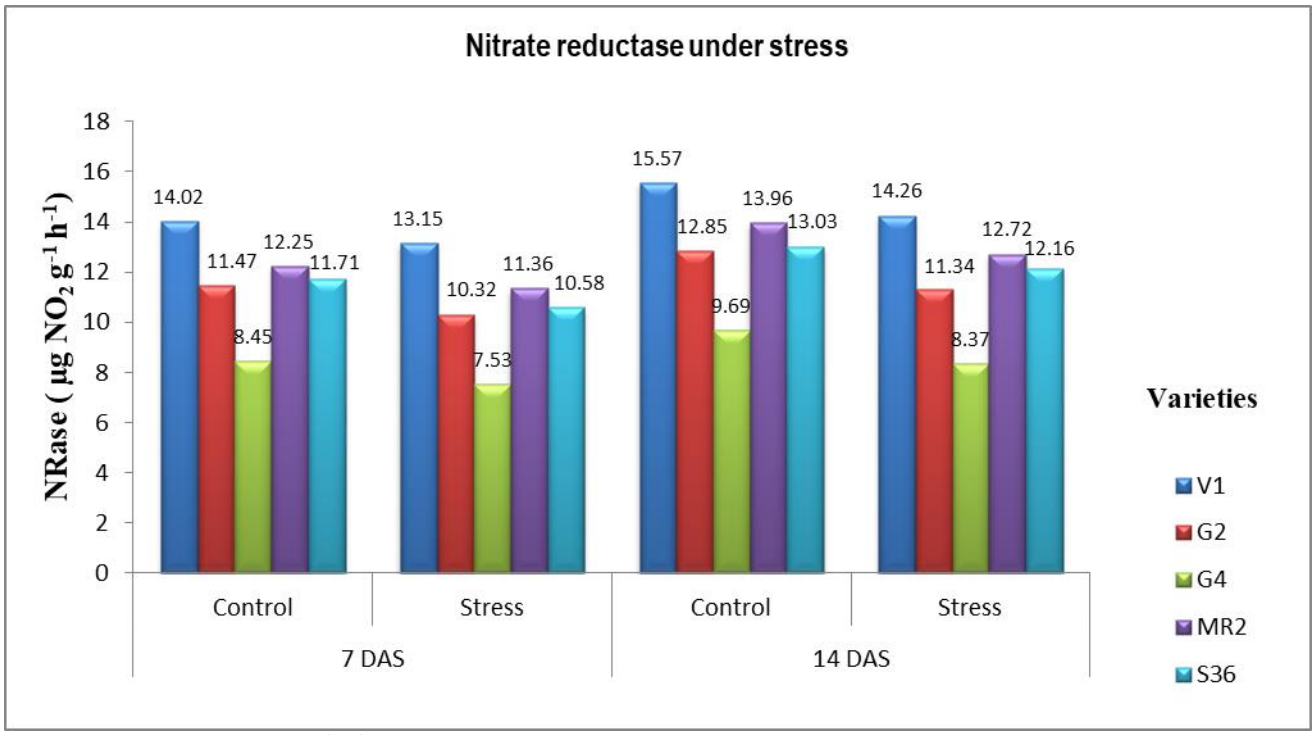

Figure 2 NRase (µg NO_2 g^{-1} h^{-1}) activity in mulberry varieties exposed to high temperature stress for two weeks interval

Table 2 Catalase (μg H_2O_2 $min^{-1}g^{-1}$) activity in mulberry varieties exposed to high temperature stress for two weeks intervals

Mulberry Varieties	7th DAS		14th DAS	
	Control	Stress	Control	Stress
V1	2.98±0.01	3.96±0.02	3.58±0.03	5.69±0.04
G2	2.58±0.02	3.38±0.01	2.78±0.02	3.56±0.02
G4	2.50±0.01	3.16±0.03	2.79±0.02	3.30±0.03
MR2	2.99±0.02	3.85±0.01	3.38±0.03	5.26±0.03
S36	2.75±0.02	3.76±0.02	3.26±0.03	5.31±0.04
P=CD(0.05) Variety	0.162**		0.043**	
Treatment	0.103**		0.0272**	
V X T	0.230(NS)		0.061**	
NS – Non significant. ** - Highly significant				

Table 3 Peroxidase (Δ 430 nm g^{-1} min^{-1}) activity in mulberry varieties exposed to high temperature stress for two weeks interval

Mulberry Varieties	7th DAS		14th DAS	
	Control	Stress	Control	Stress
V1	2.15±0.02	3.16±0.04	2.50±0.02	4.51±0.03
G2	1.98±0.01	2.54±0.03	2.16±0.01	3.57±0.02
G4	1.89±0.01	2.46±0.02	1.99±0.01	2.61±0.01
MR2	2.01±0.02	2.91±0.02	2.31±0.02	4.28±0.03
S36	2.11±0.02	3.01±0.02	2.36±0.02	4.26±0.03
P=CD(0.05) Variety	0.044**		0.038**	
Treatment	0.028**		0.024**	
V X T	0.062**		0.054**	
NS – Non significant. ** - Highly significant				

Conclusion

Mulberry is the primary host plant for silkworm *Bombyx mori* L. In today's climate change scenario, mulberry often gets exposed to high temperature stress during summer in Tamil Nadu. Hence, there is a need to develop temperature tolerant variety in mulberry for sericulture farmers. For a successful breeding programme, the basic physiological and biochemical mechanism underlying high temperature stress tolerance in mulberry has to be explored. The nitrate reductase activity of the leaf showed an increasing trend under control while it decreased under high temperature stress. The reduction in NRase activity was more in the variety G4 (15.77%). The variety S36 recorded lesser reduction in NRase activity (7.15%) followed by V1 (9.18%). Regarding the levels of catalase (CAT) and peroxidase (POX) activity is increased over control indicating the higher production of reactive oxygen radicals under heat stress. The varieties G2 and G4 showed very less enzyme activities which indicates their susceptibility to heat stress treatments.

References

[1] Hall, A. E. (2001). Crop Responses to Environment. Boca Raton, Florida: CRC Press LLC.

[2] Giaveno, C., and Ferrero, J. (2003). Introduction of tropical maize genotypes to increase silage production in the central area of Santa Fe, Argentina. Crop Breeding and Applied Biotechnology, 3(2).

[3] Singh, P., and Sawhney, S. K. (1989). Nitrate reduction in plants. Somerset, UK: earch Studies Press.

[4] Sairam, R. K., and Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci, 86, 407-421.

[5] Chaitanya, K. V., Sundar, D., Masilamani, S., and Reddy, A. R. (2002). Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. Plant Growth Regulation, 36(2), 175-180.

[6] Sairam, R. K., Srivastava, G. C., and Saxena, D. C. (2000). Increased antioxidant activity under elevated temperatures: a mechanism of heat stress tolerance in wheat genotypes. Biologia Plantarum, 43(2), 245-251.

[7] Larkindale, J., and Huang, B. (2004). Changes of lipid composition and saturation level in leaves and roots for heat-stressed and heat-acclimated creeping bentgrass (Agrostis stolonifera). Environmental and Experimental Botany, 51(1), 57-67.

[8] Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. (2004). Reactive oxygen gene network of plants. Trends in plant science, 9(10), 490-498.
[9] Bates, L. S., Waldrew, R. R., and Teare, I. D. (1973). Rapid determination of free proline for water stress studies. Plant Soil, 39, 113-127.
[10] Nicholas, J. C., Harper, J. E., and Hageman, R. H. (1976). Nitrate reductase activity in soybeans. Effect of light and temperature. Plant Physiology, 58, 731-735.
[11] Aebi, H. (1974). Catalases. In Bergmeyer HU. Methods of enzymatic analysis, 2, 673-684.
[12] Perur, N. G. (1962). Measurement of peroxidase activity in plant tissues. Curr. Sci, 31, 71-81.
[13] Angelini, R., Manes, F., and Federico, R. (1990). Spatial and functional correlation between diamineoxidase and peroxidase activities and their dependence upon etiolation and wounding in chickpea stems. Planta, 182, 89-96.
[14] Gomez, K. A., and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research. NewYork, USA.
[15] Matysik, J., Alia, Bhalu, B., and Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Current Science, 525-532.
[16] Chaitanya, K. V., Sundar, D., and Reddy, A. R. (2001). Mulberry leaf metabolism under high temperature stress. Biologia Plantarum, 44(3), 379-384.
[17] Kouadio, J. Y., H.T. Kouakou, M. Kone, M. Zouzou, and Anno, P. A. (2007). Optimum conditions for cotton nitrate reductase extraction and activity measurement. African Journal of Biotechnology, 6, 923-928.
[18] Heuer, B., Plaut, Z., and Federman, E. (1979). Nitrate and nitrite reduction in wheat leaves as affected by different types of water stress. Physiologia plantarum, 46(4), 318-323.
[19] Sairam, R. K., P.S. Desmukh, and Shukla, S. (1997). Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J. Agron. Crop Sci, 178, 171-177.
[20] Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci, 7, 405-410.

Publication History
Received 16.08.2022
Revised 29.12.2022
Accepted 29.12.2022
Online 31.12.2022