International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 5, No. 5, p. 135-147, 2014

RESEARCH PAPER OPEN ACCESS

# Diverse antioxidative effects in Pui vegetable (*Basella alba*) induced by high temperature stress

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**Article published on November 23, 2014**

**Key words:** Temperature stress, Metabolic effects, *Basella alba*, Adaptive response.

## Abstract

*Basella alba* is a green vegetable and grows in both winter and summer; however the temperature sensitivity on metabolic regulation in this species is not clarified. To identify the physiological mechanisms involved in regulation of adaptive response and anti oxidative effects in leaves of *Basella alba*, plants grown in pot were exposed to high temperature (45 $^{o}$C) for 24h, 48h and 72h periods and polyphenol oxidase (PPO) and peroxidase (POD) activities in leaves were examined. High temperature causes the higher activity of PPO and the activity was found to be potential after prolonged exposure when compared to the respective controls. Dose response characteristics of substrate on PPO activity were performed. The activity was higher at 10 mM catechol, substrate for the enzyme, than 100 mM and 200 mM concentration, however, the three doses yielded the gradual increase in activity. Conversely, POD activity in leaf was regulated reciprocally and found to be prevented up to 72h of treatment however the effects were appeared to be pronounced after 48h of exposure. The above findings demonstrate that assay of PPO and POD in response to high temperature is an index for characterization of anti oxidative effects in this species of plant and will give a new insight for adaptive response to the adverse environment.

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**Introduction**

Basic stresses such as drought, salinity, temperature and chemical pollutants are simultaneously acting on the plants causing cell injury and producing secondary stresses such as osmotic and oxidative ones (Wang *et al.*, 2003; Abu-Khadejeh *et al.*, 2012). Plants could not change their sites to avoid such stresses but have different ways and morphological adaptations to tolerate these stresses. Environmental stress can disrupt cellular structures and impair key physiological functions of plants. Drought, salinity and low temperature stress impose an osmotic stress that can lead to turgor loss. Membranes may become disorganized, proteins may undergo loss of activity or be denatured and often excess levels of reactive oxygen species (ROS) are produced leading to oxidative damage. Recent investigations reveal that high temperature induced injury is associated with the formation of oxidative stress which leads to activation of enzymes involved in the production of reactive oxygen species (ROS) (Zhu, 2002; Cushman and Bohnert, 2000). To prevent the oxidative damage caused by such abiotic stress, plants generate the different mechanism by which they survive in such critical environment. Anti oxidative enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are the most important components in the scavenging system of ROS. Several lines of evidences reveal that anti oxidative enzymes and anti oxidant molecules can neutralize ROS (Oidaira *et al.*, 2000; Lee and Lee, 2000). Polyphenol oxidase (PPO) and peroxidase (POD) have been widely recognized to be an anti oxidative causing the biosynthesis of diverse metabolites essential for diagnosis and other purposes and have been found to be involved in scavenging system of reactive oxygen species synthesized in the biological system. Polyphenol oxidases are enzymes with molecular weight of 60 kDa located in the chloroplast bound to thylakoid membranes, belonging to a group of copper containing metalloproteins and are members of oxido-reductases that catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen (Queiroz *et al.*, 2008). In presence of atmospheric oxygen and PPO, monophenol is hydroylated to o-diphenol and diphenol can be oxidized to o-quinones which then undergo polymerization to yield dark brown polymers (Chisari *et al.*, 2007). Peroxidases are a single-polypeptide chain, haemcontaining enzymes with molecular weight between 28 to 60 kDa and have been involved to oxidize a wide variety of organic and inorganic substrates by reducing $H_2O_2$ and peroxides. They are mainly located in the cell wall (Chen *et al.*, 2002) and are one of the key enzymes controlling plant growth and development. During drought or high temperature in the environment, these two enzymes might be involved in the prevention of oxidative damage in plant and therefore could be an essential index for the adaptive mechanism in adverse circumstances.

*Basella alba* (Pui) is a very soft leafy common vegetable available in Bangladesh and grows both in summer and winter and therefore, both seasons were believed to be involved in regulating metabolic alterations in this species of vegetable. The diverse clinical importance of this plant were demonstrated by recent investigations (Roshan *et al.*, 2012; Premalatha *et al.*, 2005). In response to high temperature, these species of plant have been found to survive in the atmosphere although the physiological mechanism of survival is not clarified. It has been revealed that temperature variation is a common environmental phenomenon causing diverse metabolic alterations in plants and other organisms (Janska *et al.*, 2010; Chaitanya *et al.*, 2001). Changes in environmental temperature affect the plant kingdom either by suppression of their total growth and development or by augmenting diverse physiological, metabolic and superficial changes. Moreover, high temperature has been recognized to be involved in metabolic regulation and has been shown to cause the synthesis of ROS in plants (Mahajan and Tuteja, 2005). Therefore, it is assumed that variation of temperature may affect both metabolic activities as well as its biological importance of this species of plant. The aim of this study is to examine the interrelationship between anti oxidative status and preventive mechanism against

temperature stress causing cell injury and physiological alterations in this vegetable and both PPO and POD might be involved in playing the critical role in this respect. Therefore, the current investigation has been undertaken to find the role of high temperature exposure on the regulation of metabolic functions regarding the alteration and synthesis of PPO and POD in leaf of *Basella alba* and may assist in the clarification of such stress induced mechanisms.

**Materials and methods**

*Plant materials and high temperature treatment*

For this experiment, two plastic pots were used; each pot size was 70 cm in diameter and 24 cm in height. An adequate amount of soil was taken in each plastic pot and the plastic pots were seeded with *Basella alba*. For the germination of seeds, the following points were carried out: i) the strong seeds were selected; the seeds were added to normal water and the floating seeds were discarded; ii) the seeds were kept in normal water with temperature below 37 $^{o}$C for overnight; iii) the seeds which were swollen by water absorption, were expected to be effective for germination; iv) the seeds were seeded in the pots prepared with soil and the efficiency of seed germination was 65%–75%. After 20 days of germination, the two different pots were described as control and high temperature induced plant. Control pot was used for 24h, 48h and 72h treatments in room temperature, however, the temperature was maintained 30 $^{o}$C by using air cooling system (AC) already fixed in the room and without giving any high temperature. The second pot was used similarly for 24h, 48h and 72h duration in the plastic chamber and was exposed to 45 $^{o}$C with full aeration along with sufficient water. To maintain this temperature, electric bulbs (2×200 W) were connected to the chamber. After the treatments, the leaves were collected consecutively from each pot for 24h, 48h and 72h duration and kept in 80 $^{o}$C.

*Assay of polyphenol oxidase (PPO) activity*

The leaves of the different treatments (24h, 48h and 72h) and their respective controls were homogenized with 22 mL of distilled water in a mortar kept on ice. Approximately, 1.5 g of high temperature induced and their respective control leaves were used for homogenization. The homogenates were centrifuged at 9000 rpm for 15 min and the supernatants were used as crude extract for assay of PPO activity spectrophotometrically as described by Mahadevan and Sridhar (1982) based on an initial rate of increase in absorbance at 495 nm where, catechol was used as substrate. One unit of enzyme activity is defined as a change in absorbance of 0.001 minute$^{-1}$ mL$^{-1}$ of enzyme extract. For determination of PPO activity in leaf, 3 mL of 0.1 M phosphate buffer (pH 6.0) and 2 mL of crude enzyme extract were taken in the test tube and kept on ice. The contents were mixed, placed in a spectrophotometer using a cuvette and the absorbance was adjusted to zero at 495 nm. The cuvette was removed, 1 mL of catechol (10 mM, 100 mM and 200 mM) was added, quickly mixed by inversion and the changes in absorbance at 495 nm were recorded for up to 3 minutes (1, 2, 3 min). In all experiments, three replicates were performed for each sample. The following calculation was used to assay PPO activity in a sample: change in $A_{495} = A_f - A_i$, where, $A_i$ = initial absorbance reading and $A_f$ = final absorbance reading. Change in $A_{495}$ for each sample was used to calculate the units of PPO activity and the activity is expressed as Unit g$^{-1}$ of leaf weight.

*Assay of peroxidase (POD) activity*

The leaves of the different treatments (24h, 48h and 72h) and their respective controls were homogenized with 15 mL of 0.5 M phosphate buffer (pH 7.0) in a mortar kept on ice. Approximately, 1.5 g of high temperature induced and their respective control leaves were used for homogenization. The homogenates were centrifuged at 9000 rpm for 15 min and the supernatants were used as crude extract for assay of POD activity as described by Daz *et al.* (2001). Peroxidase activity was determined spectrophotometrically at 470 nm using guaiacol as a phenolic substrate with hydrogen peroxide. The enzymatic oxidation of guaiacol changed the substrate into orange-pink product which was measured by spectrophotometer as a change in absorbance of

0.001 $min^{-1}$ and the absorbance was recorded for up to 4 min. For determination of POD activity in leaf, 2.5 mL of 0.1 M phosphate buffer (pH 7.0), 2 mL of crude enzyme extract and 0.6 mL of 1% (v/v) $H_2O_2$ were taken in a test tube and kept on ice. The spectrophotometer was adjusted to zero initially and the content of test tube was transferred into a cuvette and the absorbance was taken as initial reading. 0.6 mL of 4% (v/v) guaiacol was added to the cuvette, quickly mixed by inversion and the change in absorbance at 470 nm was measured for 1, 2, 3 and 4 min. In all experiments, three replicates were performed for each sample. The following calculation was used to assay peroxidase activity in a sample: change in $A_{470} = A_f - A_i$, where, $A_i$ = initial absorbance reading and $A_f$ = final absorbance reading. Change in $A_{470}$ for each sample was used to calculate the units of POD activity and the activity was expressed as Unit $g^{-1}$ of leaf weight.

*Statistical analysis*

Results of the experiments were expressed as mean and standard error of different groups based on three independent determinations. The differences between the mean values were evaluated by ANOVA followed by paired *t*-test using SPSS software.

**Results**

*Effect of 10 mM substrate concentration on PPO activity in leaf induced by high temperature*

To properly identify physiological responses to environmental stress such as high temperature, plants were exposed to 45 $^0$C in the temperature controlled hot chamber for 24h, 48h and 72h periods and the respective controls were kept in ambient room temperature (30 $^0$C). Polyphenol oxidase activities in leaf exposed to high temperature for the above mentioned periods were examined at 10 mM catechol, substrate for the enzyme. As shown in Table 1, the average PPO activity in leaf of vegetable in response to high temperature for 24h period was 315.28 ± 20.66 Unit $g^{-1}$ of leaf whereas for control leaf kept in ambient temperature, the PPO activity was 227.92 ± 20.24 Unit. A significant 38.33% ($p < 0.01$) increased PPO activity was observed after 24h when compared to the control plant (Figure 1A).

**Table 1.** Effect of high temperature on the regulation of PPO activity in leaf of Pui vegetable. The plants were exposed to 45 $^0$C for 24h, 48h and 72h in the experimental hot chamber. After the treatment, the plants were immediately removed from the chamber and sampling of leaf was performed. For assay of PPO activity, 10 mM concentration of catechol was used as a substrate of the enzyme. Control plants were similarly used except giving high temperature exposure.

| Treatments | Polyphenol oxidase (PPO) activity (Unit $g^{-1}$ of leaf) |
|---|---|
| Control | 227.92 ± 20.24 |
| 24h | 315.28 ± 20.66$^A$ |
| Control | 288.67 ± 9.80 |
| 48h | 346.15 ± 29.97$^B$ |
| Control | 255.55 ± 20.03 |
| 72h | 905.62 ± 189.24$^B$ |

The results are means of ± SE for three values in each group. $^A p < 0.01$, $^B p < 0.05$ versus respective control.

The results appeared to indicate that the PPO activities were affected by high temperature acclimation. Therefore, it is reasonable that an adaptive response by the species of plant was created and the higher synthesis of PPO was observed to serve as the factor in adverse environmental situation and might be sensitive to the temperature variation. Leaves of *Basella alba* were exposed to high temperature for 48h period and the average PPO activity was 346.15 ± 29.97 Unit while for the respective control plant, the enzyme activity was 288.67 ± 9.80 Unit $g^{-1}$ of leaf. The results indicated that 19.99% ($p < 0.05$) increased PPO had been found after 48h in response to high temperature compared to the control plant as illustrated in Figure 1A. The

increased synthesis of PPO in leaf in response to high temperature might be involved in the regulation of metabolic functions of this species of plant. The alteration of PPO level in leaf is an index for characterization of the sensitivity to the environmental temperature. To find the optimum effect of high temperature exposure on PPO level in leaf, the extended time was 72h. As shown in Table 1, the high temperature induced leaf had PPO level 905.62 ± 189.25 Unit while for the respective control leaf, the average PPO level was 255.55 ± 20.03 Unit $g^{-1}$ of leaf. The results showed that the PPO level in leaf had been enhanced significantly ($p < 0.05$) by 254.37% when the plants were exposed to high temperature for 72h when compared to control (Figure 1A). The results appeared to indicate that the PPO levels were severely affected by high temperature acclimation for prolonged exposure, and seem to be higher than 24h or 48h period, therefore reasonably assumed to be maximally enhanced after 72h of high temperature exposure. Of course, further extension of time may clarify the mechanism of enhancing the synthesis of PPO in response to high temperature. The results suggest that the increased PPO induced by high temperature might be caused by such abiotic stress and could be considered as the survival factor for this species of plant in critical environment.

**Table 2.** Effect of high temperature on the regulation of PPO activity in leaf of Pui vegetable. The plants were exposed to 45 °C for 24h, 48h and 72h in the experimental hot chamber. After the treatment, the plants were immediately removed from the chamber and sampling of leaf was performed. For assay of PPO activity, 100 mM concentration of catechol was used as a substrate of the enzyme. Control plants were similarly used except giving high temperature exposure.

| Treatments | Polyphenol oxidase (PPO) activity (Unit $g^{-1}$ of leaf) |
|---|---|
| Control | 2588.96 ± 94.05 |
| 24h | 2929.29 ± 30.33[A] |
| Control | 2739.62 ± 28.30 |
| 48h | 3121.15 ± 46.15[A] |
| Control | 2727.77 ± 100.15 |
| 72h | 3982.51 ± 162.15[A] |

The results are means of ± SE for three values in each group. [A] $p < 0.05$, versus respective control.

**Table 3.** Effect of high temperature on the regulation of PPO activity in leaf of Pui vegetable. The plants were exposed to 45 °C for 24h, 48h and 72h in the experimental hot chamber. After the treatment, the plants were immediately removed from the chamber and sampling of leaf was performed. For assay of PPO activity, 200 mM concentration of catechol was used as a substrate of the enzyme. Control plants were similarly used except giving high temperature exposure.

| Treatments | Polyphenol oxidase (PPO) activity (Unit $g^{-1}$ of leaf) |
|---|---|
| Control | 4482.46 ± 55.74 |
| 24h | 4809.55 ± 101.47 |
| Control | 4539.62 ± 122.58 |
| 48h | 5250.00 ± 40.38[A] |
| Control | 5061.11 ± 181.13 |
| 72h | 6941.25 ± 129.74[A] |

The results are means of ± SE for three values in each group. [A] $p < 0.05$ versus respective control.

*Effect of 100 mM substrate concentration on PPO activity in leaf induced by high temperature*

The activity of PPO is related to the concentration of substrate and therefore, to get the maximal response of enzyme, 100 mM of catechol was used. As shown in Table 2, the PPO activity in response to 100 mM catechol in leaf of *Basella alba* was recorded to determine the effect of high temperature on the regulation of this enzyme activity. After 24h of treatment, the leaf enzyme level was calculated as

2588.96 ± 94.05 Unit for control and for high temperature induced plant, the value was 2929.29 ± 30.33 Unit $g^{-1}$ of leaf. High temperature causes a significant ($p < 0.05$) increase (13.14%) in PPO level when compared to the respective control, however, the response was found to be lower than 10 mM substrate concentration for the respective period of exposure (Figure 1A and 1B). The increase in PPO activity determines the higher anti oxidative status for the prevention of high temperature induced physiological stress of the plant. It is important to note that higher the oxidative stress, higher the synthesis of the enzyme thereby the plants survive in the adverse environment. To find the maximal response, plants were exposed to high temperature (45 $^{o}$C) for 48h period and the enzyme activity in leaf was 3121.15 ± 46.15 Unit, while for the respective control plant for the above mentioned time, the activity was recorded as 2739.62 ± 28.30 Unit $g^{-1}$ of leaf. The results indicated that PPO activity in leaf had been increased by 13.92% significantly ($p < 0.05$) when compared to control; however, the activity was slightly higher than that of 24h period. Compared to 10 mm substrate concentration for the similar time, the PPO activity had been demonstrated to be lower (Figure 1A, 1B). The increased activity in response to high temperature determines the higher conversion of phenolic compounds to the desirable products as this enzyme has higher specificity for the oxidation of phenolic compounds. Higher activity of PPO is an essential parameter for producing the colored pigment substantial for industrial and other purposes. Although the higher activity of PPO in response to the increased concentration of substrate was observed, the percentage of increase in activity in response to high temperature was found to be lower (Table 1, Table 2 and Figure 1A, 1B). In response to high temperature for 72h, the PPO activity in leaf was 3982.51 ± 162.15 Unit while the respective control leaf had 2727.77 ± 100.15 Unit enzyme activity for the above mentioned time. The results (shown in Table 2 and Figure 1B) demonstrated that prolonged exposure of high temperature had been involved in synthesis of enzyme in leaf of this species of plant and the activity was increased by 45.99% significantly ($p < 0.05$) when compared to the respective control, however the efficiency of high temperature on synthesis of enzyme was found to be higher than that of 24h or 48h period (Figure 1B) and the activity of this enzyme seems to be increased time dependently.

**Table 4.** Changes of POD activity in leaf of Pui vegetable exposed to high temperature. The plants were exposed to 45 $^{o}$C for 24h, 48h and 72h in the experimental hot chamber. After the treatment, the plants were immediately removed and sampling of leaf was performed. Control plants were similarly used except giving high temperature exposure.

| Treatments | Peroxidase (POD) activity (Unit $g^{-1}$ of leaf) |
|---|---|
| Control | 2275.54 ± 311.40 |
| 24h | 1964.83 ± 53.04 |
| Control | 2558.32 ± 34.27 |
| 48h | 1755.34 ± 70.06$^{A}$ |
| Control | 3969.01 ± 37.91 |
| 72h | 3332.81 ± 41.19$^{B}$ |

The results are means of ± SE for three values in each group. $^{A}$ $p < 0.01$ versus respective control. $^{B}$ $p < 0.001$ versus respective control.

*Effect of 200 mM substrate concentration on PPO activity in leaf induced by high temperature*

To find the physiological role of high temperature on PPO activity in leaf of *Basella alba*, we further determined PPO activity after 24h, 48h and 72h periods of high temperature exposure in response to the higher dose of substrate. Table 3 shows the effect of high temperature on PPO activity after 24h of treatment. Plants exposed to high temperature had PPO level 4809.55 ± 101.41 Unit where as for control, the PPO activity 4482.46 ± 55.74 Unit $g^{-1}$ of leaf was

observed. The results indicated that the enzyme activity of *Basella* leaf had been increased by 7.29% for high temperature treatment when compared to the respective control. Acclimation to 45 °C similarly causes the enhanced PPO synthesis in prolonged time (48h) and the plants may survive in such critical environment either by synthesis of PPO in their tissues or by other phenomenon. Figure 1C also shows that the PPO level was increased significantly ($p < 0.05$) by 15.64% when they were exposed to 45 °C compared to the respective control where the values were 4539.62 ± 122.58 Unit and 5250.00 ± 40.38 Unit $g^{-1}$ of leaf respectively for control and high temperature acclimation. After 72h of treatment, high temperature causes the synthesis of PPO enzyme and the activity was 6941.25 ± 129.74 Unit while for the control leaf the value was recorded to be 5061.11 ± 181.13 Unit $g^{-1}$ of leaf showing the higher synthesis of enzyme after 72h period and enhanced significantly ($p < 0.05$) by 37.14% when compared to the respective control however the activity was not declined after 72h of exposure rather increased than the 24h or 48h of exposure of high temperature and time dependently. The results are also illustrated in Figure 1C when expressed as percentage of control. Although the higher PPO level in leaf in response to higher dose of substrate was demonstrated during the experiment (Table 1, Table 2 and Table 3), the efficiency of high temperature on enhancing increased activity of PPO was assumed to be optimal and higher at 10 mM substrate concentration for 72h of exposure. Therefore, the faster increase in enzyme activity in leaf in response to high temperature acclimation might be dependent on the substrate concentration as well as the period of exposure and might be involved in regulation of metabolism of phenolic substrates by which the plants survive in such a critical environment and gives a new insight for the prevention of the oxidative stress and might be involved to give a signal to the physiological level.

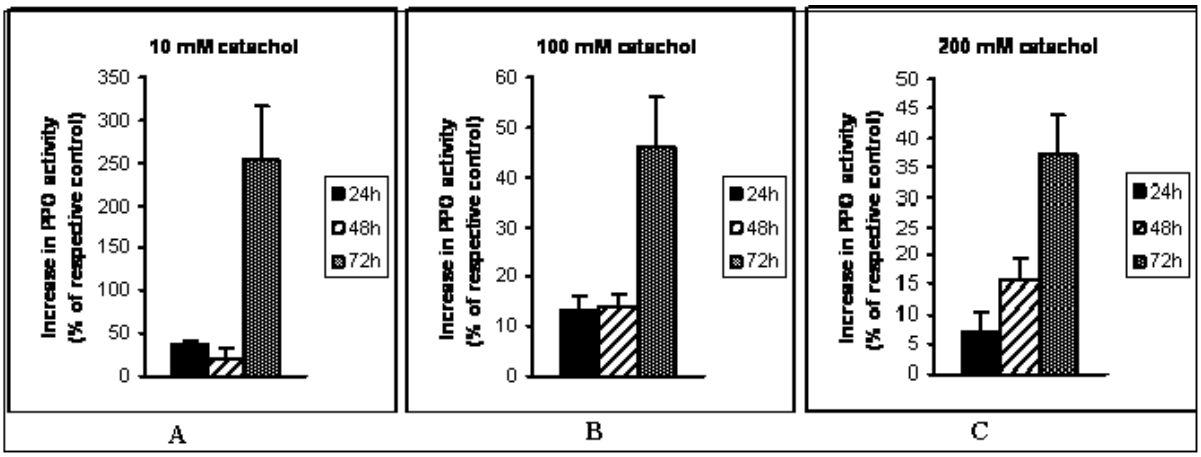


**Fig. 1.** Alteration of PPO activity in response to 10 mM [A], 100 mM [B] and 200 mM [C] catechol in leaves of *Basella alba* during high temperature acclimation. The plants were exposed to 45 °C for 24h, 48h and 72h in hot chamber, however, the respective controls were used without any high temperature exposure. After the treatments, the leaves of plants and their respective controls were used for the assay of PPO activity. The results are expressed as percentage of the respective controls.

*Time course effect of high temperature on POD activity in leaf of Basella alba*

Anti oxidative enzymes involved in scavenging system of reactive oxygen species (ROS) include peroxidase. They are a family of isoenzymes found in almost all plants; they are heme-containing monomeric glycoproteins that utilize either $H_2O_2$ or $O_2$ to oxidize a wide variety of molecules. Peroxidase is an oxidoreductase that is directly involved in many plant functions such as hormone regulation, defense mechanisms, indolacetic degradation and lignin biosynthesis. Therefore, during high temperature acclimation, peroxidase might be involved in enzymatic defense of plant cells. To examine the role

of high temperature on the regulation of POD activity in leaf of *Basella alba*, plants in the pot were exposed to 45 °C in temperature controlled chamber for 24h period and the respective control was kept in ambient room temperature (30 °C). The average POD activity in response to high temperature was 1964.83 ± 53.04 Unit $g^{-1}$ of leaf whereas for control leaves, the POD activity was 2275.54 ± 311.40 Unit. The results demonstrated that POD activity in leaf had been reduced (13.65%) by high temperature compared to the respective control (shown in Table 4 and Figure 2). The decrease in activity in response to high temperature might be the regulatory mechanism of enhancing the synthesis of compounds responsible for browning color since the enzyme has higher specificity for the phenolic substrate guaiacol. The higher the activity of this enzyme, higher the conversion of the phenolic substrate to colored o-quinones.

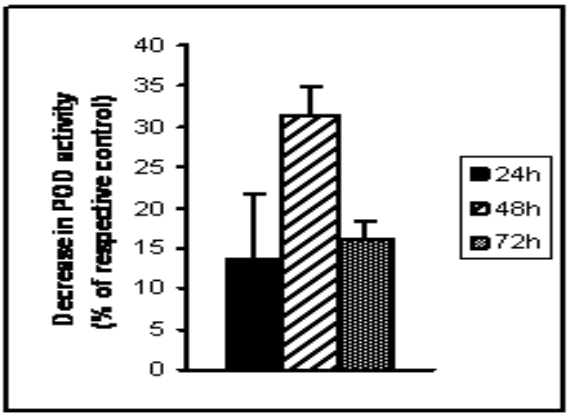


**Fig. 2.** Alteration of POD activity in leaves of *Basella alba* during high temperature acclimation. The plants were exposed to 45 °C for 24h, 48h and 72h in hot chamber, however, the respective controls were used without any high temperature exposure. After the treatments, the leaves of plants and their respective controls were used for determination of POD activity. The results are expressed as percentage of the respective controls.

As shown in Table 4, the POD activity in leaves of plant was recorded to determine the effect of high temperature on POD synthesis for prolonged exposure. After 48h of treatment, the leaf POD level was estimated as 2558.32 ± 34.27 Unit for control and for high temperature induced plant, the value was 1755.34 ± 70.06 Unit $g^{-1}$ of leaf. High temperature causes a significant and more pronounced decrease in POD activity in leaf by 31.38% ($p < 0.01$) (Figure 2) when compared to the respective control. The decrease of POD activity in leaf was found to be higher than the previous 24h of exposure as demonstrated in Figure 2. Therefore, the activity of this enzyme in leaf is assumed to be regulated by the variation of temperature and be strictly followed by the extension of time.

Table 4 also shows the effect of high temperature on POD activity in leaf of plant after 72h of exposure. Plants acclimated to high temperature had leaf POD level 3332.81 ± 41.19 Unit, whereas 3969.01 ± 37.91 Unit $g^{-1}$ of leaf for control plant was observed during the experiment. As the time extended, the average POD activity in leaf had been enhanced in response to 45 °C and was increased both for control and high temperature induced plant. High temperature for prolonged exposure causes decreased synthesis of POD significantly ($p < 0.001$) by 16.02% compared to the control (Figure 2), however, the effect was lower than the previous 48h of exposure and higher than 24h. Although the optimum reduced activity of POD in leaf was observed after 48h of exposure in response to high temperature, the gradual increase in activity for both the control and high temperature acclimated plant was noted as the time extended. Therefore, the synthesis of POD in leaf was found to be augmented time dependently however, high temperature causes the deactivation of this enzyme in this species. The results suggest that the reduced POD activity in leaf might be due to the higher sensitivity of temperature and caused by temperature stress in the environment where they survive and could be considered as the survival factor as well as index for characterization of physiology of leaf of this species of plant.

**Discussion**

To understand the mechanism of plant species responses to high temperature regarding the physiological and adaptive responses, assay of PPO and POD activity in leaf of *Basella alba* was performed. In this respect, plants were grown in pot

and exposed to 45 ° C for 24h, 48h and 72h periods. In the present study, high temperature has been found to be involved in causing higher PPO activities in leaf however the effects were more pronounced after prolonged exposure. On the contrary, POD activity in leaf was reversibly regulated in response to high temperature and found to be deactivated, however the effects were more pronounced after 48h of the exposures. The regulatory mechanism of activation and deactivation of these enzymes in response to the temperature stress is not known in this species of plant, however, several lines of evidences might be involved to clarify and recognize the formation of these molecules in such adverse situation. It has been shown that high temperature causes the higher oxidative stress inducing the synthesis of reactive oxygen species (ROS) (Mahajan and Tuteja, 2005) and increases tolerance to ROS in plants and with an increase in anti oxidative enzymes (Zhu, 2002). Anti oxidative enzymes can neutralize ROS and thereby prevents the cellular membranes and organelles from the damaging effects of ROS. It is reasonable that fluctuation of temperature can cause stress to the normal physiological functions of plants, and hence alteration of metabolic activities in leaf of the plant might be observed. High temperature induced ROS can cause extensive peroxidation and de-esterification of membrane lipids, as well as lead to protein denaturation and mutation of nucleic acids (Bowler *et al*., 1992), therefore the reduced POD in response to high temperature in leaf in the present study might be due to the above reasons. Moreover, peroxidase inactivation was observed in some species of plants and was faster at the higher temperature (Muftugil, 1985). *Basella alba* is a common green vegetable grown in Bangladesh and other countries during both winter and summer seasons. Therefore, the plants have the higher sensitivity to these temperatures; however, the plants survive in very hot environment although the mechanism is not clarified. Since high temperature causes the significant alteration in metabolic functions of plant and has been revealed to cause reactive oxygen species, therefore might be involved in causing the synthesis of diverse metabolites essential for various purposes. The reactive oxygen species have been shown to cause the injury in plants during the critical circumstance and therefore, to survive in this environment, plants generate different mechanisms and synthesize the compounds.

To find the optimum effect of catechol, dose response characteristics of substrate for the enzyme PPO have been adopted in this study. Among the different concentration of catechol, 10 mM concentration has been found to cause the higher response although the three different concentrations of catechol (10 mM, 100 mM and 200 mM) produced the gradual increase in PPO activity for both the control and high temperature induced plants showing the validity of the substrate effect. Accordingly, the higher dose of catechol enhanced the PPO activity maximally in leaf extract therefore, higher colored quinones were synthesized which might be an effective approach for producing the essential pigments. Polyphenol oxidase catalyzes the oxidation of phenol to quinone which can covalently modify and crosslink various cellular nucleophiles, undergo melanin-forming auto oxidation reactions, or participate in other reactions. The enzymes are found in higher plants and responsible for the enzymatic browning of raw fruits and vegetables. Such reactions are generally considered to be undesirable in food preservation and processing because of the unpleasant appearance and the concomitant development of a substandard flavor. Several lines of evidences revealed that PPO had been considered to be an important reagent for clinical diagnosis and micro-analytical immunoassays because of its high sensitivity (Siers, 1991) while many fruits and vegetables contain POD in amounts that contribute to browning-like reactions (Vamos-Vigyazo, 1981). Although PPO is involved primarily in the degradation of phenolics, they can also be degraded by POD (Thypyapong *et al*., 1995) and the activities of both enzymes have been found to be increased in response to biotic and abiotic stresses (Kwak *et al*., 1996). Both PPO and POD have been considered in defensive mechanisms for plants against stress (Vamos-Vigyazo, 1981). Oxidative

stress can arise from an imbalance between the generation and elimination of reactive oxygen species (ROS), leading to excess ROS levels that causes indiscriminate damage to virtually all biomolecules, leading, in turn, to various diseases and cell death (Scandalios, 2005). Reactive species can be eliminated by a number of enzymatic and non-enzymatic antioxidant defense mechanisms (Boullier *et al.*, 2001) therefore, the higher activity of PPO in response to high temperature in species of Pui vegetable might be linked to the defense mechanisms and the results are consistent with their findings.

Abiotic stress leads to a series of molecular, biochemical, physiological and morphological changes that adversely affect plant growth and productivity. High temperature is a major factor limiting the productivity and geographical distribution of many species, including important agricultural crops. Higher plants manifest a unique capability of the synthesis of a large amount of diverse molecules so-called secondary metabolites, such as phenolic compounds and the synthesis and release of phenolics are induced by various biotic and abiotic factors (Makoi and Ndakidemi, 2007). It has been demonstrated in the previous study that these enzyme activities are regulated in response to different types of stress, both biotic and abiotic (Yadegari *et al.*, 2007). More specifically, both enzymes have been related to the appearance of physiological injuries caused by thermal stress. During the experiment, it was observed that both high temperature induced and the respective controls caused the color pigmentation quickly, however high temperature induced leaves had lower pigmentation than the control during the peroxidase assay therefore, it is reasonable that high temperature acclimation causes the lower oxidation of phenolic compounds and might be an effective approach for the regulation of the synthesis of colored pigment essential for the several purposes. Of course, the phenomenon is a substantial mechanical and physiological way by which the plants survive in the adverse environment. The oxidation of phenolic compounds might be related to the temperature variation in the environment.

Metabolic adjustments in response to unfavorable conditions are dynamic and not only depend on the type and strength of the stress, but also on the cultivar and the plant species. Some metabolic changes are common to salt, drought, and temperature stress, whereas others are specific. Enzymatic browning is a significant problem in a number of fruits and vegetables such as strawberry (Chisari *et al.*, 2007), grape (Munoz *et al.*, 2004), potato (Lee and Park, 2007) and lettuce (Gawlik-Dziki *et al.*, 2007). The discoloration in fruits and vegetables by enzymatic browning, resulting from conversion of phenolic compounds to o-quinones which subsequently polymerize to be a brown or dark pigment and the enzymes involved these processes are PPO and POD (Jiang *et al.*, 2004). Because PPO and POD are the main enzymes involved in phenolic oxidation of many fruits and vegetables, their activities have attracted much attention. The relationship between the degree of browning and PPO activity were studied in processing apple varieties to provide reference for raw material selection (Ye *et al.*, 2007). Recent study reveals that high temperature acclimation adversely affects physiological and morphological structures of plants (Almeselmani *et al.*, 2006) and the nutritional deficiency has been observed in response to high temperature. Therefore, it is reasonable that adverse oxidative effects caused by high temperature acclimation might be correlated to the alteration of physiology of leaf of *Basella alba* and also to the nutritional deficiencies particularly the uptake of essential nutrients from the soil and also from the environment. Further studies are needed to clarify the mechanisms linked to the above approaches. Measurement of PPO and POD in leaf of *Basella alba* might be an essential approach and will give a new insight to clarify the mechanism of diverse metabolic functions of plant as well as help in analysis of physiology of *Basella alba*. Moreover, regulation of these enzymes is not only mediated by hot environment but also might be by other chemical mediators in the environment.

**Conclusion**

It is obvious from the current investigation that high

temperature induces diverse metabolic alterations regarding the enhancement of polyphenol oxidase and prevention of peroxidase activity in leaf therefore, assumes to be involved in the alteration of physiology of plants *Basella alba*. The adverse environment caused by high temperature produced the severe effect on the plants and thereby plants face the stress to physiological and molecular level and therefore, different regulatory metabolic alterations were observed in the circumstances. High temperature induced oxidative stress and injury is frequently observed in the critical environment and to overcome these effects, some enzymes are over expressed where PPO might be involved. Moreover denaturation of enzymes and proteins in response to high temperature is commonly observed, thereby peroxidase enzyme has been assumed to be deactivated in this species, therefore, the anti oxidative enzymes are reversibly regulated by high temperature which is an essential parameter for characterization for the prevention of the anti oxidative effects in this species of vegetables. Species adapted by natural selection to hot environments have evolved a number of physiological and morphological means to improve survival in the face of extended hot periods. During high temperature acclimation, plants possess nutritional or energy deficiency as they survive in such critical circumstances however, the complications might be due to the higher oxidative effects and the enzymes may play the critical role in this respect.

**Acknowledgement**

This study was carried out in the Department of Biochemistry and Molecular Biology, Rajshahi University and was supported by the University Grant Commission (UGC), Bangladesh.

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