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ORIGINAL ARTICLE

A regulatory approach on low temperature induced enzymatic and anti oxidative status in leaf of Pui vegetable (*Basella alba*)

Md. Shahidul Haque [a,*], Md. Monirul Islam [a], Md. Abdur Rakib [a], Md. Asraful Haque [b]

[a] *Department of Biochemistry and Molecular Biology, Laboratory of Protein and Enzyme Research, University of Rajshahi, Rajshahi 6205, Bangladesh*
[b] *Department of Biotechnology, BSMRAU, Salna, Gazipur, Bangladesh*

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Abstract *Basella alba* is a soft green vegetable, survives in adverse environmental circumstances, for example, very cold temperature although the mechanism and the temperature sensitivity in this species are not clarified. Pot experiment for cultivation of *B. alba* was carried out to examine the effects of low temperature on the synthesis of two enzymes, polyphenol oxidase (PPO) and peroxidase (POD) in leaf of this plant. They were exposed to 8 °C for 24 h, 48 h and 72 h periods and the respective controls were kept in ambient room temperature for the above mentioned time. Low temperature causes the higher activity of PPO and the threshold level was found after 48 h period when compared to the respective controls. The activity was higher at 10 mM catechol, substrate for this enzyme, than 100 mM and 200 mM concentration, however, the three doses yielded the gradual increase in activity. Similar stimulatory effects on peroxidase (POD) activity in leaf were observed whenever the plants were exposed to cold for 24 h, 48 h and 72 h periods and maximal after 48 h period. Our findings demonstrate that the higher activity of these enzymes in leaf might be an index for the regulatory mechanism of the survival of these species in such adverse environment.

1. 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

* Corresponding author. Tel.: +880 721 711109; fax: +880 721 750064.
E-mail address: mshaque1967@rocketmail.com (Md. Shahidul Haque).
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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 chilling induced injury is associated with the formation of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$) (Basra, 2001; Lee and Lee, 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 (PRX) 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 the 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 the presence of atmospheric oxygen and PPO, monophenol is hydroxylated 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, heme containing enzymes with molecular weight between 28 and 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 the cold 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 was demonstrated by recent investigations (Roshan et al., 2012; Premalatha and Rajgopal, 2005). In response to low 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). 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, low temperature has been recognized to be a major stimulatory effector 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 cold acclimation on the regulation of metabolic functions regarding the alteration and synthesis of PPO and POD in leaf of *B. alba* and may assist in the clarification of such stress induced mechanisms.

2. Materials and methods

2.1. Plant materials and low 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 *B. 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 °C 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 30 days of germination, the two different pots were described as control and low temperature induced plants. Control pot was used for 24 h, 48 h and 72 h treatments in the room temperature without cold acclimation. The second pot was used for 24 h, 48 h and 72 h duration in the temperature controlled cooling chamber and given cold exposure (8 °C) with full aeration. After the treatments, leaves were collected consecutively from each pot for 24 h, 48 h and 72 h duration and kept in −80 °C.

2.2. Assay of polyphenol oxidase (PPO) activity

The leaves of the different treatments (24 h, 48 h and 72 h) and their respective controls were homogenized with 22 mL of distilled water in a mortar kept on ice. Approximately, 1.5 g of low 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 min^{-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 min (1, 2, 3 min). In all experiments, three replicates were performed for each sam-