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REVIEW PAPER

Polyphenol oxidase and peroxidase activity in leaf of Pui vegetable induced by low and high temperature - a regulatory and comparative study

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Key words: Pui vegetable, polyphenol oxidase, temperature.

Abstract

Both biotic and abiotic stresses cause metabolic regulation and coordination in plants. To clarify its mechanism, a comparative study was performed where B alba grown in pot exposed to 8oC for 24h, 48h and 72h periods. For high temperature induced plants, they were exposed to 45oC with full aeration along with sufficient water. Plants exposed to 8 oC for 24h, 48h and 72h had higher PPO level and maximal after 48h of exposure. A dose response characteristic of substrate on PPO activity was observed up to 72h and the activity was maximal at 10 mM concentration although 100 and 200 mM concentration show higher specificity. The enhanced peroxidase activity was found and maximal at 48h of exposure. Similar stimulatory effects on PPO activity were observed whenever plants were exposed to high temperature for the above mentioned periods. The different doses of substrate (10, 100, 200 mM) caused the higher PPO activity in response to high temperature, however the effects were more pronounced after prolonged exposure and assumed to be lower than low temperature induced plants. On the contrary, POD activity was reciprocally regulated and deactivated compared to the respective control. The Km and Vmax values were altered in response to these stresses among different groups with different doses of substrates for PPO. The review article will give a new insight for adaptive and anti-oxidative response to low and high temperature and might be a regulatory mechanism for survival of this species.

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Introduction

Both biotic and abiotic stresses cause metabolic regulation and coordination in plants although the mechanism is not clarified. A comparative study was performed to examine the effects of low and high temperature on the antioxidative and enzymatic status in leaf of *B. alba*. Plants grown in pot were exposed to 8°C for 24h, 48h and 72h periods and the respective controls were kept in ambient room temperature while for high temperature induced plants they were exposed to 45°C with full aeration along with sufficient water. Plants exposed to 8 °C for 24h, 48h and 72h had higher polyphenol oxidase (PPO) level and maximal after 48h of exposure. A dose response characteristic of substrate on PPO activity was observed up to 72h and the activity was maximal at 10 mM concentration although 100 and 200 mM concentration show higher specificity. The enhanced peroxidase activity was found and maximal at 48hof exposure. Similar stimulatory effects on PPO activity were observed whenever plants were exposed to high temperature for the above mentioned periods. The different doses of substrate (10, 100, 200 mM) caused the higher PPO activity in response to high temperature, however the effects were more pronounced after prolonged exposure and assumed to be lower than low temperature induced plants. On the contrary, peroxidase (POD) activity was reciprocally regulated and deactivated compared to the respective control. The K_m and V_{max} values were altered in response to these stresses among different groups with different doses of substrates for PPO. The review article will give a new insight for adaptive and antioxidative response to low and high temperature and might be a regulatory mechanism for survival of this species.

Extreme low and high temperature are harmful to the plants and other organisms causing adverse effects in physiological and molecular level however plants survive in such adverse environment through different mechanism and is not clarified well. Recent study reveals that 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 impairs key physiological functions of plants. 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. Similarly, high temperature stress causes some physiological, biochemical and molecular changes in plant metabolism such as protein denaturation, lipid liquefaction or perturbation of membrane integrity. Plant responses to environmental stress like high temperature have also been associated with activated forms of oxygen, including hydrogen peroxide, singlet oxygen, superoxide, and the hydroxyl radical (Anderson, 2002). Heat stress induces or enhances the active oxygen species-scavenging enzymes like superoxide dismutase, catalase, peroxidase and several antioxidants (Chaitanya *et al*., 2002). Therefore, it is well recognized that both low and high temperature is critically involved in diverse metabolic regulation and the oxidative stress caused by low or high temperature therefore, is involved in inducing the synthesis of ROS which damages and injury to the plants. However, plants adapt to this adverse situation by synthesis of some enzymes which may play the critical role in metabolic regulation and coordination in the biosphere and the biochemical process is not understood well.

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 (ROS) 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).

Table 1. Determination of K_m and V_{max} of PPO in leaf of Pui vegetable after 24h of treatment. The plants were exposed to 8 °C or 45°C for 24h separately. After the treatment, the plants were immediately removed from the experimental chamber and sampling of leaf was performed. For assay of PPO activity, 10, 100 and 200 mM concentration of catechol were used as a substrate of the enzyme. Control plants were similarly used except giving low or high temperature exposure.

Treatments	K_m (mM)	V_{max}(Unit)	Substrate concentration (mM)
Control	804.00	2050.37	10, 100, 200
Low temperautre	505.45	2045.45	10, 100, 200
Control	543.3	1666.58	10, 100, 200
High temperautre	440.00	1588.23	10, 100, 200

Table 2. Determination of K_m and V_{max} of PPO in leaf of Pui vegetable after 48h of treatment. The plants were exposed to 8 °C or 45°C for 48h separately. After the treatment, the plants were immediately removed from the experimental chamber and sampling of leaf was performed. For assay of PPO activity, 10, 100 and 200 mM concentration of catechol were used as a substrate of the enzyme. Control plants were similarly used except giving low or high temperature exposure.

Treatments	K_m (mM)	V_{max}(Unit)	Substrate concentration (mM)
Control	2.53	150.23	10, 100, 200
Low temperautre	0.49	223.19	10, 100, 200
Control	689.3	2021.27	10, 100, 200
High temperautre	433.0	1665.62	10, 100, 200

In 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, haem-containing 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.

Table 3. Determination of K_m and V_{max} of PPO in leaf of Pui vegetable after 72h of treatment. The plants were exposed to 8 °C or 45°C for 72h separately. After the treatment, the plants were immediately removed from the experimental chamber and sampling of leaf was performed. For assay of PPO activity, 10, 100 and 200 mM concentration of catechol were used as a substrate of the enzyme. Control plants were similarly used except giving low or high temperature exposure.

Treatments	K_m (mM)	V_{max}(Unit)	Substrate concentration (mM)
Control	2.74	333.50	10, 100, 200
Low temperautre	1.54	366.49	10, 100, 200
Control	1472.5	3801.28	10, 100, 200
High temperautre	60	642.85	10, 100, 200

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 adverse environment like low or high temperature, 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.

Table 4. Changes of POD activity in leaf of Pui vegetable exposed to low or high temperature. The plants were exposed to 8 °C or 45°C for 24h, 48h and 72h separately. After the treatment, the plants were immediately removed and sampling of leaf was performed. Control plants were similarly used except giving low or high temperature exposure.

Low temperature induced POD activity (Unit/g of leaf weight)		
Treatment	Control	Low temperature
24h	2144.82 ± 51.93	3324.18 ± 185.05^{A}
48h	2942.72 ± 68.01	4961.71 ± 4.68^{B}
72h	2979.76 ± 118.22	4530.46 ± 211.92^{B}
High temperature induced POD activity (Unit/g of leaf weight)		
Treatment	Control	High temperature
24h	2275.54 ± 311.40	1964.83 ± 53.04
48h	2558.32 ± 34.27	1755.34 ± 70.06^{B}
72h	3969.01 ± 37.91	3332.81 ± 41.19^{C}

The results are means of ± SE for three values in each group. $^{A}p<0.05$ versus respective control. $^{B}p<0.01$ and $^{C}p<0.001$ versus respective control.

The diverse clinical importance of this plant was demonstrated by recent investigations (Roshan *et al.*, 2012; Premalatha and Rajgopal, 2005). In response to low or 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). 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. Therefore, it is assumed that these enzymes might be involved in the regulation of metabolic activities during cold or heat stress and play the dominant role in the coordination between cellular damage and survive maintaining the adaptive response to the environment. The present study has been therefore undertaken to clarify the role of cold acclimation or heat stress on the regulation of these enzymes in this species of vegetable and will enhance in clarification of the understanding of the regulatory and comparative study of the respective metabolic aspects.

Reactive oxygen species, sites of production and their effects

Reactive oxygen species

ROS are a group of free radicals, reactive molecules, and ions that are derived from O_2. It has been estimated that about 1% of O_2 consumed by plants is diverted to produce ROS (Asada and Takahashi, 1987) in various subcellular loci such as chloroplasts, mitochondria, peroxisomes. ROS are well recognized for playing a dual role as both deleterious and beneficial species depending on their concentration in plants. At high concentration ROS cause damage to biomolecules, whereas at low/moderate concentration it acts as second messenger in intracellular signaling cascades that mediate several responses in plant cells. The ROS capable of causing oxidative damage include superoxide, perhydroxy radical, hydrogen peroxide, hydroxy radical, alkoxy radical, peroxy radical, organic hydro peroxide, singlet oxygen, excited carbonyl etc.

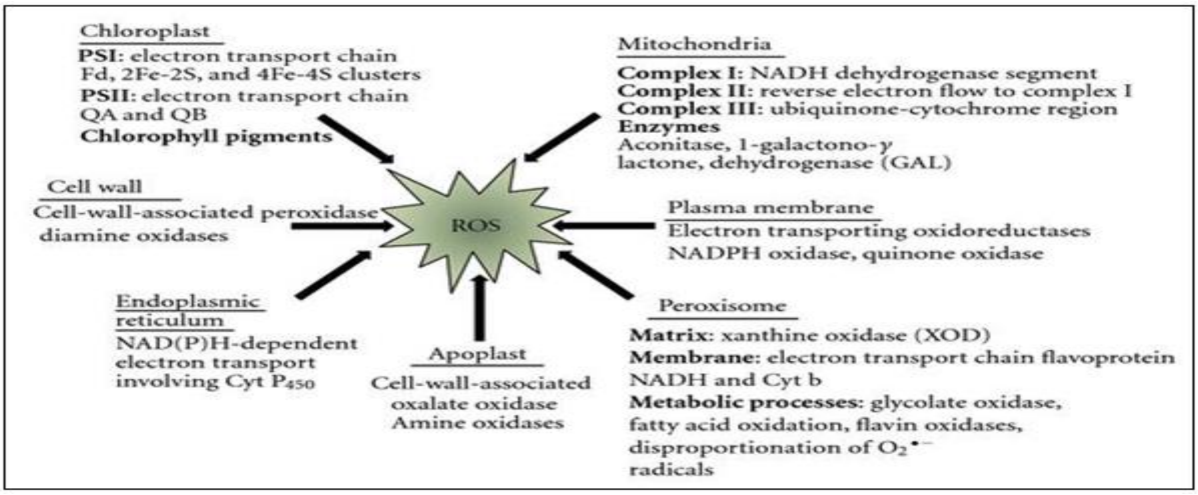

Fig. 1. Sites of production of reactive oxygen species (ROS) in plants. ROS are produced at several locations in the cell-like chloroplast, mitochondria, plasma membrane, peroxisomes, apoplast, endoplasmic reticulum, and cell wall.

Sites of production

ROS are produced in both unstressed and stressed cells at several locations in chloroplasts, mitochondria, plasma membranes, peroxisomes, apoplast, endoplasmic reticulum, and cell walls (Fig. 1). ROS are always formed by the inevitable leakage of electrons onto O_2 from the electron transport activities of chloroplasts, mitochondria, and plasma membranes or as a byproduct of various metabolic pathways localized in different cellular compartments.

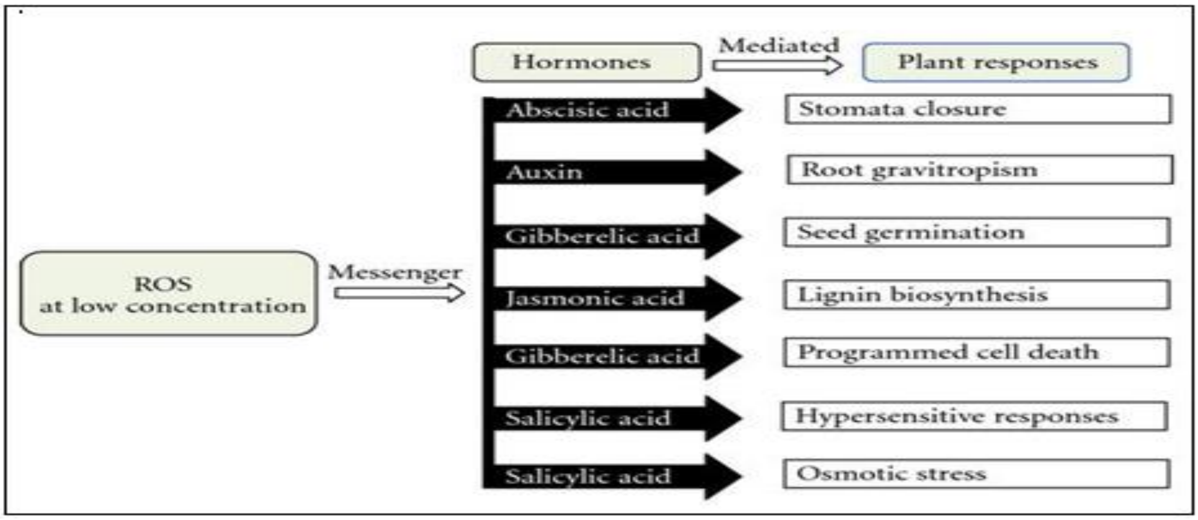

Fig. 2. Reactive oxygen species (ROS) as second messengers in several plant hormone responses, including stomatal closure, root gravitropism, seed germination, lignin biosynthesis, programmed cell death, hypersensitive responses, and osmotic stress.

Cellular effects of ROS

At low/moderate concentration, ROS have been implicated as second messengers in intracellular signaling cascades that mediate several plant responses in plant cells, including stomatal closure (Yan *et al.*, 2007), programmed cell death (Mittler, 2002), gravitropism (Joo *et al.*, 2001) and acquisition of tolerance to both biotic and abiotic stresses (Miller *et al.*, 2008). Fig. 2 shows the role of ROS as second messenger in hormone mediated cellular responses in plants. Production and removal of ROS must be strictly controlled in order to avoid oxidative stress. When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of "oxidative stress". However, the equilibrium between production and scavenging of ROS is perturbed under a number of stressful conditions such as salinity, drought, high light, toxicity due to metals, pathogens, and so forth. Enhanced level of ROS can cause damage to biomolecules such as lipids, proteins and DNA (Fig. 3).

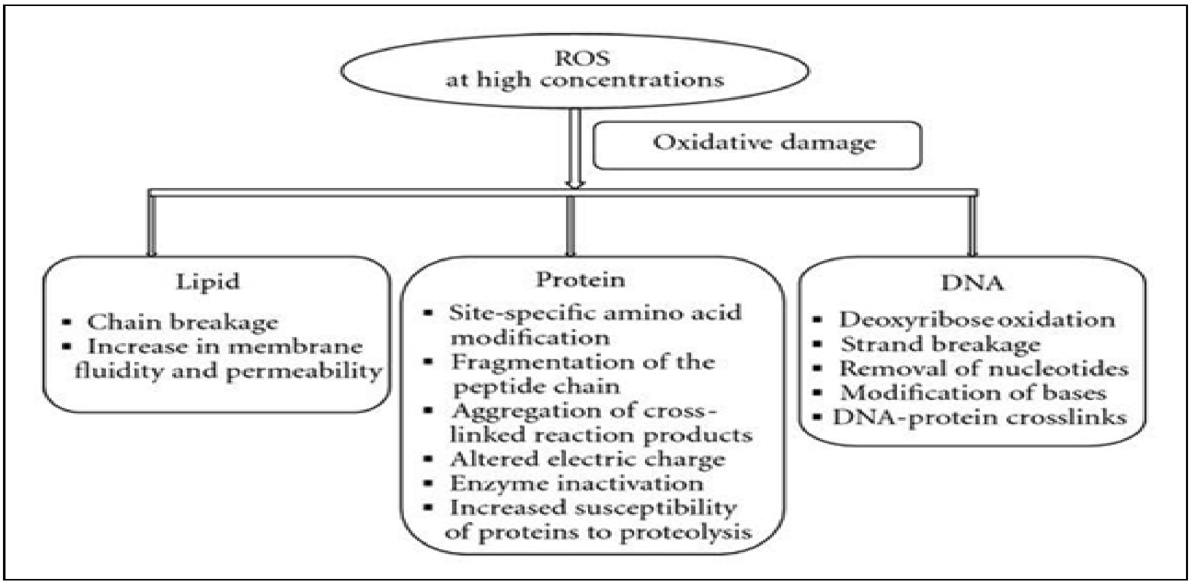

Fig. 3. Reactive oxygen species (ROS) induced oxidative damage to lipids, proteins, and DNA.

These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage, and so forth ultimately resulting in cell death.

Antioxidative defense system in plants

Plants possess complex antioxidative defense system comprising of nonenzymatic and enzymatic components to scavenge ROS. In plant cells, specific ROS producing and scavenging systems are found in different organelles such as chloroplasts, mitochondria, and peroxisomes. ROS-scavenging pathways from different cellular compartments are coordinated (Pang and Wang, 2008). Under normal conditions, potentially toxic oxygen metabolites are generated at a low level and there is an appropriate balance between production and quenching of ROS.

The balance between production and quenching of ROS may be perturbed by a number of adverse environmental factors, giving rise to rapid increases in intracellular ROS levels (Sharma *et al.*, 2010), which can induce oxidative damage to lipids, proteins, and nucleic acids. In order to avoid the oxidative damage, higher plants raise the level of endogenous antioxidant defense (Sharma *et al.*, 2010). Various components of antioxidative defense system involved in ROS scavenging have been manipulated, over expressed or down regulated to add to the present knowledge and understanding the role of the antioxidant systems.

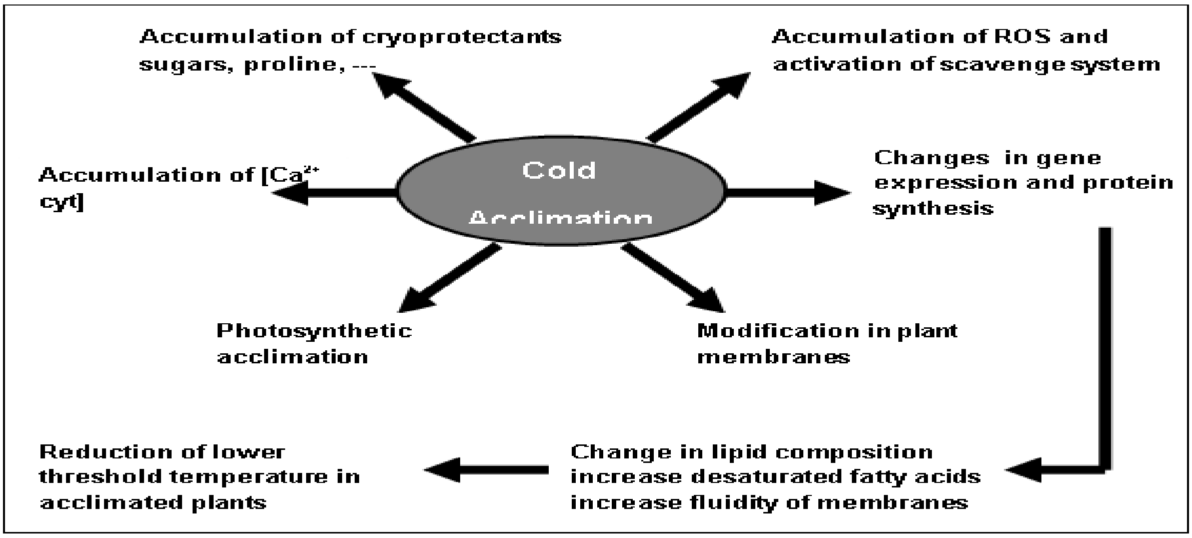

Fig. 4. Different cellular processes induced as a consequence of plant acclimation to cold.

Nonenzymic components of the antioxidative defense system include the major cellular redox buffers ascorbate (AsA) and glutathione (γ-glutamyl-cysteinyl-glycine, GSH) as well as tocopherol, carotenoids, and phenolic compounds. They interact with numerous cellular components and in addition to crucial roles in defense and as enzyme cofactors, these antioxidants influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and cell death (De Pinto and De Gara, 2004). Mutants with decreased nonenzymic antioxidant contents have been shown to be hypersensitive to stress (Semchuk *et al.*, 2009).

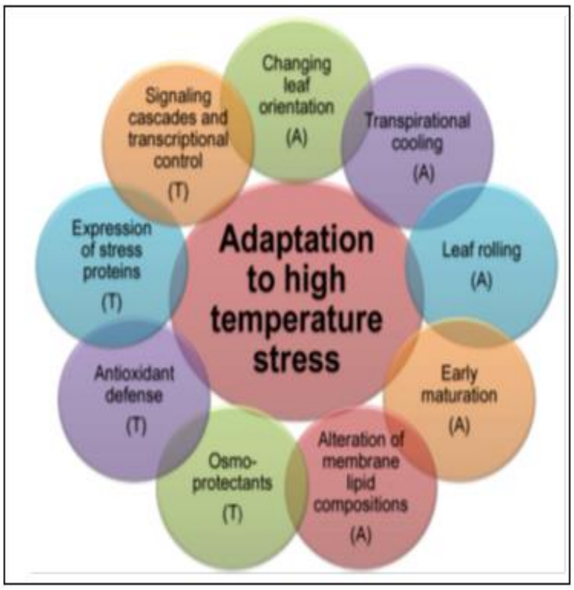

Fig. 5. Different adaptation mechanisms of plants to high temperature. A: Avoidance, T: Tolerance.

The enzymatic components of the antioxidative defense system comprise of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-GSH) cycle, ascorbate peroxidase (APX), polyphenol oxidase (PPO), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Noctor and Foyer, 1998).

These enzymes operate in different subcellular compartments and respond in concert when cells are exposed to oxidative stress.

Environmental stimuli and its role on metabolic regulation chilling

Chilling stress is a key environmental factor limiting growth and productivity of crop plants. Low temperatures, defined as low but not freezing temperatures (0–15°C), are common in nature and can damage many plant species.

In order to cope with such conditions, several plant species have the ability to increase their degree of freezing tolerance in response to low, non-freezing temperatures, a phenomenon known as cold acclimation. It is well established that some of the molecular and physiological changes that occur during cold acclimation are important for plant cold tolerance (Hsieh *et al.*, 2002; Zhu *et al.*, 2007). Accordingly, it has been concluded that cold tolerance that develops in initially insensitive plants is not entirely constitutive and at least some of it is developed during exposure to low temperatures.

The primary mechanisms involved in cold acclimation are related to a number of processes discussed below. These include molecular and physiological modifications occurring in plant membranes, the accumulation of cytosolic Ca^{2+}, increased levels of ROS and the activation of ROS scavenger systems, changes in the expression of cold related genes and transcription factors, alterations in protein and sugar synthesis, proline accumulation, and biochemical changes that affect photosynthesis (Fig. 4).

Role of reactive oxygen species in acclimation to low temperature

The role of ROS in abiotic stress management has become a subject of considerable research interest, particularly since ROS have been reported to be involved in processes leading to plant stress acclimation (Suzuki *et al.*, 2011).

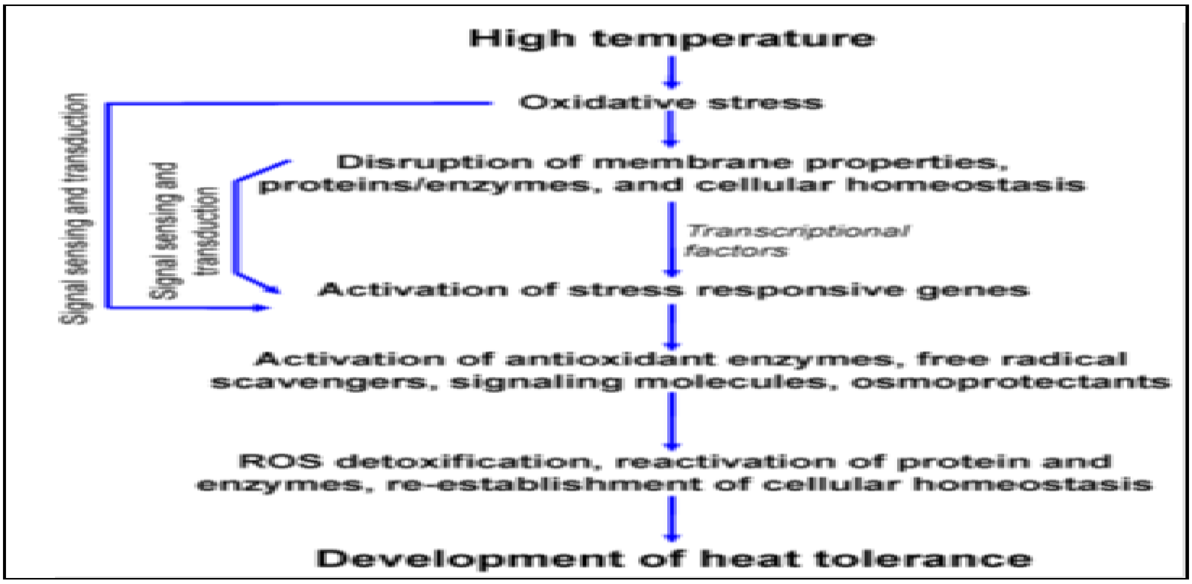

Fig. 6. Schematic illustration of heat induced signal transduction mechanism and development of heat tolerance in plants.

This finding indicates that ROS are not simply toxic by-products of metabolism, but act as signaling molecules that modulate the expression of various genes, including those encoding antioxidant enzymes and modulators of H_2O_2 production (Suzuki *et al.*, 2011) (Fig. 2).

In addition, low temperature stress has been reported to cause significant increases in the levels of the soluble non-enzymatic antioxidants ascorbate and glutathione, as well as the activity of the main NADPH-generating dehydrogenases (Airaki *et al.*, 2011).

High temperature (HT) stress

High temperature (HT) stress is a major environmental stress that limits plant growth, metabolism, and productivity worldwide. Plant growth and development involve numerous biochemical reactions that are sensitive to temperature. Plant responses to HT vary with the degree and duration of HT and the plant type. HT is now a major concern for crop production and approaches for sustaining high yields of crop plants under HT stress are important agricultural goals. Plants possess a number of adaptive, avoidance, or acclimation mechanisms to cope with HT situations. In addition, major tolerance mechanisms that employ ion transporters, proteins, osmoprotectants, antioxidants, and other factors involved in signaling cascades and transcriptional control are activated to offset stress-induced biochemical and physiological alterations. Plant survival under HT stress depends on the ability to perceive the HT stimulus, generate and transmit the signal, and initiate appropriate physiological and biochemical changes. HT-induced gene expression and metabolite synthesis also substantially improve tolerance.

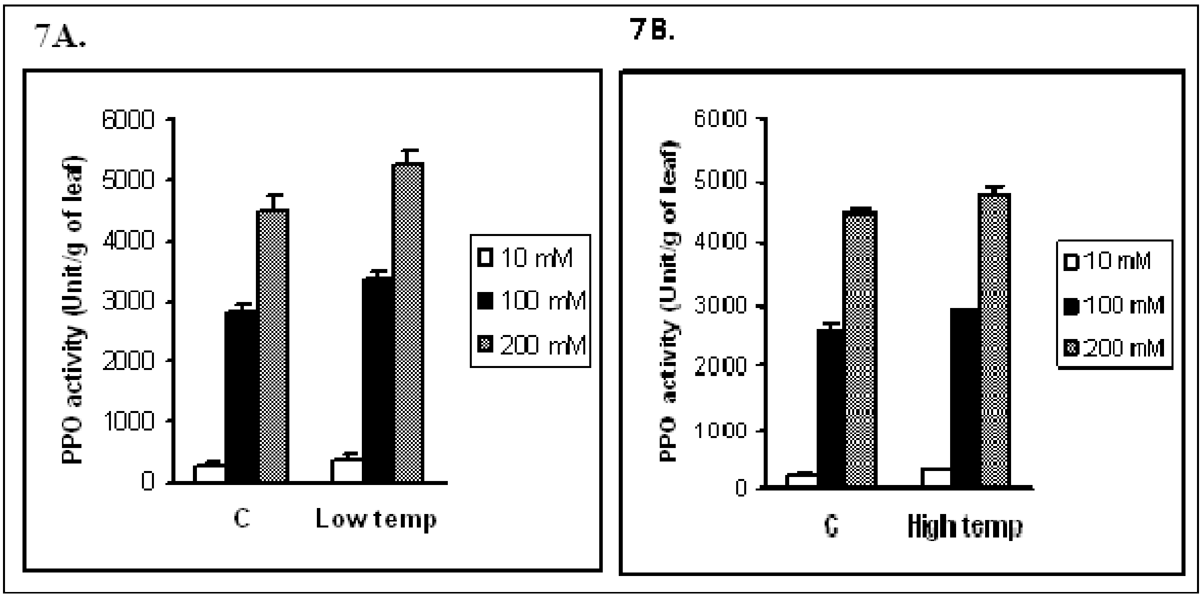

Fig. 7. Alteration of PPO activity in response to 10, 100 and 200 mM catechol in leaves of *B alba*during low [7A] and high temperature [7B] acclimation. The plants were exposed to 8 °C or 45 °C for 24h separately, however, the respective controls were used without low or high temperature exposure. After the treatments, the leaves of treated and their respective control plants were used for the assay of PPO activity.

The physiological and biochemical responses to heat stress are active research areas, and the molecular approaches are being adopted for developing HT tolerance in plants.

This article reviews the recent findings on responses, adaptation, and tolerance to HT at the cellular, organellar, and whole plant levels and describes various approaches being taken to enhance thermotolerance in plants.

Heat stress induced oxidative stress

Different metabolic pathways are depended upon enzymes which are sensitive to various degrees of HTs. It has been suggested that, like other abiotic stress, heat stress might uncouple enzymes and metabolic pathways which cause the accumulation of unwanted and harmful ROS most commonly singlet oxygen, superoxide radical, hydrogen peroxide and hydroxyl radical which are responsible for oxidative stress (Asada, 2006). Various physiological damages occur in plants upon exposures to varying levels of heat stress (Halliwell, 2006). Hydroxyl radicals can potentially react with all biomolecules, like pigments, proteins, lipids and DNA, and almost with all constituents of cells (Moller *et al.*, 2007).

Singlet oxygen can directly oxidize protein, polyunsaturated fatty acids and DNA (Huang and Xu, 2008).

Thermal stress can induce oxidative stress through peroxidation of membrane lipids and disruption of cell membrane stability by protein denaturation (Rodríguez *et al.*, 2005).

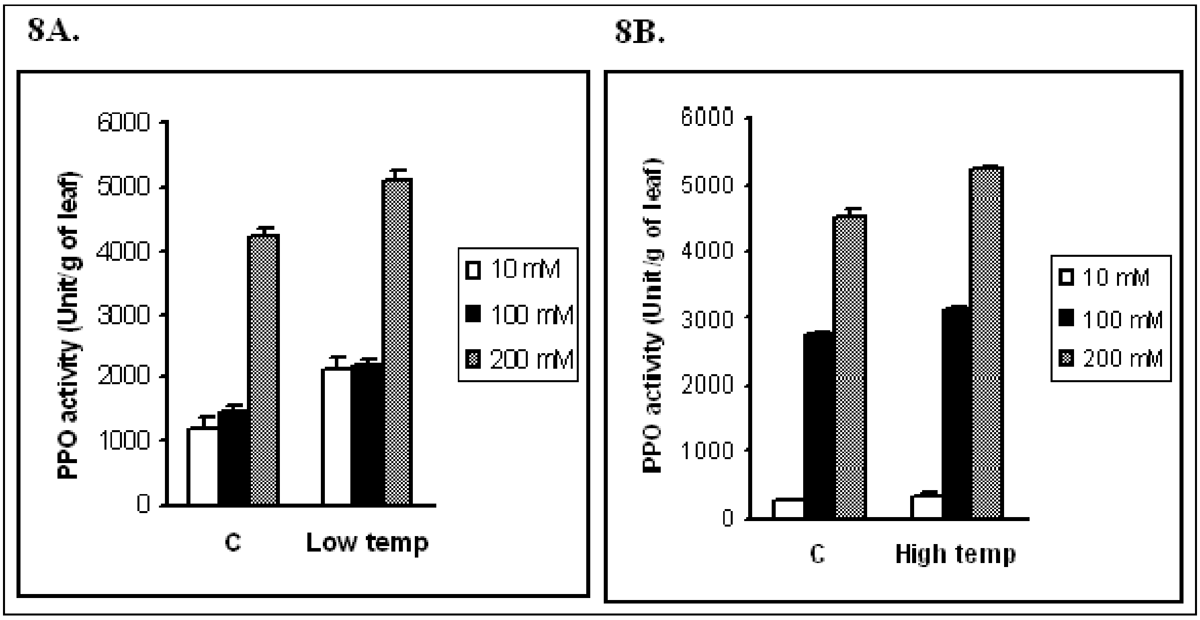

Fig. 8. Alteration of PPO activity in response to 10, 100 and 200 m M catechol in leaves of *B alba* during low [8A] and high temperature [8B] acclimation. The plants were exposed to 8 °C or 45 °C for 48h separately, however, the respective controls were used without low or high temperature exposure. After the treatments, the leaves of treated and their respective control plants were used for the assay of PPO activity.

Plant adaptation to high temperature stress

Survival in hot, dry environments can be achieved in a variety of ways, by combinations of adaptations (Fitter and Hay, 2002). Plant adaptation to heat stress includes avoidance and tolerance mechanisms which employ a number of strategies (Fig. 5). Under high temperature conditions, plants exhibit various mechanisms for surviving which include long-term evolutionary phenological and morphological adaptations and short-term avoidance or acclimation mechanisms such as changing leaf orientation, transpirational cooling, or alteration of membrane lipid compositions. Closure of stomata and reduced water loss, increased stomatal and trichomatous densities, and larger xylem vessels are common heat induced features in plant (Srivastava *et al.*, 2012).

In many crop plants, early maturation is closely correlated with smaller yield losses under high temperature, which may be attributed to the engagement of an escape mechanism (Rodríguez *et al.,* 2005; Adams *et al.*, 2001).

Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under high temperature.

This is a highly specific trait, and closely related species, even different organs and tissues of the same plant, may vary significantly in this respect. Plants have evolved various mechanisms for thriving under higher prevailing temperatures. They include short term avoidance/acclimation mechanism or long term evolutionary adaptations. Some major tolerance mechanisms, including ion transporters, late embryogenesis abundant (LEA) proteins, osmoprotectants, antioxidant defense, and factors involved in signaling cascades and transcriptional control are essentially significant to counteract the stress effects (Rodríguez *et al.*, 2005; Wang *et al.*, 2004). In case of sudden heat stress, short term response, *i.e.*, leaf orientation, transpirational cooling and changes in membrane lipid composition are more important for survival (Rodríguez *et al.*, 2005). Smaller yield losses due to early maturation in summer shows possible involvement of an escape mechanism in heat stress tolerance (Adams *et al.*, 2001). Different tissues in plants show variations in terms of developmental complexity, exposure and responses towards the prevailing or applied stress types (Queitsch *et al.*, 2000). The stress responsive mechanism is established by an initial stress signal that may be in the form of ionic and osmotic effect or changes in the membrane fluidity. This helps to reestablish homeostasis and to protect and repair damaged proteins and membranes (Vinocur and Altman, 2005).

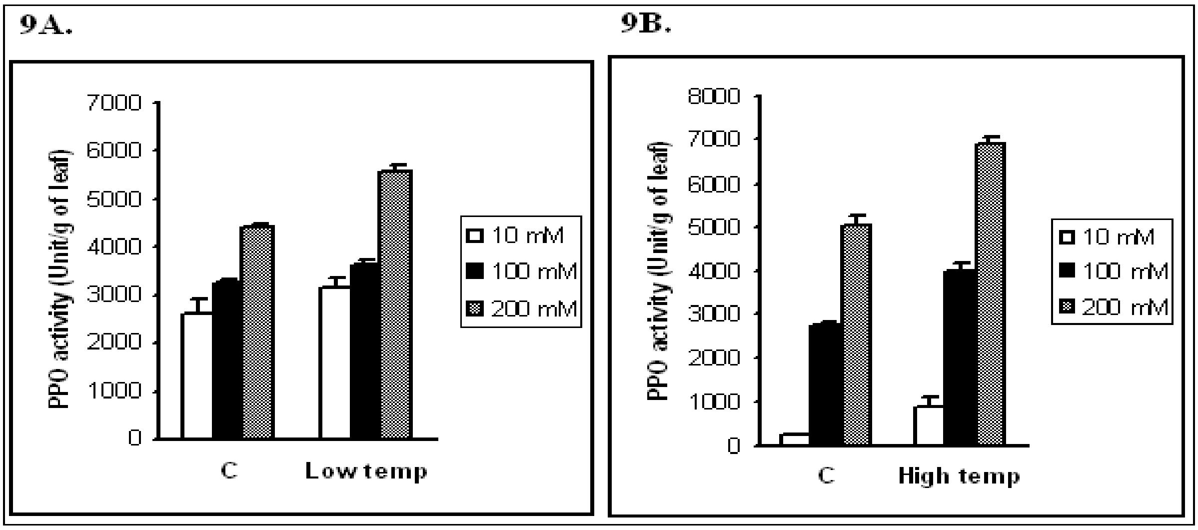

Fig. 9. Alteration of PPO activity in response to 10, 100 and 200 m M catechol in leaves of *B alba* during low [9A] and high temperature [9B] acclimation. The plants were exposed to 8 °C or 45 °C for 72h, however, the respective controls were used without low or high temperature exposure. After the treatments, the leaves of plants and their respective controls were used for the assay of PPO activity.

Upregulation of many genes has been reported to help the plant to withstand the stress conditions which leads to plant adaptation (Tuteja, 2009). Upon stress plants perceive the external and internal signals through different independent or interlinked pathways which are used to regulate various responses for its tolerance development (Kaur and Gupta, 2005) (Fig.6). Plant responses to stress are complex integrated circuits within which multiple pathways are involved. To generate response in specific cellular compartments or tissues against a certain stimuli, interaction of cofactors and signaling molecules are required. Signaling molecules are involved in activation of stress responsive genes. There are various signal transduction molecules related to stress responsive gene activation depending upon plant type, types of stresses. Some broad group of those are the Ca dependent protein kinases (CDPKs), mitogen-activated protein kinase (MAPK/MPKs), NO, sugar (as signaling molecule), phytohormones (Ahmad *et al.*, 2012). These molecules together with transcriptional factors activate stress responsive genes. Once the stress responsive genes activate, these help to detoxify the ROS (by activating detoxifying enzymes, free radical scavengers); reactivate the essential enzymes and structural proteins (Ciarmiello *et al.*, 2011) and all the above stated processes help to maintain the cellular homeostasis (Fig. 6).

Experimental plant sample

Basella alba (Pui vegetable) and its chemical constituents

Analysis of the dried leaves revealed the following composition (per 100 g of dried leaves): Protein (20%), Fat (3.5%), Carbohydrate (54%), Fibre (9%) and Ash (19%). The leaves contain a high level of calcium (3000 mg) and are rich in vitamin A (50mg), Thiamine (B1) (0.7mg), Rriboflavin (B2) (1.8mg), Niacin (7.5mg) and Vitamin C (1200mg); betacyanins, oxalic acid, flavonoids such as acacetin,7,4-dimethoxy kaempferol and 4'-methoxy isovitexin and phenolic acids like vanilla, syringic and ferulic acids. The fruits contain betacyanins, gomphrenin I, II &III (Daniel, 2006).

Medicinal importance of Basella alba

Recently, the ethanobotanical properties of *Basella alba* has been recognized. Various parts of the plant are used for treatment of the diseases as well as for different healing activities of human beings and animals across the globe especially in India and China. Its use has been discovered as asperient, rubefacient and for catarrhl infections. Some of the compounds available especially in the plant are *basellasaponins*, kaempherol, betalin etc. Several extracts like aqueous, chloroform, ethanol and petroleum has been used for different pharmaceutical activities (Roshan *et al.*, 2012).

Low and high temperature induced metabolic regulation in pui vegetable

The abiotic stresses, particularly low and high temperature have been found to be involved in metabolic regulation in leaves of *B. alba*. The plants were grown in pot and were exposed to 8°C or 45 °C for 24h, 48h and 72h periods. The plants exposed to low temperature had enhanced anti oxidative enzymes particularly PPO and POD and therefore, it is reasonable that low temperature might be involved in inducing higher antioxidative effects in this species of plant. It has been observed from the previous study that low temperature has been found to cause oxidative stress (Wang *et al.*, 2003) and augments the synthesis of ROS thereby causing cell injury and damage. To prevent the oxidative damage, plants generate different mechanical ways by which they survive in such adverse circumstances. It is reasonable that plants may induce the synthesis of some molecules responsible for survival in the atmosphere. In leaves of this species of plant, the PPO activities were much increased at 48h rather than 24h or 72h periods when the plants were exposed to cold temperature (8°C) compared to the respective controls kept in ambient temperature (Fig. 7, 8, 9). Moreover, the dose response characteristics of the substrate catechol for this enzyme have been clarified and found to be maximal at 10 mM concentration of substrate rather than 100 or 200 mM concentration. It is therefore recognized that this enzyme has higher sensitivity to the temperature along with the higher specificity to the substrate and may act time dependently in the biological system. The K_m for this enzyme for the three substrate concentrations (10, 100, 200 mM) were 505.45, 0.49 and 1.54 mM for 24h, 48h and 72h periods respectively for low temperature induced plants while 804.0, 2.53 and 2.74mM for control respectively. The V_{max} were 2045.45, 223.19 and 366.49 Unit for low temperature induced plants and 2050.37, 150.23 and 333.50 Unit for control for the above mentioned periods respectively (Table 1, 2, 3). Similarly dose response characteristics of catechol for the enzyme PPO was clarified during high temperature treatment (Fig. 7, 8, 9). The K_m for this enzyme for the three substrate concentrations (10, 100, 200 m M) were 440.0, 433.0 and 60 mM for 24h, 48h and 72h periods respectively for high temperature induced plants while 543.3, 689.3, and 1472.5 mM for control respectively. The V_{max} values were 1588.23, 1665.62 and 642.85 Unit for the high temperature induced plants and 1666.58 Unit, 2021.27 Unit and 3801.28 Unit for the control respectively for the above mentioned periods (Table 1, 2, 3). The parameters determined for the enzyme PPO for both low and high temperature treatment clearly demonstrate that the K_m and V_{max} were influenced by the temperature stress and are an essential tool for characterization of the enzyme under these circumstances.

Although low and high temperature cause the higher activities of PPO for different doses of catechol, however the activity of PPO was pronounced after prolonged exposure of high temperature. Several lines of evidences reveal that heat stress causes oxidative effects similar to cold acclimation causing the synthesis of ROS (Anderson, 2002; Yin *et al.*, 2008), therefore both low and high temperature is the critical to the plants where they survive. Higher activity of PPO might be involved in the regulation of oxidative effects caused by temperature stress and play the mechanism of survival in such circumstances. The up regulation of PPO activity in leaf might be linked to the signaling cascade induced by low or high temperature. The stress is first perceived by the receptors present on the membrane of the plant cells, the signal is then transduced downstream and this results in the generation of second messengers including calcium, reactive oxygen species (ROS) and inositol phosphates. These second messengers such as inositol phosphates, further modulate the intracellular calcium level. This perturbation in cytosolic Ca^{2+} level is sensed by calcium binding proteins, also known as Ca^{2+} sensors. These sensors apparently lack any enzymatic activity and change their conformation in a calcium dependent manner. These sensory proteins then interact with their respective interacting partners often initiating a phosphorylation cascade and target the major stress responsive genes or the transcription factors controlling these genes. The products of these stress genes ultimately lead to plant adaptation and help the plant to survive and surpass the unfavorable conditions. Thus, plant responds to stresses as individual cells and synergistically as a whole organism. Stress induced changes in gene expression in turn may participate in the generation of hormones like ABA, salicylic acid and ethylene. These molecules may amplify the initial signal and initiate a second round of signaling that may follow the same pathway or use altogether different components of signaling pathway.

Time course effects of low and high temperature on the regulation of POD were done in this species of plant (Table 4). The stimulatory effects on POD activity in leaf were observed during low temperature and the activity was higher at 48h of treatment. Because of the higher activity of this enzyme, higher conversion of phenolic substrates to quinoles might be happened and these colored compounds are essential for the industrial purposes. During the experiment, we observed that both low temperature induced and the respective controls caused the color pigmentation quickly, however low temperature induced leaves had higher pigmentation than the control, therefore, it is reasonable that cold acclimation causes the higher oxidation of phenolic compounds and might be an effective approach for producing the 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 POD activity in leaf was also clarified for high temperature treatment and found to be prevented up to 72h periods (Table 4). Therefore, the reciprocal regulation of this enzyme was demonstrated during high temperature acclimation. It is reasonable that the formation of quinoles in response to high temperature is regulated and might be an index for characterization of the synthesis of such colored compounds in response to both low and high temperature. The results are good agreement with some evidences. It has been demonstrated that peroxidase inactivation was observed in some species of plants and was faster at the higher temperature (Muftugil, 1985; Chakraborty and Pradhan, 2011). Collectively, the diverse regulation of synthesis of these enzymes will give a new insight for adaptive response to the environment and might be involved in prevention of oxidative damage to cells during the situation.

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.*, 2008). 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 is 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 *B. 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 *B. 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 *B alba*. Moreover, regulation of these enzymes is not only mediated by hot environment but also might be by other chemical mediators in the environment.

Each plant has its unique set of temperature requirements, which are optimum for its proper growth and development. A set of temperature conditions, which are optimum for one plant may be stressful for another plant. Many plants, especially those, which are native to warm habitat, exhibit symptoms of injury when exposed to low non-freezing temperatures (Lynch, 1990). These plants including maize (*Zea mays*), soybean (*Glycine max*), cotton (*Gossypium hirsutum*), tomato (*Lycopersicon esculentum*) and banana (*Musa* sp.) are in particular sensitive to temperatures below 10–15 °C and exhibit signs of injury (Lynch, 1990; Guy, 1990). The symptoms of stress induced injury in these plants appear from 48 to 72h, however, this duration varies from plant to plant and also depend upon the sensitivity of a plant to cold stress. Various phenotypic symptoms in response to chilling stress include reduced leaf expansion, wilting, chlorosis (yellowing of leaves) and may lead to necrosis (death of tissue). Chilling also severely hampers the reproductive development of plants for example exposure of rice plants to chilling temperature at the time of anthesis leads to sterility in flowers (Jiang *et al.*, 2002).

Conclusion

Oxidative stress caused by extreme low and high temperature has been found to result the regulation of biosynthesis of enzymes PPO and POD in leaves of the species of plant. These enzymes are assumed to play the critical role in prevention of the oxidative effects which are recognized to be involved in cell injury and damage. Although low temperature causes the up regulation of these enzymes, however down regulation of POD in response to high temperature might be the regulatory mechanism of the antioxidative effects. Therefore, diverse regulation of these enzymes in response to these abiotic stresses was observed. The regulatory mechanism of these enzymes by variation of temperature gives essential metabolic aspects in adaptive response to the adverse circumstances and also gives a new insight in the regulation of phenolic oxidation to compounds essential for drugs and other purposes. During the adverse environmental circumstances, the antioxidative enzymes would play the role in maintaining the metabolic regulation particularly the prevention of cellular damage and injury thereby the plants survive in the adverse circumstances.

Elucidation of the regulatory mechanism of PPO and POD in *B. alba* in response to low and high temperature also gives some future perspectives. These includes the measurement of hydrogen peroxide (H_2O_2) in leaf because of the higher oxidative stress caused by these abiotic stresses. The enhanced H_2O_2 level during exposure of low and high temperature may induce activation of PPO and POD level. For the prevention of oxidative stress, the effects of calcium channel blocker on the regulation of these enzymes in presence of low and high temperature might be employed. Moreover, calcium dependent protein, calmodulin expression might be altered when the plants will be exposed to low and high temperature. It is assumed that higher expression of this protein in leaf may affect these two enzyme activities. The formation of H_2O_2 may affect calcium channel in leaves and might be coupled to the higher expression of calmodulin as well as Ca^{2+} concentration. Therefore, further studies are needed to clarify the mechanism.

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