



COMPARATIVE RESPONSE ON THE ACCUMULATION OF INORGANIC PHOSPHATE IN LEAF OF *BASELLA ALBA* TO LOW AND HIGH TEMPERATURE

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Abstract

Context: *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.

Objectives: To find the role of low and high temperature induced regulation of metabolic functions particularly alteration and accumulation of inorganic phosphate in leaf.

Materials and Methods: Plants grown in pot were exposed to cold (8°C) for 24h, 48h and 72h periods and the respective controls were kept in ambient room temperature for the above mentioned time. For high temperature induced plants, they were exposed to 45°C with full aeration along with sufficient water. Control pot was used similarly in room temperature; however, the temperature was maintained 30°C by using air cooling system (AC).

Results: Low temperature causes the higher inorganic phosphate level in leaf and was increased by 33.6%, 34.7% and 62.8% respectively when compared to the respective controls. Therefore, it is assumed that short term exposure has mild effect on phosphate accumulation in leaf; conversely as the time extended, the synthesis assumed to be higher and increased time dependently. Similarly, the inorganic phosphate concentrations were increased by 27.8%, 37.5% and 74.9% respectively whenever the plants were exposed to high temperature (45°C). Taken together, a faster increase in accumulation of phosphate in leaf was mediated by high temperature for prolonged exposure.

Conclusion: The plants exposed to both abiotic stresses cause the release of inorganic phosphate which may participate in the survival of the species in the adverse environment.

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

Introduction

Environmental stress might disrupt cellular structures and impair key physiological functions of plants (Larcher 2003). Drought, salinity and low temperature stresses impose an osmotic stress that result in 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. The previous investigations reveal that chilling induced injury is associated with the formation of ROS, such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH⁻) and singlet oxygen (¹O₂) (Basra 2001, Lee and Lee 2000). To prevent the oxidative damage caused by such abiotic stress, plants generate different mechanism by which they can survive in such critical environment. Antioxidative 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 antioxidative enzymes and anti oxidant molecules can neutralize ROS (Oidaira *et al.* 2000, Lee and Lee 2000). These antioxidant molecules might be involved to regulate the metabolic sequences, for example, degradation of high energy rich

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molecules like ATP, GTP to produce inorganic phosphate and the enzymes involved in this process might be influenced. Phosphorus is an essential nutrient for plants and an important component in cell metabolism. It has a vital functional role in energy transfer, and acts as modulator of enzyme activity and gene transcription; hence its assimilation, storage and metabolism are of major importance to plant growth and development. Hydrolytic breakdown of phosphate esters is brought about by phosphatases, which is a critical process in energy metabolism, metabolic regulation and a wide range of signal transduction pathways in plants. It is assumed that the oxidative stress caused by low and high temperature might be involved in the hydrolytic degradation of phosphates esters. The released inorganic phosphate may serve as the essential molecule in such adverse environmental situations like cold and draught.

Basella alba is a very soft leafy vegetable available in Bangladesh and grows both in summer and winter. The diverse clinical importance of this plant was demonstrated by recent investigations (Roshan *et al.* 2012, Premalatha *et al.* 2005). Therefore, it is assumed that variation of temperature may affect both metabolic activities as well as its biological importance. The aim of this study is to examine the interrelationship between important nutrients of plant metabolism, such as phosphorus and the physiological mechanisms such as cold acclimation or high temperature stress in *B. alba*. The current investigation has also been undertaken to find the role of cold acclimation and high temperature treatment on the regulation of metabolic functions, particularly, alteration and accumulation of leaf inorganic phosphate of *Basella alba* and may assist in the clarification of such stress-induced mechanisms.

Materials and Methods

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 *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°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 30 days of germination, the two different pots were described as control and low temperature induced plants. Control pot was used for 24h, 48h and 72h treatments in the room temperature without cold acclimation. The second pot was used for 24h, 48h and 72h 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 24h, 48h and 72h duration and kept in -80°C.

Plant materials and high temperature treatment: In separate experiments, another two plastic pots were prepared with soil and seeded similarly with *Basella alba*. After 30 days of germination, the two different pots were described as control and high temperature induced plants. Control pot was used for 24h, 48h and 72h treatments in room temperature; however, the temperature was maintained 30°C by using air cooling system (AC) already fixed in the room. The second pot was used similarly for 24h, 48h and 72h duration in the plastic chamber and was exposed to 45°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 °C.

Assay of inorganic phosphate (Pi): The leaves of the different treatments (24h, 48h and 72h) and their respective controls were homogenized with water in a mortar kept on ice. Approximately, 3-4 g of cold exposed and their respective control leaves were used for homogenization while approximately 2-3 g of high temperature induced leaves and their respective controls were similarly used. The homogenates were centrifuged at 8000 rpm for 10 minutes and the supernatants were used as crude extract for assay of inorganic phosphate (Pi) as described by Ramnik (1999). 200 µl tissue extract was diluted to 5 ml with water

and was mixed vigorously with 5 ml of 5% TCA (Trichloroacetic acid) and centrifuged at 6000 rpm for 10 min. 5 ml supernatant was transferred to another tube and kept on ice. 1 ml molybdate reagent (10 g of ammonium molybdate in 100 ml water was taken and 100 ml of 5N H₂SO₄ was added to prepare 200 ml solution) was added and mixed. The solution was mixed with 0.4 ml of aminonaphtholsulphonic acid reagent. An amount of 3.6 ml water was added and after mixing, the tube was kept standing for 10 min for the complete development of color. For blank, 5 ml of 5% TCA and 5 ml water were mixed only. Absorbance was taken at 690 nm against the blank. The Pi contents in leaf extract were calculated using standard KH₂PO₄ solution.

Statistical analysis: Results of the experiments were expressed as mean and standard error of different groups. The differences between the mean values were evaluated by ANOVA followed by paired *t*-test using SPSS software.

Results

Effects of low temperature on Pi content in leaf of Basella alba:

To properly identify the physiological responses to environmental stress such as low temperature, plants were exposed to 8°C in a temperature controlled cooling chamber for 24h. As shown in Fig. 1A, the average inorganic phosphate content in leaf in response to low temperature for 24h period was 105.32 ± 0.88 mg/100 g of leaf whereas for the control leaf kept in ambient temperature, the amount of Pi was 78.83 ± 3.92 mg. A significant (33.6%, $P < 0.05$) increased Pi was observed after 24h when compared with control plant (Fig. 3). The results appeared to indicate that the Pi contents were affected by cold acclimation. Therefore, it is reasonable that an adaptive response by the species was created and the accumulation of Pi was observed to serve as the factor responsible for the adverse environmental condition.

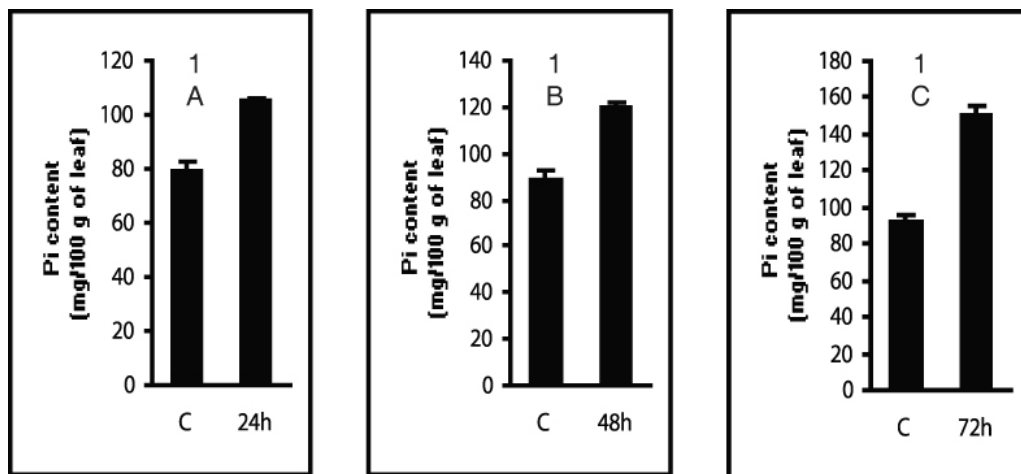


Fig. 1. Low temperature induced regulation of Pi accumulation in leaves of *Basella alba*. The plants exposed to cold (8 °C) for 24h (1A), 48h (1B) and 72h (1C) in cold chamber, however, the respective controls were used without any cold acclimation. The leaves of plants of different treatments and their respective controls were used for determination of Pi. The results are means of \pm SE for 3 values in each group.

Leaves of *Basella alba* were exposed to low temperature for 48h and the amount of Pi was 119.99 ± 1.48 mg while for the respective control plant, the Pi content was 89.02 ± 3.91 mg/100 g of leaf. The results indicated that 34.78% ($P < 0.01$) increased Pi had been found after 48h in response to cold compared to the control plant (Fig. 1B and Fig. 3). The increased accumulation of Pi in leaf in response to cold environment might be involved in the regulation of metabolic functions of this species of plant. The alteration of Pi concentration in leaf is an index for characterization of the sensitivity to the environmental temperature.

To find the optimum effect of cold acclimation on Pi content in leaf, the extended time was 72h. As shown in Fig. 1C, the low temperature induced leaf had Pi content 150.56 ± 4.20 mg while for the respective control leaf, the average Pi content was 92.45 ± 3.41 mg/100 g of leaf. The results showed that the Pi content in leaf had been enhanced significantly ($P < 0.001$) by 62.85% when they were exposed to cold for 72h, compared to the control (Fig. 3). The results appeared to indicate that the Pi contents were affected by cold acclimation and was assumed that the prolonged cold exposure had higher stimulatory effects on Pi release in leaf of the plant than 24h and 48h periods. The results suggest that the increased Pi induced by low temperature might be caused by such abiotic stress and could be considered as the survival factor for this species of plant in critical environment.

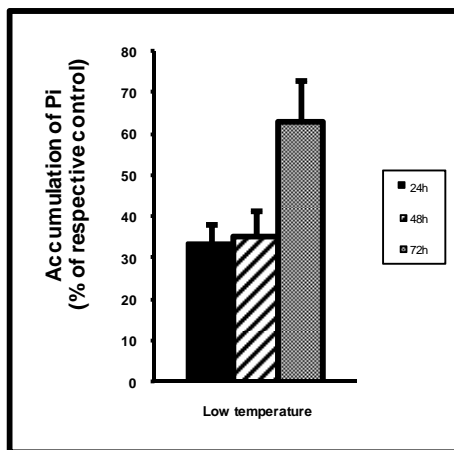


Fig. 3. Accumulation of Pi in leaves of *Basella alba* during cold acclimation. The plants exposed to cold (8 °C) for 24h, 48h and 72h in cold chamber, however, the respective controls were used without any cold acclimation. After the treatments, the leaves of the plants and their respective controls were used for determination of inorganic phosphate. The results are expressed as percentage of the respective controls

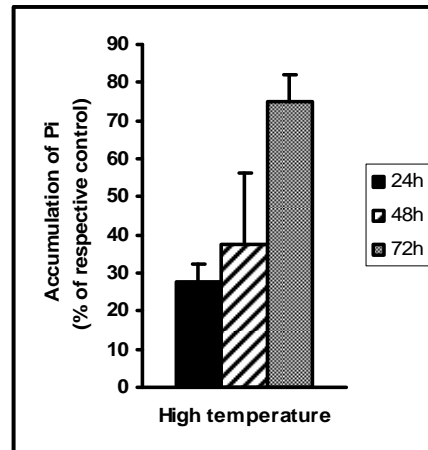


Fig. 4. Accumulation of Pi in leaves of *Basella alba* during high temperature acclimation. The plants were exposed to 45 °C for 24h, 48h and 72h periods in the high temperature producing chamber. The respective controls were kept in the room where the temperature remains 30 °C and was maintained by air cooling system. After the treatments, the leaves of the plants and their respective controls were used for determination of inorganic phosphate. The results are expressed as percentage of the respective controls.

Effect of high temperature on Pi content in leaf of Basella alba:

To examine the role of high temperature on the regulation of inorganic phosphate accumulation in leaf of *Basella alba*, plants in the pot were exposed to 45°C in plastic chamber equipped in the laboratory for 24h period and the respective control was kept in 30°C. The amount of Pi in response to high temperature was 68.36 ± 0.81 mg/100 g of leaf, whereas for control leaves, the Pi content was 53.46 ± 2.37 mg. The results

demonstrated that Pi contents had been significantly enhanced and stimulated (27.87%, $P < 0.05$) by high temperature compared to the respective control. However, the accumulation of Pi was appeared to be lower than the low temperature shown in Fig. 2A, Fig. 1A and Fig. 4.

As shown in Fig. 2B, the amount of Pi in leaves of plant was recorded to determine the effect of high temperature on inorganic phosphate accumulation. After 48h of treatment, the leaf Pi was estimated as 86.25 ± 5.36 mg for control and 62.70 ± 5.36 mg/100 g of leaf for high temperature induced plant. High temperature caused a significant and more pronounced increase in Pi in leaf by 37.55% ($P < 0.01$) (Fig. 4) when compared to the respective control. The increase of Pi in leaf was found to be higher than the previous 24h of exposure as demonstrated in Fig. 1B, 2A, 2B. Therefore, the Pi content in leaf is assumed to be regulated by the variation of temperature and will be strictly followed by the extension of time.

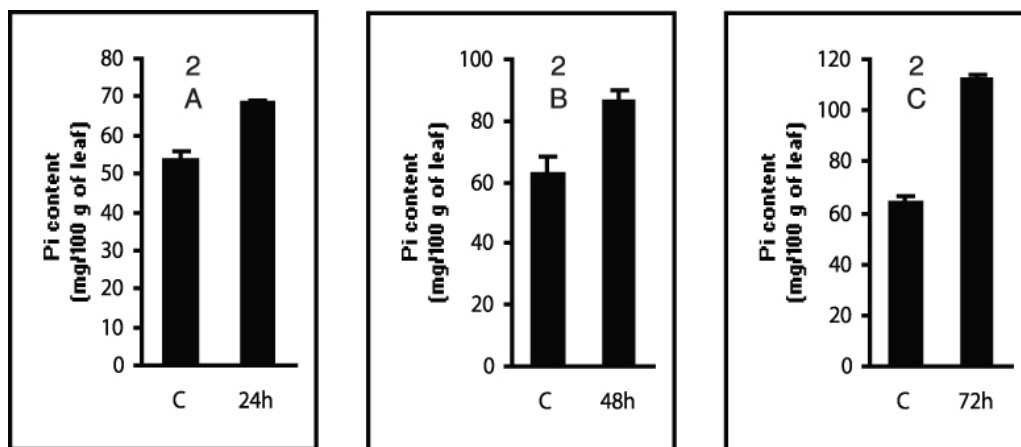


Fig. 2. High temperature induced regulation of Pi accumulation in leaves of *Basella alba*. Leaves of *Basella alba* were exposed to 45 °C for 24h (2A), 48h (2B) and 72h (2C) periods. The respective controls were kept in the room where the temperature remains 30 °C and was maintained by air cooling system. After the treatments the leaves of the plants and their respective controls were used for determination of Pi. The results are means of \pm SE for 3 values in each group.

Fig. 2C shows the effect of high temperature on Pi accumulation in leaf of plant after 72h of exposure. Plants acclimated to high temperature had leaf Pi content of 112.31 ± 1.92 mg whereas 64.20 ± 2.13 mg/100 g of leaf for control was observed. As the time extended, the amount of Pi in leaf had been enhanced in response to 45°C. High temperature caused increased accumulation of Pi significantly ($P < 0.001$) by 74.93% compared to the control (Fig. 4), however, the effect was higher than the previous 24h and 48h exposure. The results suggested that the increased Pi in leaf might be due to the higher sensitivity of temperature and caused by temperature stress in the environment where they survived and could be considered as the survival factor as well as index for characterization of physiology of leaf of this species.

Discussion

In the present study, both low and high temperature has been found to be involved in the higher accumulation of inorganic phosphate in leaf of *Basella alba*, however the effects were more pronounced in high temperature induced leaf than that of low temperature. The mechanism of formation of inorganic phosphate in response to the temperature stress is not yet clearly known. However, several lines of evidences might be involved to clarify and recognize the formation of the molecule inorganic phosphate in such adverse situation. It has been shown that low temperature causes the higher oxidative stress inducing

the synthesis of active oxygen species (AOS) (Lee and Lee 2000) and increases tolerance to AOS in cereals and with an increase in anti oxidative enzymes (Mahajan and Tuteja 2005, Kuk 2003). Anti oxidative enzymes can neutralize AOS (Lee and Lee 2000, Odaira *et al.* 2000) and thereby prevents the cellular membranes and organelles from the damaging effects of AOS (Foyer *et al.* 1991). Similarly, heat stress or high temperature is also involved in the synthesis of AOS (Mei and Song 2010). High temperature affects the metabolism and structure of plants, especially cell membranes and many basic physiological processes such as photosynthesis, respiration and water relations (Wahid *et al.* 2007). On the molecular level, this effect of heat stress reflects the temperature dependences of Michaelis-Menten constant (K_m) of every enzyme participating in the process (Mitra and Batia 2008). Plants must cope with heat stress for survival, so they developed different mechanisms including the maintenance of cell membrane, stability, capturing the reactive oxygen species (ROS), synthesis of antioxidants, accumulation and osmoregulation of osmoticum, induction of some kinases that respond to stress. Therefore, the higher synthesis of Pi in the present study in response to both low and high temperature might be linked to the formation of ROS in plant organelles. As the plants survive in such adverse situations, they need the use of Pi as released from the substrate. During low or high temperature exposure, plants may have nutritional deficiency and the accumulated Pi may serve as the molecule giving phosphorus which is very essential element for plant nutrition. Moreover, the energy released for hydrolysis of the energy rich compounds like adenosine triphosphate (ATP) or guanosine triphosphate (GTP) might be utilized to drive the metabolic processes in such adverse environmental circumstances. Since $\text{Na}^+\text{-K}^+$ ATP-ase activity has been demonstrated to be increased by cold exposure (Videla *et al.* 1975), therefore, the increased Pi in leaf in response to cold temperature after 24h, 48h and 72h periods might be due to the higher activity of this enzyme and higher degradation of cellular energy rich compounds such as ATP. The released Pi may play the role for survival of the species during cold acclimation

It is reasonable that the 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. Previous studies revealed that low temperature had been associated with pronounced modifications in the ultrastructure of leaf cells, disorganization of cellular compartments (Stefanowska *et al.* 2002, Stefanowska *et al.* 1999) and therefore, may induce the synthesis of new enzymes and proteins. The structural change, therefore, may cause the alteration of metabolic processes, for example, protein phosphorylation-dephosphorylation reactions. It has been demonstrated that activation of the protein phosphatases of the type 2A is mediated by cold acclimation resulting enhancement of dephosphorylation process (Yaneva *et al.* 2002, Antonio *et al.* 1998). The increased accumulation of Pi in leaf of plant in response to low temperature, therefore, might be due to the higher phosphorylation-dephosphorylation process. The energy rich molecules such as ATP might be degraded or hydrolyzed to produce Pi giving the energy which may be used to serve the reactions, so that the plants survive in the circumstances. Similarly, heat treatment may cause phosphorylation, dephosphorylation or degradation of ATP and other energy rich compounds to produce Pi. Therefore, the concentration of Pi might be increased. Behind this phenomenon, obviously increased oxidative stress should be connected since accumulated Pi in leaf was appreciably higher than the low temperature induced accumulation. However, all these phenomena may assist so that the plants survive in the environment.

During low temperature acclimation, increase of phosphorus playing an important role in plant metabolism is expected since the energy requirement for the circumstances can be obtained from phosphorus containing compounds. Moreover, low temperature storage is used to prolong the shelf life of fruit and vegetables. Muñoz *et al.* (2001) found that storage of fruit at 6 °C was associated with an increase in the total amount of Pi and phosphorylated metabolites and the preferential accumulation of Pi in the cytoplasm in chilled tissues have been confirmed by their experiments. During illumination at low temperature, pea leaves accumulated

high quantities of sucrose, glucose-6-phosphate, fructose-6-phosphate, mannose-6-phosphate and phosphoglycerate (PGA) whereas ATP/ADP-ratios decreased (Streb *et al.* 2003). Therefore, the availability of inorganic phosphate plays a significant role for acclimation to such adverse conditions. High temperature stress directly or indirectly affects plant photosynthetic functions by changing the structural organization and physico-chemical properties of thylakoid membranes (Lichtenthaler *et al.* 2005). The rate of photorespiration increases with increasing temperature which reduces net photosynthesis and probably the seed yield of the crop. Rapid and effective measures of plant treatment are necessary so that deterioration of crops due to high temperature can be countered successfully. The application of plant growth regulators is known to play an important role in plant response to stress (Chakrabarti and Mukherjee 2003). The increased accumulation of inorganic phosphate in leaf in response to high temperature may serve as the molecule during this critical phenomenon like reduced photosynthesis.

Conclusion

During low or high temperature acclimation, plants possess nutritional or energy deficiency as they survive in such critical circumstances. The increased or accumulated inorganic phosphate in leaf may serve as the molecule released from the degradation of energetic compounds and the energy released by this process might be used in this case. The accumulated phosphate might be due to the higher oxidative stress which causes the synthesis of reactive oxygen species (ROS). Therefore, assay of inorganic phosphate during the temperature stress might be a useful parameter to the physiological responses or could be an index for characterization of the physiological and metabolic status of *Basella alba*.

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