

Peroxidase Activity and Lipid Peroxidation in Strawberry (*Fragaria X ananassa*) Plants Under Low Temperature

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ABSTRACT

The activity of peroxidase (PRX) isozyme, lipid peroxidation (Malondialdehyde, MDA content) and cell membrane injury were studied during low temperature treatment for different periods in strawberry (*Fragaria x ananassa* cv. Camarosa) leaf tissues. Seedlings were grown for six weeks (plants had 4-5 leaves) in a greenhouse then the plants were transferred to a climate chamber with constant 5°C, 60% relative humidity, 14/10 h (light/dark) photoperiod regime and 4 LS light intensity for 1, 4, 7 or 10 days to impose a low temperature stress. In general, low temperature application during 10 days caused a linear increase in MDA content. Native polyacrylamide gel electrophoresis (PAGE) of both acidic and basic peroxidase (PRX) isozymes yielded a single sharp protein band with Rf=0.23 and Rf=0.17, respectively. In addition data indicated a strong relationship between band intensities and the duration of the low temperature treatment. However, the considerable increase of PRX activities could not stop the deleterious effects of low temperature, but reduced severity of stress, thus showing a reduction in the percentage of injury on the 7th day which is correlated with cold-acclimation of strawberry leaf tissues under low temperature.

Key Words: Cell membrane injury, lipid peroxidation, low temperature, peroxidase, strawberry (*Fragaria x ananassa*).

INTRODUCTION

Many temperate fruit crops are in the *Rosaceae* and their distribution is restricted by low temperature stress. However, commercially floral damage is often the main form of freeze damage, particularly in several of the early blooming members of the *Rosaceae* such as strawberry, cherry, peach (Rodrigo 2000, Owens et al 2003). One of the biochemical changes occurring when plants are subjected to low temperature stress is the production of reactive oxygen species (ROS). ROS are highly reactive and in the absence of any protective mechanism they can disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids (Allen 1995). Antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (PRX) are the most important components in the scavenging system of ROS. Peroxidases are a family of isozymes found in all plants; they are heme-containing monomeric glycoproteins that utilize either H₂O₂ or O₂ to oxidize a wide variety of molecules (Yoshida et al 2002). So peroxidases are known one of the important parts of the enzymatic defense of plant cells under stress conditions (Gaspar et al 1982).

Plant species differ greatly in their ability to develop cold-tolerance through a process known as cold-acclimation. Biochemical changes that have been associated with cold-acclimation include alterations in lipid composition, increased sugar and soluble protein content, expression of specific proteins, the appearance of new isozymes and so on (Thomashow 1999; Sarnighausen et al 2004; Cansev et al 2005; Eris et al 2007). In this respect, enhancement of cold-tolerance of species would be of considerable interest for preventing cold damage. Therefore figuring out the mechanism of cold-acclimation of species is of a great importance even on the cultivar basis.

In this study, changes in the qualitative activities of PRX were analyzed in strawberry cv. Camarosa plants during low temperature treatments. The experiment was also conducted to examine the effect of low temperature stress on leaf injury and lipid peroxidation (malondialdehyde=MDA content) in cellular level. The main purpose of the study was to provide preliminary information for later, more detailed, studies on strawberry cold-acclimation and freezing tolerance.

MATERIALS AND METHODS

Plant material and low temperature treatments

Cold stored (frigo) seedlings of strawberry (*Fragaria x ananassa* cv. Camarosa) were planted in 12 cm pots using perlite:torf:soil (1:1:1) mixture. Plants were grown for six weeks (plants had 4-5 leaves) in a greenhouse with day/night mean temperature of 25/10°C day/night temperature, average relative humidity of 70%, average photoperiod of 14 h and watered on need basis to avoid any water stress by Actagro (7-7-7) (Actagro LLC, Biola, CA, USA) nutrient solution. The plants were transferred to a climate chamber

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(SANYO MLR-351H, IL, USA) with constant 5°C, 60% relative humidity, 14/10 h (light/dark) photoperiod regime and 4 LS light intensity for 1, 4, 7 or 10 days to impose a low temperature stress. Plants were kept watered by the nutrient solution during the low temperature treatments in the growth chamber. Also, control plants were kept in the greenhouse during the treatments.

Measurement of injury

Membrane thermostability was measured by using the procedure of Arora et al (1998), with some modifications of Gulen and Eris (2003) for strawberry leaf tissue. Leaf discs in 2 cm diameter were cut from fully expanded, uniform leaves from each of three plants (replicates) per treatment [unstressed- control, low temperature (5°C) for 1, 4, 7 or 10 days]. Discs were lightly rinsed in distilled water, gently blotted with paper, and placed in test tubes (one disc per tube). Then, 20 ml of distilled water was added to each test tube. Samples were then vacuum infiltrated to allow uniform diffusion of electrolytes and incubated for 4 h at room temperature. After incubation, electrical conductivity of each solution was measured using a conductivity meter (WTW Cond 315i, Weilheim, Germany). Leaf discs were then killed in the same solution by autoclaving, and total conductivity was measured at room temperature. Percentage injury of each treatment was calculated from ion leakage data using the equation: %injury=[(% L_(t)-% L_(c)) / (100-% L_(c))] x 100 (Arora et al 1992), where % L_(t) and % L_(c) are percentage ion leakage data for the treatments or control samples, respectively.

Lipid peroxidation (malondialdehyde=MDA content)

Lipid peroxidation was estimated by determining the malondialdehyde (MDA) contents in the leaves according to method of Rajinder et al (1981). A hundred milligram of leaf samples was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 × g for 5 min at 4 °C. Aliquot of 0.3 ml supernatant was mixed with 1.2 ml of 0.5% thiobarbituric acid (TBA) prepared in TCA 20%, and incubated at 95 °C for 30 min. After stopping the reaction in an ice bath for 5 min, samples were centrifuged at 10 000 × g for 10 min at 25°C. The supernatant absorbance at 532 nm was then measured using a Beckman UV-DU 520 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA). After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient 155 mM⁻¹ cm⁻¹.

PRX extraction and Native polyacrylamide gel electrophoresis (PAGE) of acidic and basic isoperoxidases

Fully expanded leaf material was collected from each plant group of each treatment. Triplicate samples of leaf tissues were right away frozen and ground in liquid N₂ and stored at -80°C until used. PRX was extracted from leaf tissues using the extraction methods described by Gulen et al (2002). Ground leaf tissues (0.1 g) were homogenized at 4°C in 0.6 ml extraction buffer [0.1 M potassium phosphate pH 7.5, 30 mM boric acid, 50 mM L-ascorbic acid, 17 mM sodium metabisulfite, 16 mM dithiocarbamic acid, 1 mM EDTA and 4% (w/v) PVP-40 and final pH was readjusted to 7.5 with NaOH]. Homogenates were centrifuged at 15 000 rpm for 20 min and supernatant was used for electrophoresis.

Discontinuous PAGE was performed with a PROTEAN III vertical electrophoresis unit (Bio-Rad, Hercules, Calif.) for acidic and basic PRX, respectively, according to Davis (1964) and Reisfeld et al (1962). Five percent stacking gels and 10% separating gels were prepared for both systems. For each sample 20 µl of crude extract was loaded to the gel. Electrophoresis was performed at 20 mA for 30 min, followed by 40 mA for 3h. Gels were stained for PRX using the method of Wendel and Weeden (1989). The relative distance (Rf value) of the bands on the gel was calculated as described by Manganaris and Alston (1992) using Rf=1.0, distance to the fastest band and Rf=0.0, the starting point.

Statistics

The experiment was arranged in a randomized block design with three replications. Data were tested by SPSS 13.0 for Windows program.

RESULTS

The percentage of injury (based on electrolyte leakage) in leaf discs as a function of low temperature for different duration was shown in Figure 1. The percentage injury increased until the 4th day of the low temperature treatment, followed by a decrease on the 7th day. Then the injury sharply increases over 50% level on the 10th day of the low temperature treatment.

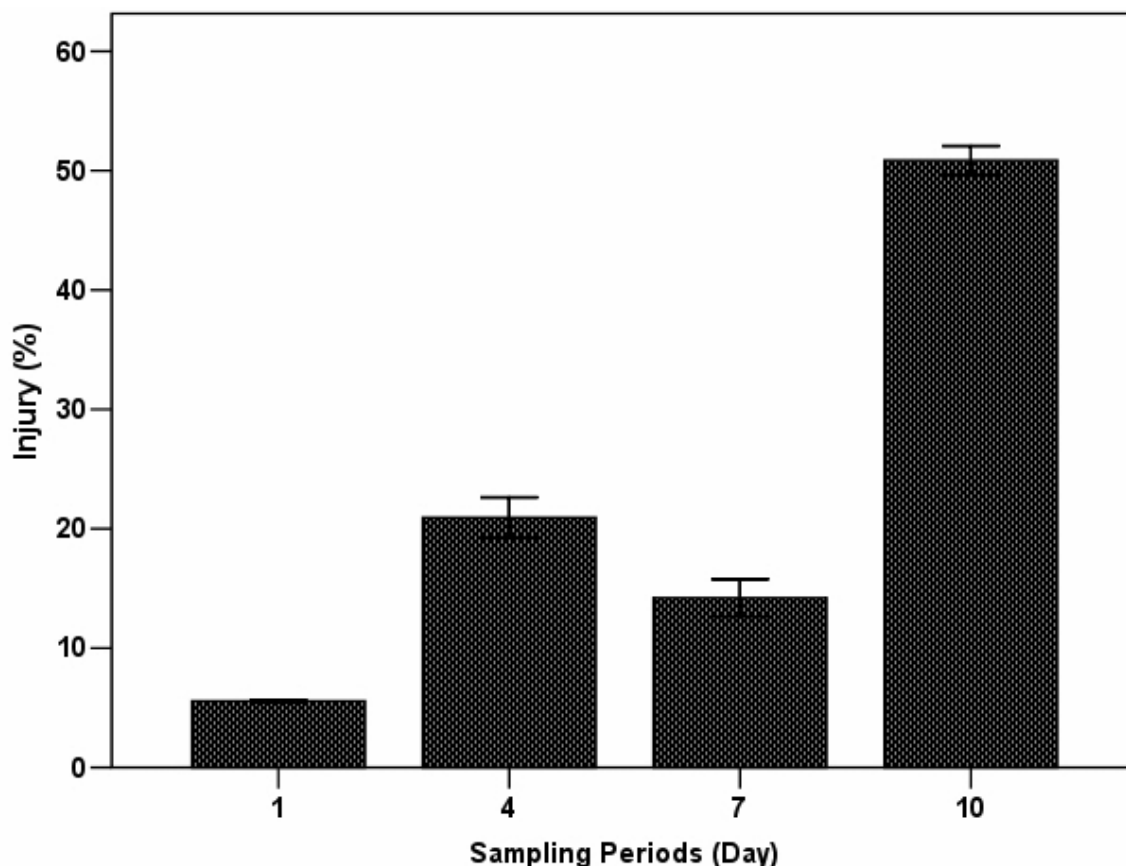


Figure 1. The percentage of injury (based on electrolyte leakage) in the leaf discs of strawberry cv. Camarosa plants under low temperature (5°C) treatment for the periods of 1, 4, 7 or 10 days, and unstressed (control) plants. Error bars represent \pm SE of three replications.

The time courses of MDA content in the leaf tissues subjected to low temperature were given in Figure 2. In general, low temperature application for 10 days caused a linear increase in MDA content. Even though there was no statistical difference between the 1st and the 4th day of the low temperature treatment, MDA content increased linearly and reached to the highest level (~ 115 nmol g FW⁻¹) on the 10th day of low temperature in comparison with control plants (~ 70 nmol g FW⁻¹).

Native PAGE analysis of the samples was repeated at least three times with similar results. Data from a single, representative analysis are presented herein. Native PAGE of PRX was performed to obtain acidic and basic isozyme profiles of leaf tissues subjected to the low temperature for various durations. Native PAGE of both acidic and basic isozymes yielded a single sharp protein band with Rf=0.23 and Rf=0.17, respectively (Figure 3). Although both acidic and basic bands were faint and barely observed in control (unstressed) sample, the bands were observed commonly with different band intensities in all over the low temperature treatment. The intensities of the bands significantly increased depending on the low temperature durations and reached to highest levels on the 10th day in both acidic and basic PRX.

DISCUSSION

Cell membrane stability has been widely used to express stress tolerance, and higher membrane stability could be correlated with abiotic stress tolerance (Premachandra et al 1992). Uemura et al (2006) recently indicated the necessity of an increase in membrane stability during cold-acclimation both under natural and artificial conditions. Moreover, it was stated that there are compositional, structural and functional changes occurring in the plasma membrane, which are increased stability of the plasma membrane under cold conditions. It is known that many of the changes during acclimation to temperature stress are reversible, but if the stress is too great, irreversible changes can occur and these can lead to death (Lester 1985). Indeed,

Shilpi and Narendra (2005) suggested that the symptoms of stress induced injury in plants appear from 48 to 72 h later; however, this duration varies from plant to plant and also depends upon the sensitivity of individual plant to cold-stress. In this respect, percentage injury increased depending on the duration of low temperature treatment with an exception in present study (Figure 1). The decrease in injury on the 7th day of the low temperature treatment was correlated to cold-acclimation of the leaf tissues. Longer period of low temperature treatment which is 10 days had more deleterious effect on the cell membrane, since the injury was over 50% on the 10th day.

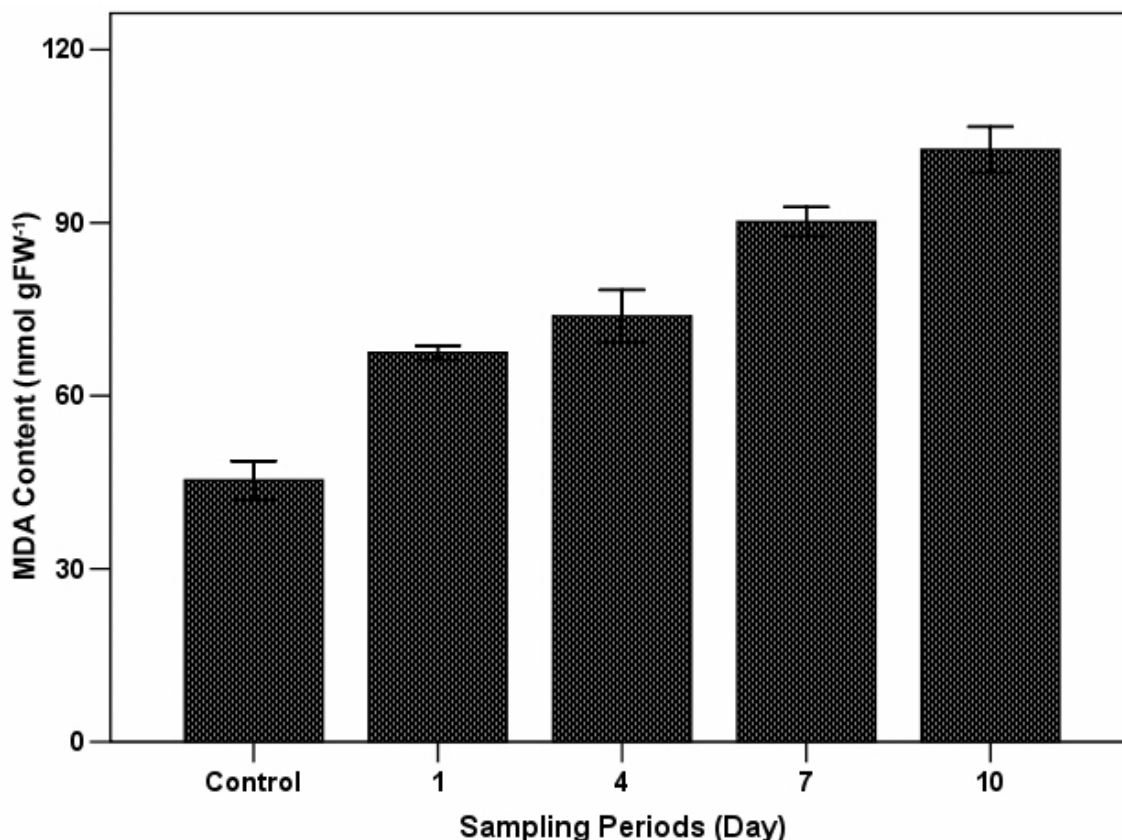


Figure 2. Lipid peroxidation (malondialdehyde=MDA content) in the leaf tissues of strawberry cv. Camarosa plants under low temperature (5°C) treatment for the periods of 1, 4, 7 or 10 days, and unstressed (control) plants. Error bars represent \pm SE of three replications. FW indicates fresh weight.

The peroxidation reactions differ among the fatty acids depending on the number and position of the double bounds on the acyl chain. Oxidation of unsaturated fatty acids by singlet oxygen produces distinctly different products such as MDA (Bradley and Min 1992). MDA is a common product of lipid peroxidation and a sensitive diagnostic index of oxidative injury (Janero 1990). In this respect increase in lipid peroxidation was reported in many plants under various environmental stresses (Moran et al 1994; Prasad 1996). In the present study, time course of MDA content as an expression of lipid peroxidation was increased by low temperature treatment (Figure 2). These data also indicated to severity of cell membrane injury.

Peroxidases are a large group of isoenzymes with an extreme range of isoelectric points, serving a multitude of functions (van Huystee 1987). Each group is thought to have a different function in the cell. Acidic peroxidases are the isoenzymes most likely involved in lignin formation and wall-associated, whereas function of basic isoenzymes has been suggested that they might provide H₂O₂ for other peroxidases (Walter 1992). Regarding the temperature stress, one basic isoperoxidase band (Rf=0.22) was correlated with lignification and recovery of cell membrane damage under heat stress in strawberry leaf (Gulen and Eris 2004). Recently, Cansev et al (2005) reported expression of acidic peroxidase bands with different band intensities which are responsible for tolerance to freezing stress of 15 olive cultivars. In the present study, data from native PAGE indicated one acidic and one basic isoperoxidases with different intensities (Figure

3). Since data indicated a linear relationship between band intensities and the duration of the low temperature treatment, it is correlated to cold-acclimation of strawberry leaf tissues.

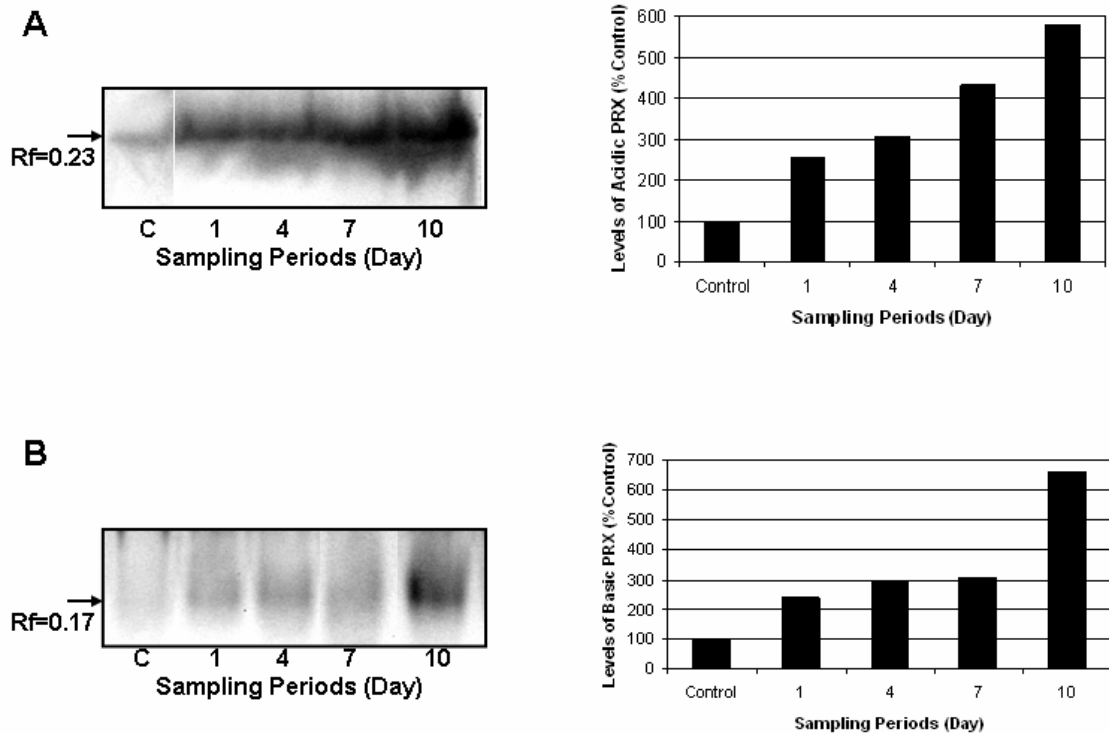


Figure 3. Native PAGE of acidic (A) and basic (B) PRX activities of the leaf tissues of strawberry cv. Camarosa plants under low temperature (5°C) treatment for the periods of 1, 4, 7 or 10 days, and unstressed (control) plants and their band intensities based on the quantitative measurements. Equal volumes of the crude extracts, 20 μ l, were loaded in each lane. Arrows to the left mark indicate the Rf=23 (A) and Rf=17 (B) isoperoxidases.

In conclusion, our data indicated a correlation between PRX activities and cellular damage provoked by low temperature treatment. The considerable increase of PRX activities could not stop the deleterious effects of low temperature, but reduced stress severity thus showing a reduction in the percentage of injury on the 7th day followed by a sharp increase in the 10th day. This fluctuation in the percentage of injury was correlated with cold-acclimation of the plants. In addition MDA content significantly increased under low temperature, which is another indication of cellular damage. One acidic (Rf=0.23) and one basic (Rf=0.17) PRX bands was observed commonly in all samples with different band intensities, and, therefore, might be associated with cell membrane recovery and cold-acclimation in strawberry cv. Camarosa plants under low temperature stress.

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