Alleviation of Chilling Injury in Tropical and Subtropical Fruits

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Abstract

Chilling injury in tropical and subtropical fruits can be alleviated by low temperature preconditioning, intermittent warming, heat treatment, controlled atmosphere storage, treatments with calcium or other chemicals, waxing, film packaging, genetic modification, or applications with ethylene, abscisic acid, polyamines, methyl jasmonate, methyl salicylate, or other natural compounds. The effectiveness of each treatment varies with the commodity, the maturity of the fruit, and the dosage of the treatment. Low temperature conditioning and intermittent warming maintain high levels of phospholipids, increase the degree of unsaturation of fatty acids, increase the levels of spermidine and spermine, and stimulate the activities of free radical scavenging enzymes. Heat treatment induces heat shock proteins (HSP), suppresses oxidative activity, and maintains membrane stability. Methyl jasmonate and methyl salicylate stimulates the synthesis of some stress proteins, such as HSP, pathogenesis-related (PR)-proteins, and alternative oxidase (AOX) and can activate lipoxygenase gene expression and induce synthesis of abscisic acid and polyamines. Polyamines may act as free radical scavengers and membrane stabilizers. All of these processes can enhance chilling tolerance of tissues and alleviate chilling injury of tropical and subtropical fruits.

INTRODUCTION

Most fruits originated from the tropical or subtropical regions are chilling-sensitive (Gross et al., 2002). These crops are injured after a period of exposure to chilling temperatures below 10 to 15°C but above their freezing points. Some horticultural crops of temperate origin are also susceptible to low temperature injury. Those temperate crops have lower critical threshold temperatures (generally below 5 to 10°C). At these chilling temperatures, the tissues weaken because they are unable to carry on normal metabolic processes. Various physiological and biochemical alterations and cellular dysfunctions occur in chilling-sensitive species in response to chilling stress (Wang, 1982). These alterations include stimulation of ethylene production, increase in respiratory rate, interference in energy production, increase in activation energy, slowing of protoplasmic streaming, increase in permeability, reduction in photosynthesis, enzyme inactivation, membrane dysfunction, and alteration of cellular structure. If chilling stress is prolonged, these alterations and dysfunctions will lead to the development of a variety of chilling injury symptoms such as surface lesions, internal discoloration, water-soaking of the tissue, off-flavor, decay and failure to ripen normally (Saltveit and Morris, 1990).

CHILLING INJURY SYMPTOMS

Common symptoms of chilling injury in tropical and subtropical crops are pitting, discoloration, water-soaked appearance, internal breakdown, failure to ripen, off-flavor, and decay (Paull, 1990; Saltveit and Morris, 1990). Symptom development depends not only on species and cultivars, but also on maturity, types of tissues, and environmental factors. Surface pitting is the most common form of chilling injury in many tropical and subtropical fruits and vegetables. Pitting can occur on citrus fruits, cucumbers, eggplant, melons, okra, papayas, pomegranates, sweet peppers, sweetpotatoes, and tamarillos.

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Failure to ripen is another common symptom of chilling injury in tropical and subtropical crops. It can occur in chilled avocados, bananas, mangos, melons, papayas, sapodilla, and tomatoes. Some specific chilling injury symptoms occur in certain commodities, such as subepidermal brown streaking of vascular tissues in bananas, membranous staining in lemons, and mahogany browning in potatoes. Another common chilling injury symptom is internal discoloration. This symptom can occur in such tropical and subtropical crops as avocados, pineapples, sweet potatoes and taro.

FACTORS AFFECTING CHILLING INJURY SUSCEPTIBILITY

Factors affecting the susceptibility to chilling injury include the origin of the crop, genetic makeup of the commodity, stage of development or maturity, metabolic status of the tissue, and a number of environmental factors such as temperature, light, relative humidity, and atmospheric composition.

The origin of a crop or the genetic makeup of a plant determines whether the species is sensitive or resistant to chilling (Patterson and Reid, 1990). In chilling-sensitive species, the critical threshold temperature may vary with the stage of development or maturity. For example, avocados, honeydew melons, mangos, papayas, and tomatoes are more sensitive to chilling when they are less mature (Paull, 1990).

The metabolic status and the chemical composition of the tissue at the time of chilling can affect the resistance of tissue to chilling. Chilling-resistant tissues tend to have a higher degree of unsaturation of fatty acids in the membrane lipids than chilling-sensitive tissues (Tabacchi et al., 1979). High levels of reducing sugars and proline were also found to correlate positively with the resistance to chilling injury (Purvis, 1981; Purvis and Grierson, 1982).

Among the environmental factors, temperature is obviously the main element which can influence the severity of chilling injury. Light can also enhance chilling injury under certain circumstances (van Hasselt, 1990). Chilling-induced structural alteration of proteins associated with photosystem II might be the primary event for light-induced damage (Moll and Steinback, 1986). Relative humidity in the storage environment is another factor affecting the severity of chilling injury. Generally, chilling injury symptoms are more severe under low relative humidity than under high relative humidity.

RESPONSE OF TROPICAL AND SUBTROPICAL FRUITS TO CHILLING TEMPERATURE

Prolonged exposure of tropical and subtropical crops to chilling temperature results in various physiological and biochemical alterations which lead to a variety of chilling injury symptoms. There are two hypotheses to explain how chilling temperatures induce various responses and cause detrimental effects. The first hypothesis suggests that there is a single unifying primary response to chilling temperature for all chilling-sensitive species. This primary response would lead to secondary events and then develop into a variety of chilling injury symptoms. Several events have been proposed as likely candidates for the primary response including a phase transition in membrane lipids, an alteration in the kinetics or substrate specificity of a regulatory enzyme, a change in the cytoskeletal structure, or an increase in cytosolic calcium (Raison and Orr, 1990). After prolonged exposure of sensitive species to chilling, the primary response would lead to secondary events which include loss of membrane integrity, leakage of solutes, loss of compartmentation, decrease in the rate of mitochondrial oxidative activity, increase in the activation energy of membrane-associated enzymes, cessation of protoplasmic streaming, reduction in energy supply and utilization, decrease in photosynthetic rate, disorganization of cellular and subsellular structure, dysfunction and imbalance of metabolism, accumulation of toxic substances, stimulation of ethylene production, increase in respiration rate, and manifestation of a variety of chilling injury symptoms.

The second concept puts forth the idea that chilling injury originates from a multitude of responses to low temperature. Because of the diversity of tropical and subtropical fruits and vegetables in the structure and expression of chilling injury symptoms, it is hard to believe that a single universal primary response can set in motion a chain of events and cascade into a wide range of chilling symptoms. Therefore, the second concept suggests that there are multiple responses over a wide range of conditions (Lyons and Breidenbach, 1987; Shewfelt, 1992; Wade, 1979).

ALLEVIATION OF CHILLING INJURY IN TROPICAL AND SUBTROPICAL FRUITS

The ultimate goal of chilling injury research is to find effective techniques to alleviate injury induced by chilling. The reduction of chilling injury can be achieved either by increasing the tolerance of commodities to chilling stress or by retarding the development of chilling injury symptoms in the chilling-sensitive tropical and subtropical fruits and vegetables. Several methods have been shown to reduce chilling injury including high or low temperature conditioning, intermittent warming, controlled atmosphere, applications of growth regulators or other chemicals, waxing and other coatings, and packaging (Wang, 1993). The first three approaches manipulate and modify the storage environment while the others involve direct treatment to the commodities. Some techniques are more effective in alleviating chilling injury in certain crops than others, and the optimum treatment conditions vary with different commodities.

Temperature Conditioning

1. Low Temperature Conditioning. Exposure of chilling-sensitive tropical and subtropical fruits and vegetables to temperatures slightly above the critical chilling range increases the tolerance of these commodities to chilling during subsequent low temperature storage and delays the development of injury symptoms. Apparently, low temperature conditioning induces an adaptive response in fruits and vegetables to chilling stress. This adaptation to lower temperatures is the result of various physiological and biochemical modifications induced by the conditioning treatment. These modifications include reducing the loss of membrane phospholipids; increasing sugar, starch and proline content; maintaining high levels of polyamines, squalene, and long-chain aldehydes; and increasing the ratio of unsaturated to saturated fatty acids. All of these modifications contribute to the increase in chilling tolerance. Low temperature conditioning has been shown to be effective in alleviating chilling injury in cucumbers, eggplants, grapefruit, lemons, limes, mangos, papayas, sweet peppers, sweet potatoes, tomatoes, watermelons, and zucchini squash (Hatton, 1990).

2. High Temperature Conditioning. Short term treatment with temperatures higher than 35°C can reduce chilling injury of some commodities. It has been hypothesized that exposure to one type of stress may induce some factors which can protect against another type of stress (Klein and Lurie, 1991). Heat treatment can be applied as hot water dip, hot forced air, or vapor heat. High temperatures promote healing of cuts and bruises, and increase antifungal compounds, provide protection against invasion of pathogens. Heat treatment also induces increase in transcripts for a low-molecular weight (KD 17) and a 70 KD heat-shock protein, reduces chromatin condensation and DNA breakdown, and suppresses oxidative activity (Wang et al., 2001). Heat treatment at proper temperatures and duration has been shown to alleviate chilling injury of avocados, citrus fruits, cucumbers, mangos, sweet peppers, persimmons, tomatoes, and zucchini squash.

Intermittent Warming

Intermittent warming is the interruption of low temperature storage with one or more short periods of warm temperature. This brief warm temperature treatment can increase the storage life of some chilling-sensitive commodities, but must be applied before chilling injury becomes irreversible. Therefore, it is important to detect chilling injury early. Early detection of chilling injury can be achieved by measuring the stimulation of ethylene production (Field, 1990), the changes in Fourier transform infrared spectra (Buta and Wang, 1993), or the increase in nuclear magnetic resonance imaging signals (Wang and Wang, 1992).

There are several hypotheses concerning the mechanisms of intermittent warming. Raising the temperature temporarily could induce higher metabolic activities which would allow tissues to metabolize excess intermediates and replenish deficiencies that developed during chilling. Warming of chilled tissues for short periods helps to repair damage to membranes, organelles, or metabolic pathways (Lyons and Breidenbach, 1987). Warming and cooling of tissues could also increase the synthesis of polyunsaturated fatty acids. Intermittent warming has been found to alleviate chilling injury in cucumbers, grapefruit, lemons, nectarines, peaches, sweet peppers, tomatoes, and zucchini squash (Forney and Lipton, 1990).

Controlled Atmospheres

The effectiveness of controlled atmosphere in reducing chilling injury varies with commodities. Controlled atmosphere may be beneficial, detrimental or have no effect in alleviating chilling injury. Most commodities respond favorably to a decrease in oxygen level and an increase in carbon dioxide concentration. However, for certain crops, controlled atmosphere aggravates the symptoms of chilling injury. Tropical and subtropical crops which can benefit from controlled atmosphere include avocados, grapefruit, Japanese apricots, nectarines, okra, peaches, pineapples, potatoes, and zucchini squash.

Growth Regulators and Natural Products

Growth regulators influence a wide range of biochemical and physiological processes in plant tissues. The modifications of these processes may in turn alter the chilling tolerance. The level and balance of certain growth regulators can also affect the susceptibility of plant tissues to chilling injury (Ismail and Grierson, 1977).

Abscisic acid (ABA) stabilizes the microtubular network, suppresses ion leakage, and prevents loss of reduced glutathione and membrane phospholipids (Rikin et al., 1979). ABA applications reduce chilling injury in grapefruit and zucchini squash. Polyamines are a group of polycationic organic compounds and are ubiquitous in cells. Postharvest treatments with exogenous polyamines increase internal polyamine levels and reduce chilling injury (Kramer and Wang, 1989). The reduction of chilling injury by polyamines may be related to their antioxidant activity and stabilizing effects on membrane. Methyl jasmonate occurs naturally in a wide range of higher plants and has been shown to affect a number of biological processes. Treatment of chilling-sensititve crops with methyl jasmonate induces the synthesis of some stress proteins, such as heat shock proteins, pathogenesis-related proteins, and alternative oxidase (Ding et al., 2001, 2002; Fung et al., 2004). Postharvest application of methyl jasmonate was found to reduce chilling injury of avocados, grapefruit, sweet peppers, and zucchini squash (Meir et al., 1996; Wang and Buta, 1994).

Calcium and Other Chemicals

There have been some good correlations between calcium content in tissues and the susceptibility of fruits or vegetables to chilling injury. Lime fruit with the lowest calcium content in their juice develop the highest percentage of chilling injury (Slutzky et al., 1981). Application of calcium significantly reduced the severity of chilling injury in avocados, okra, peaches, and tomatoes. Some chemicals that possess the properties of antioxidants or free radical scavengers have been reported to reduce chilling injury. For example, ethoxyquin and sodium benzoate applied to cucumbers and sweet peppers were found to maintain a high degree of unsaturation of fatty acids in polar lipids and reduce chilling injury (Wang and Baker, 1979). Postharvest treatment with dimethylpoly-siloxane, safflower oil, or mineral oil prevented chilling-induced underpeel discoloration of bananas (Jones et al., 1978). These compounds may act as antioxidants and reduce oxidative damage induced by chilling temperature.

Packaging

The packaging of fruits and vegetables with plastic films helps to maintain high relative humidity and modify the concentrations of oxygen and carbon dioxide in the atmospheres surrounding the commodity. The prevention of chilling injury by film packaging is likely related to these factors. The reduction of water loss from the tissue apparently inhibits the collapse of epidermal and underlying cells and prevents pitting formation. Packaging with low density polyethylene film alleviated chilling injury in cucumber fruit (Wang and Qi, 1997). Wrapping fruit individually in heat-shrinkable film also drastically reduced pitting and scald in chilled grapefruit (Miller et al., 1990). Packaging has also been shown to delay chilling injury in bananas, pineapples, Japanese apricots, lemons, and tomatoes.

Waxing and Other Coatings

Waxing not only improves the appearance of fruits and vegetables but also restricts gas exchange and retards transpiration of fresh produce. Waxing has been proven to reduce chilling injury in grapefruit, oranges, pineapples, and cucumbers. Coating with vegetable oil, safflower oil, mineral oil, or dimethylpolysiloxane also has been shown to prevent the development of underpeel discoloration in bananas stored at chilling temperatures. Similar to film packaging, the effectiveness of waxing in alleviating chilling injury is related to reducing moisture loss and modifying internal atmosphere.

Genetic Modification

Great genetic variation in plants provides opportunity for modifying chilling sensitivity by transferring resistant genes to sensitive species. Species from high altitudes or latitudes such as wild tomatoes and potatoes which grow naturally in Ecuador and Peru are usually less sensitive to chilling injury than those from low altitudes or latitudes (Patterson and Reid, 1990). Therefore, these wild species are likely sources of chilling resistant genes for possible use in conventional breeding or genetic engineering. Identifying and isolating the genes responsible for chilling resistance and the subsequent transfer of these genes into susceptible species provide a viable avenue for alleviating chilling injury. Structural information for cDNA clones for mRNA coding of chilling resistant genes or the use of classical genetic mapping is required to narrow down the genome and the region on the chromosome which carries the resistant trait. Immunological screening or antisense RNA technology may be applied for characterization of the genes. The success of these molecular biological techniques would also depend upon proper transfer of the genes and the successful expression of the transferred genes.

CONCLUSIONS

The great diversity in shape, size, and physiology of various tropical and subtropical fruits and vegetables contributes to the variations in the degree of sensitivity or tolerance to chilling injury and the resulting injury symptoms. In addition, environmental conditions under which the crops are grown have great influence on the susceptibility to chilling injury. Chilling injury is responsible for substantial postharvest losses in tropical and subtropical horticultural commodities. Several approaches have shown promise in alleviating chilling injury as described in this paper. The effectiveness of these methods in reducing chilling injury often varies with the commodity and is affected by the maturity and physiological state of the commodity as well as a number of environmental factors. Some techniques described in this paper are applicable for several commodities. However, the optimum conditions of a treatment are often specific for a given species or cultivar. Further research is needed to determine how chilling injury develops in tropical and subtropical crops, when the damages become irreversible, and how the cellular metabolism can be modified to increase its capacity to withstand chilling stress. The more we understand the mechanisms of chilling injury, the better we can devise a more effective method to alleviate chilling-induced postharvest losses of tropical and subtropical fruits and vegetables.

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