LOW-TEMPERATURE TOLERANCE OF PRICKLY PEAR CACTI

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Abstract

Freezing temperatures are one of the main factors limiting the cultivation of prickly pear cacti for cattle fodder and human consumption in the United States. Agronomically important *Opuntias* such as *Opuntia ficus-indica* generally are irreversibly injured at -5°C to -10°C, whereas various widely distributed native species such as *Opuntia fragilis* and *O. humifusa* can tolerate -20°C to -40°C. As ice crystals grow between cells when stems are subjected to freezing temperatures, the cells become dehydrated and are killed by a loss of internal water. When environmental temperatures decrease, the stem water content also tends to decrease, especially for winter-hardy species, and plants are able to tolerate lower temperatures. During such low-temperature acclimation, the cellular content of sugars increases, and mucilage sometimes increases. Spraying the stress hormone abscisic acid on *Opuntias* also increases their tolerance to low temperatures. Further hormone studies as well as comparisons between sensitive and winter-hardy species should help elucidate the cellular changes that occur for prickly pear cacti during acclimation to low temperatures.

Introduction

Prickly pear cacti have been used as fodder and forage for cattle in southern Texas for over 125 years. A few hundred hectares (1 hectare = 2.47 acres) are now planted with *Opuntia ficus-indica, O. streptacantha, O. rastrera*, and *O. robusta* for feeding cattle in times of drought. Spines deter cattle from eating these prickly pear when other plants are available as forage; when drought commences, the spines can be burned off with propane burners, exposing the succulent stems that cattle readily eat (Russell and Felker, 1987a,b; Nobel, 1994). In addition, native prickly pear, such as *Opuntia engelmannii, O. lindheimeri, O. macrorhiza, O. phaeacantha*, and *O. violacea*, occur on 500,000 hectares in Texas. These species can also be eaten by range cattle, greatly increasing the importance of the members of the genus *Opuntia* as a forage source of nutrients and water. Furthermore, over 600,000 hectares of *Opuntias* are cultivated for fodder worldwide (Nobel, 1994). Prickly pear cacti are also raised for fruits in over 20 countries, including a small region in west-central California. The fruits, commonly called "cactus pears" (Pimienta-Barrios et al., 1993), can be purchased in supermarkets in the southwestern United States (these fruits come mainly from Mexico) as well as in specialty sections of stores elsewhere in the U.S. In addition, young cladodes are eaten as a vegetable in Mexico and increasingly in other countries. In 1992, approximately 1.2 million people worldwide derived their main livelihood from cacti (Nobel, 1994).

The survival and productivity of cacti grown as cattle fodder and forage or for human consumption are often limited by the episodic occurrence of freezing temperatures (Russell and Felker, 1987b). Agronomically important prickly pear are generally irreversibly injured at temperatures of -5°C to -10°C (14°F to 23°F; Nobel, 1988, 1990). Indeed, susceptibility to freezing is the primary factor limiting the expansion of prickly pear as fodder and forage in cattle-producing regions of the United States. Yet, over half of the approximately 200 cactus species occurring north of Mexico are native to regions where they experience freezing temperatures every year (Benson, 1982). Many of these species tend to exhibit substantial cold acclimation ability and freezing tolerance (Nobel, 1988). For example, the extremely cold tolerant *Opuntia fragilis* when acclimated to day/night air temperatures of 10°C/0°C for about 10 days can survive a 1-hour treatment at -40°C (-40°F), consistent with its occurrence at high elevations in United States and as far north as 57° north latitude in Canada (Benson, 1982; Loik and Nobel, 1993a). This review examines the low-temperature responses of *Opuntias* and the accompanying changes in cellular properties.

Low-temperature Injury

The events occurring at freezing temperatures that lead to the death of prickly pear stems can be determined using a combination of microscopic and thermal techniques. Typically, small samples of stem tissue are cooled gradually to a specific freezing temperature at which they are maintained for 1 hour (Nobel, 1988). They are then slowly warmed to 5°C, immersed in the vital stain neutral red, and stored at about 5°C for 24 hours. Tissue sections approximately three cells thick are examined under a microscope. The cells that survive the subzero treatment accumulate neutral red and appear dark red whereas those that died do not accumulate stain. By plotting the percent of cells that accumulate stain as a function of treatment temperature (Fig. 1), the temperature at which cell death occurs can be determined. The temperature leading to 50% stain accumulation (compared to accumulation at 0°C) is often considered the lethal temperature, LT_{50} (Loik and Nobel, 1993a).

Monitoring changes in the temperature of stem tissue during a freezing episode can be used to interpret the interaction between ice formation and cell death. As air temperature is decreased below 0°C, usually at a rate similar to that observed in the field (Nobel, 1981, 1982; Nobel and Loik, 1990), water in the stem tissue decreases below 0° but does not immediately freeze. Such supercooling of cellular water continues until about -5°C, when a sudden increase in tissue temperature occurs. This increase in temperature is due to the release of heat as water freezes (melting of ice requires an equal supply of heat). Microscopic observation reveals that such ice formation occurs in the spaces between cells (Nobel, 1988). The amount of water that moves out of cells to the intercellular ice crystals depends on the cooling rate, the subzero temperature achieved, and the original water content of the cells. When intracellular water loss is excessive, cell death occurs due to dehydration. Indeed, cells that have been killed by freezing appear greatly shrunken due to their loss of water.
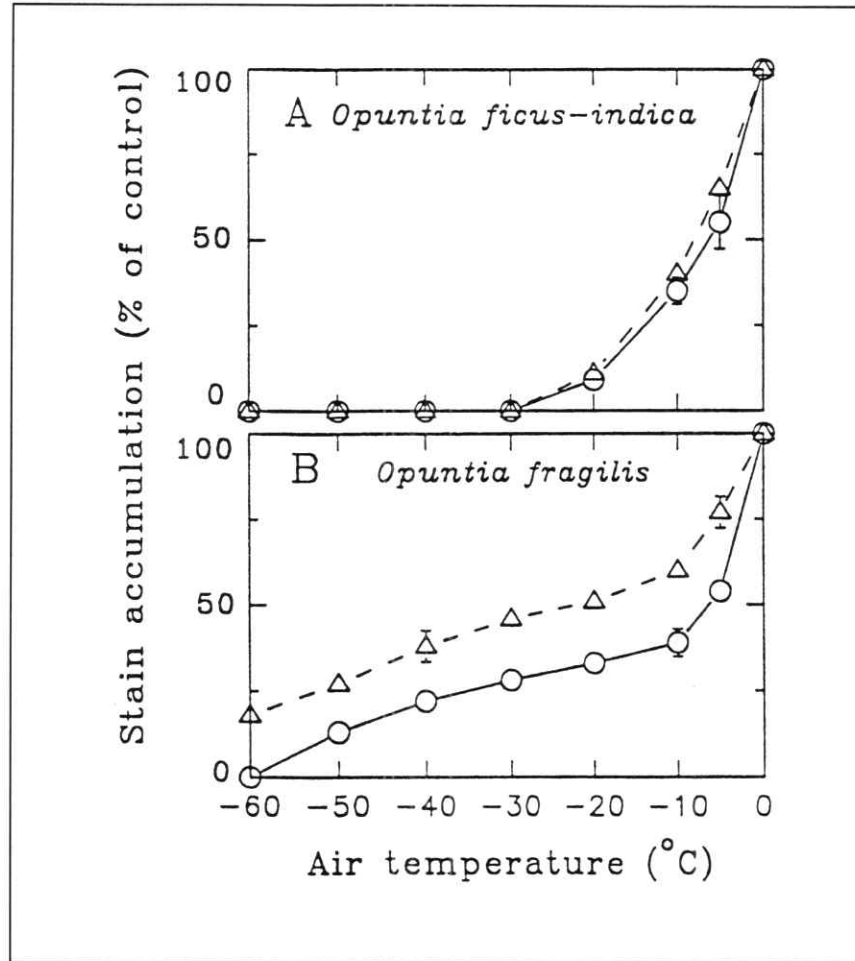
Figure 1. Viability of chlorenchyma cells of *Opuntia ficus-indica* (A) and *Opuntia fragilis* (B) measured by stain accumulation at freezing temperatures. Uptake of a vital stain for stem samples treated for 1 hour at the indicated temperature is expressed relative to stain uptake at 0°C. Plants were maintained at day/night air temperatures of 30°C/20°C (O) or for 14 days at 10°C/0°C (Δ). Data are unpublished observations of M.E. Loik and P.S. Nobel.

The lethal temperature LT_{50} and the low-temperature acclimation (the change in LT_{50} following a decrease in day/night air temperatures) can be determined using the vital stain technique (Figure 1). For *O. ficus-indica* maintained at day/night air temperatures of 30°C/20°C, half of the cells succumb to a 1 hour treatment at -7.7°C (Figure 1A). When maintained at day/night temperatures of 10°C/0°C for 14 days, the LT_{50} is -8.8°C, so the low-temperature acclimation is 1.1°C (Nobel, 1988). This technique has been used to identify species that can survive extremely low temperatures, such as *O. fragilis* (Figure 1B). Such winter-hardy species may be important for future breeding efforts to increase the freezing tolerance of agronomically important prickly pears.

Variation in Low-temperature Tolerance

Tolerance of freezing episodes varies greatly among prickly pear cacti (Table 1). Over many generations, the local environment influences prickly pear populations. This variation helps determine the geographic distribution of naturally occurring species and limits the use of many prickly pears planted as cattle food in regions of the United States that experience appreciable freezing temperatures each winter. Moreover, the potential for genetic change may be important for future efforts at enhancing the freezing tolerance of these plants.

Measurements of vital stain uptake have shown that cacti from high latitudes and high elevations are generally more freezing tolerant than those from predominantly tropical or subtropical regions (Table 1). For example, *O. stricta* from Brazil is not very cold tolerant, presumably related to its origin in tropical regions where freezing does not occur. For *O. streptacantha* from Arizona, where freezing episodes occur every few years, LT_{50} is -12°C. On the other hand, for species that experience appreciable subzero temperatures each year, such as *O. humifusa* from the Great Lakes region of Ontario, Canada, and *O. polyacantha* from 2230 meters (7300 feet) in the Rocky Mountains of Wyoming, LT_{50} is about -20°C (Table 1).

Table 1. Low-temperature tolerances (LT_{50}) for economically important and widely distributed species of *Opuntia*. Data are for plants acclimated to day/night air temperatures of 10°C/0°C and are adapted from Russell and Felker (1987a), Nobel (1988, 1990), Nobel and Loik (1990), and Loik and Nobel (1993a).

Species	Origin	LT_{50} (°C)
O. ficus-indica	Mexico	-9
O. fragilis	Alberta, Canada	-40
	New Mexico, USA	-28
	Ontario, Canada	-20
O. humifusa	Ontario, Canada	-19
	Virginia, USA	-8
O. hyptiacantha	Mexico	-9
O. megacantha	Mexico	-9
O. polyacantha	Wyoming, USA	-21
O. streptacantha	Arizona, USA	-12
O. stricta	Brazil	-8

Freezing tolerance can also vary within a species for plants from different geographic locations. For example, *O. fragilis* and *O. humifusa* are widely distributed in western and eastern North America, respectively (Benson, 1982). Comparisons of LT_{50} for plants of these species from different locations show that the lethal temperature differs according to the climatic condition where the plants grow (Nobel and Loik, 1990; Loik and Nobel, 1993a). For *O. fragilis* from New Mexico, LT_{50} is about -28°C, whereas for plants from northern Alberta, LT_{50} is -40°C (Table 1). For a population in eastern Ontario with an annual average snowfall of 385 mm (15 inches), LT_{50} is -20°C, presumably due to the insulating nature of snow. Other climatic features that influence survival of freezing temperatures include wind, the presence of neighboring vegetation, the duration of low-temperature episodes, and the date of snowmelt. Based on a study of *O. fragilis* from 20 populations in North America, LT_{50} for plants from a particular location is most correlated with the extreme minimum air temperatures at the location (Loik and Nobel, 1993a).

Water-Relations Changes During Low-Temperature Acclimation

Cold-acclimated cacti generally have a reduced water content. For the winter-hardy *Opuntia humifusa*, water content is reduced by 24% in Iowa in January (Koch and Kennedy, 1980) and by 42% for the green photosynthetic chlorenchyma of plants in environmental growth chambers at day/night air temperatures of 5°C/-5°C (Loik and Nobel, 1991). On the other hand, the water content of the freezing-sensitive *O. ficus-indica* remains virtually constant over a 13-day period at 10°C/0°C (Goldstein and Nobel, 1991). Artificial maintenance of high water content by injections of water into stems apparently reverses the acclimation process (Loik and Nobel, 1993b). Whether this is a result of the high water content per se or to the reversal of biochemical changes is not known.

At present, the cause of the reduced water content at low temperatures is unclear, although water may simply be frozen in the soil and therefore be unavailable for uptake. In addition, root water uptake for *O. ficus-indica* is more than halved when the temperature is decreased from 30°C to 1°C (Lopez and Nobel, 1991). Despite reduced water uptake, continued stomatal opening and substantial stem water loss occurs for 14 days after a shift to 5°C/-5°C for *O. humifusa* (Nobel and Loik, 1990). Therefore, reduced water content may occur through a combination of reduced water uptake by the roots and maintenance of appreciable water loss from the cladodes.

The reduction in water content during low-temperature acclimation is accompanied by changes in several water relations parameters that can enhance freezing tolerance. For example, the osmotic pressure (an indication of the solute content) of the chlorenchyma increases by 0.13 megapascal for *O. ficus-indica* and by 0.55 megapascal for *O. humifusa* a few weeks after reducing the temperature by 20°C (Goldstein and Nobel, 1991; Loik and Nobel, 1991). The van't Hoff relation indicates that this increase in osmotic pressure lowers the freezing point of water less than 0.3°C and so, by itself, does not appreciably enhance survival (Koch and Kennedy, 1980; Littlejohn and Williams, 1983; Loik and Nobel, 1991; Nobel, 1991). Changes in cell wall flexibility, which may influence the rate of water loss during extracellular freezing, also can occur during low-temperature acclimation.

Furthermore, the amount of water inside cells relative to that in the extracellular spaces decreases after 14 days at day/night air temperatures of 5°C/-5°C (Loik and Nobel, 1991).

Solute Changes During Low-Temperature Acclimation

The accumulation of organic solutes, particularly sugars such as glucose, fructose, and sucrose, occurs for many plant species during low-temperature acclimation (Franks, 1985; Guy, 1990; Goldstein and Nobel, 1991). Solutes may act as noncolligative cryoprotectants that maintain the structure and function of membranes, proteins, and nucleic acids by substituting for water molecules. Sugars may also be important osmotically in the cellular retention of water and thus the prevention of plasmolysis during extracellular freezing (Levitt, 1980). In addition, sugars may dilute toxic compounds as the cell sap becomes concentrated during extracellular freezing (Steponkus and Lanphear, 1978). Whether and how the accumulation of sugars and other solutes reduces freezing damage to cells of prickly pear cacti during low-temperature acclimation remain to be determined.

Mucilage, which is produced in large cells and then exuded into the extracellular spaces, often occurs in copious amounts in prickly pear stems (Gibson and Nobel, 1986). The mucilage content approximately doubles after 14 days at 5°C/-5°C for *O. humifusa* (Loik and Nobel, 1991) but decreases for *O. fragilis* (Loik and Nobel, 1993a). Mucilage has a high capacity to bind water, suggesting that it may play a role in regulating the movement of water out of cells during freezing. Mucilage may also provide extracellular protection during freezing by interfering with ice crystal growth or by enhancing ice nucleation (Franks, 1985; Goldstein and Nobel, 1991; Loik and Nobel, 1991). Changes in the ion content of mucilage can affect water movement between cells and mucilage during drought (Nobel *et al.*, 1992) and mucilage may act as a carbon reserve (Sutton *et al.*, 1981), both of which could influence the low-temperature acclimation of *Opuntias*.

When various C_3 and C_4 plants are exposed to low temperatures, the stress hormone abscisic acid (ABA) increases (Chen and Gusta, 1983; Reaney *et al.*, 1989). Exogenously applied ABA can increase the freezing tolerance by 6 to 12°C (Chen and Li, 1982; Xin and Li, 1992). Localized increases in ABA induced by low temperature are generally followed by changes in gene expression (Johnson-Flanagan *et al.*, 1991), which can lead to increases in glucose, fructose, sucrose, and proline content (McLaren and Smith, 1976; Stewart, 1980). Other effects of ABA application include changes in membrane structure and ion transport, in xylem differentiation and the ability of roots to take up water, and in cell structure and tissue development (Reaney *et al.*, 1989). Exogenous ABA shifts *Portulacaria afra* from C_3 to CAM (Ting, 1981) but does not enhance the expression of genes unique to CAM for *Mesembryanthemum crystallinum* (Thomas *et al.*, 1992). The relation of ABA to freezing tolerance for cacti, most of which are CAM plants, is unclear (Gibson and Nobel, 1986; Nobel, 1988; Loik and Nobel, 1993a). In this regard, spraying *O. ficus-indica* and *O. fragilis* with ABA at day/night air temperatures of 30°C/20°C causes their LT_{50} to be similar to that for plants maintained at 10°C/0°C (Loik and Nobel, 1993b).

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Concluding Remarks

Although some progress has been made recently (Goldstein and Nobel, 1991; Loik and Nobel, 1993a, b), much remains to be learned about the cellular changes that occur during acclimation to low temperatures for prickly pear cacti. For example, does abscisic acid lead to the production of cryoprotectants, as it appears to do for many other agronomically important species? Do membranes, proteins, and other cellular constituents change during low-temperature acclimation? A recent approach has been to compare the low-temperature responses of *O. ficus-indica* with those of winter-hardy species such as *O. fragilis* and *O. humifusa*. This strategy may identify features unique to winter-hardy species. Breeding and management efforts can then be used to increase the tolerance of the agronomically important prickly pear cacti to freezing temperatures.

Acknowledgement

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