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Low-temperature resistance in *Polylepis tarapacana*, a tree growing at the highest altitudes in the world

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ABSTRACT

The *Polylepis tarapacana* forests found in Bolivia are unique with respect to their altitudinal distribution (4200–5200 m). Given the extreme environmental conditions that characterize these altitudes, this species has to rely on distinct mechanisms to survive stressful temperatures. The purpose of this study was to determine low-temperature resistance mechanisms in *P. tarapacana*. Tissue was sampled for carbohydrate and proline contents and micro-climatic measurements were made at two altitudes, 4300 and 4850 m, during both the dry cold and wet warm seasons. Supercooling capacity (–3 to –6 °C for the cold dry and –7 to –9 °C for the wet warm season) and injury temperatures (–18 to –23 °C for both seasons), determined in the laboratory, indicate that *P. tarapacana* is a frost-tolerant species. On the other hand, an increase in supercooling capacity, as the result of significant increase in total soluble sugar and proline contents, occurs during the wet warm season as a consequence of higher metabolic activity. Hence, *P. tarapacana*, a frost-tolerant species during the colder unfavourable season, is able to avoid freezing during the more favourable season when minimum night-time temperatures are not as extreme.

Key-words: cold resistance; freezing avoidance; frost tolerance; tree line; tropical Andes.

INTRODUCTION

The upper altitudinal limit of continuous forest is one of the most distinct ecological boundaries. In tropical mountains this limit varies around 3000 m, depending on local conditions. *Polylepis tarapacana*, a tree line species found at Sajama Volcano in Bolivia, is unique because of its altitudinal distribution. The lower altitudinal limit of this species lies at 4200 m and the upper limit reaches 5200 m. This upper distribution limit represents the maximum altitude in the world for any arborescent form (Braun 1997; Liberman-Cruz, Gaffta & Pedrotti 1997). All *Polylepis* species are restricted to the Andean Cordillera, ranging from Venezuela to northern Argentina. The different species of this high mountain genus must rely on impressive adaptive features that permit them to survive extreme environmental conditions, mainly low temperatures (Goldstein, Meinzer & Rada 1994).

Different mechanisms by which *Polylepis sericea* survives low night-time temperatures at 4100 m in the Venezuelan Andes have been described previously (Rada *et al.* 1985). Avoidance mechanisms are sufficient to ensure survival of this species under these environmental conditions where little seasonal thermal variation occurs and minimum temperatures have not been reported below –10 °C (Azócar & Monasterio 1980). On the other hand, Squeo *et al.* (1991, 1996) working with different tropical and subtropical plants, representing different life forms, along altitudinal gradients have described a very close relationship between environmental conditions and resistance mechanisms. As suggested by other authors (Levitt 1980; Sakai & Larcher 1987), they found that in less extreme environments at lower altitudes all species depend exclusively on avoidance mechanisms, whereas plants growing in more extreme environments at higher altitudes all survive through the frost tolerance of some or all of their tissues.

The purpose of this study was to determine low-temperature resistance mechanisms in *Polylepis tarapacana*, a tree species growing at the upper altitudinal limit of this life form. Two questions may be addressed concerning this objective. How does this species, growing under stressful conditions, compare with other tropical tree line species in terms of cold resistance mechanisms? Are there seasonal differences in its low-temperature resistance mechanisms because of its distribution at latitudes where marked temperature differences occur between seasons?

MATERIALS AND METHODS

Site characteristics and plant material

The *Polylepis tarapacana* open forest forms a continuous belt around the Sajama Volcano in Oruro, Bolivia (18°7′ S, 68°57′ W). The studies were carried out on lateral moraines of the southern slope of the volcano at altitudes of 4300 and 4850 m. This semi-arid, tropical, high mountain environment has an annual mean temperature of 3·4 °C and pre-

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cipitation of 347 mm. Striking seasonal changes are observed in both temperature and water conditions between the dry cold and wet warm season. Maximum and minimum temperatures registered at 4200 m were 21 °C and −19 °C, respectively (Liberman-Cruz 1986). Field studies and collection of samples for laboratory work were made during the end of the dry cold and middle of the wet warm season. Adult trees ($n = 5$) were chosen for both field and laboratory studies.

Field studies

Measurements were made over three 24 h periods during both the dry cold season at 4300 m (7–10 September 1998) and the wet warm season (19–22 February 1999). In all periods the air temperature (copper–constantan thermocouples) and relative humidity (aspirated psychrometer) were measured at 2 h intervals during daylight hours, and also at midnight and pre-dawn. Plant material for carbohydrate and proline content was collected at approximately 6 h intervals during these same periods (0630, 1300, 1830 and 2330 h).

Laboratory studies

To determine the temperature at which freezing of leaf and stem tissue occurred (i.e. supercooling capacity), branches bearing leaves taken from five different trees, at both 4300 and 4850 m, were cut under water in the field, covered with polyethylene bags and brought to the laboratory. In the laboratory, leaf and stem sections were placed in tightly sealed 6 cm^3 tubes to avoid changes in tissue water content that could affect supercooling capacity (Rada *et al.* 1987). Copper–constantan thermocouples were inserted into the leaf and stem samples and temperature changes were continuously monitored with a chart recorder. The tubes were immersed in a refrigerated alcohol bath and temperature was lowered from 5 °C to −25 °C at a rate of 8 °C h^{-1}. The temperature at which freezing of the samples occurred was determined by the marked increase in temperature resulting from the exothermic process of ice formation.

Triphenyl tetrazolium chloride (TTC) dye was used to determine tissue injury (Steponkus & Lanphear 1967). Leaves and stem sections were placed in sealed tubes and immersed in an alcohol-refrigerated bath and the temperature was lowered as described above. Five replicates of each sample were taken from the bath at 5 °C intervals and incubated at room temperature for 12 h. After this incubation period, the TTC solution was applied to 50 mg of each sample, which was then left in the dark, at room temperature, for 24 h. An ethanol extract was then made and the absorbance at 530 nm measured. Freezing injury was defined as the amount of TTC reduced by the samples that resulted in 50% absorbance of the amount of TTC reduced by the unfrozen reference sample at 5 °C.

For the determination of carbohydrate and proline content, leaf samples were collected in the field, placed in hermetically sealed tubes and submerged in liquid nitrogen. These tubes were placed on dry ice and transported back to the laboratory. Sugars were extracted from leaf tissues as follows (Prado *et al.* 1998): a 500 mg fresh mass (FW) of each sample was homogenized with 2 cm^3 of 80% ethanol solution using a pestle and mortar. Following heating of the homogenate in a water bath at 75 °C for 10 min, the insoluble material was separated by centrifugation at 5000 × *g* for 10 min. The precipitate was re-extracted with 2 cm^3 of 80% ethanol at 75 °C and centrifuged again. The supernatants were pooled and dried under a stream of hot air and the dry residue was re-suspended in 1 cm^3 of water and desalted through a column of ion-exchange resin (Amberlite MB3). The filtrate was used for soluble sugar determinations. Total soluble sugars were determined by the phenol–sulphuric acid method (Dubois, Guilles & Hamilton 1956). The procedures of Roe & Papadopoulos (1954) and Cardini, Leloir & Chiriboga (1955) were used to determine free fructose and sucrose, respectively. Glucose was estimated by the difference between total soluble sugars and (sucrose + free fructose). The insoluble fraction remaining after the ethanol extraction was used for starch determinations (Rose *et al.* 1991). The precipitate was re-suspended in 2 cm^3 of 2·5 N NaOH and boiled for 5 min. After cooling, the pH was adjusted to 4·5 using 2 N HCl. The resulting gelatinized starch was hydrolysed by buffered amyglucosidases from *Rhizopus* mould (15 IU cm^3 in 0·1 M sodium acetate, pH 4·5). After 10 min at 50 °C, aliquots were withdrawn and glucose assayed by Nelson's reagent (Somogyi 1945). Appropriate blanks and standards using soluble starch were included.

Proline was determined from aliquots of carbohydrate supernatant, prior to the desalting procedure (Bates, Waldren & Teare 1973).

RESULTS

There were no significant differences in injury temperature between seasons, between tissues (i.e. leaves and stems) or between altitudes, for temperatures ranging from −18 to −23 °C (Table 1). Significant differences were found in supercooling capacity between seasons, with values of −3 to −5 °C in the cold season, and −7 to −9 °C in the warm season.

There was a significant increase in total soluble sugar content from the dry cold to the wet warm season, as shown in Fig. 1a for representative daily cycles. During all the 24 h measurement periods, the lowest total soluble sugar contents were recorded at 0630 h, coinciding with the minimum temperatures registered during the cycles (temperature data not shown). In terms of individual sugars, sucrose showed the largest relative difference (up to × 5) between the daily courses of the two seasons, even though the contents of glucose and fructose were larger (Fig. 2).

In comparison with amounts of both total and individual sugars, starch content showed an opposite pattern between seasons (Fig. 1b), whereas proline showed a significant increase in content from the dry cold to the wet warm season, similar to the soluble sugars(Fig. 3).

	Altitude	T_{min}	PS	SC	IT
September (dry cold)	4300 m	–	Leaf	−3·5 ± 0·5	−20 ± 0·5
			Stem	−3·8 ± 1·1	−23 ± 0·8
	4800 m	−13·1	Leaf	−5·4 ± 0·9	−21 ± 1·8
			Stem	−4·4 ± 0·2	−18 ± 1·4
February (wet warm)	4300 m	–	Leaf	−9·2 ± 0·6	−21 ± 1·2
			Stem	−7·6 ± 0·6	−23 ± 0·6
	4800 m	−6·1	Leaf	−7·7 ± 0·6	−21 ± 0·7
			Stem	−7·8 ± 0·5	−18 ± 1·3

Table 1. Minimum air temperature measured at 5 cm (T_{min}, °C) above ground level (Liberman-Cruz 1986), supercooling capacity (SC, °C) and injury temperature (IT, °C) for different plant sections (PS) for *P. tarapacana* at two different altitudes during the dry cold (September 1998) and wet warm seasons (February 1999)

DISCUSSION

Although the study site is considered to have a tropical high mountain environment, the 7 °C difference in mean and minimum temperature between seasons (Table 1, Liberman-Cruz 1986) determines a clear seasonality to which the plants may respond.

The different results for injury temperature and supercooling capacity indicate that *P. tarapacana* mainly relies on frost tolerance to endure extreme temperature conditions in the Bolivian high mountains.

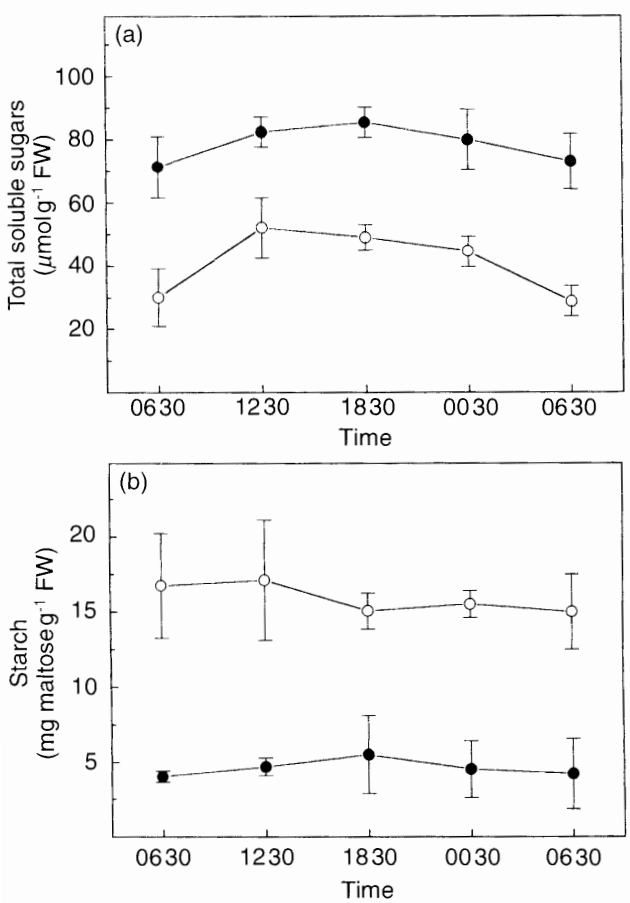

Figure 1. (a) Total soluble sugar and (b) starch content in leaves of *P. tarapacana* during a 24-h cycle in the wet warm (●) and dry cold (○) seasons.

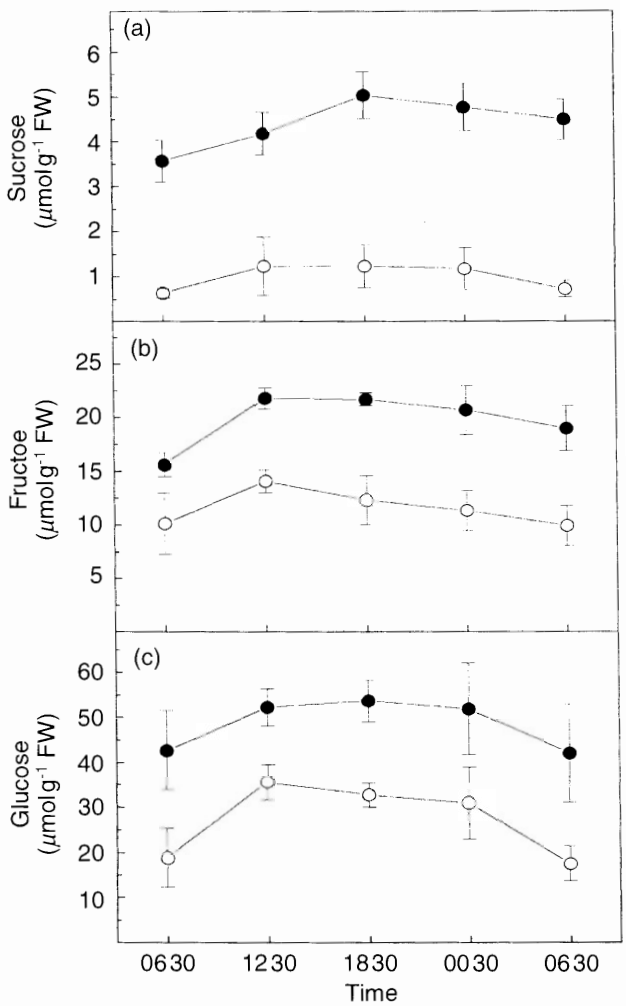

Figure 2. Individual sugar content: (a) sucrose; (b) fructose; (c) glucose in leaves of *P. tarapacana* during a 24-h cycles in the wet warm (●) and dry cold (○) seasons.

As soluble sugar contents were lower in the pre-dawn hours at the times when minimum temperatures occur, we can exclude daily lowering of the freezing point by accumulation of osmotically active solutes as a freezing avoidance mechanism in this species. These results contrast with those obtained for *Polylepis sericea*, which depends exclusively on avoidance mechanisms through daily osmotic adjustment, together with a certain degree of supercooling

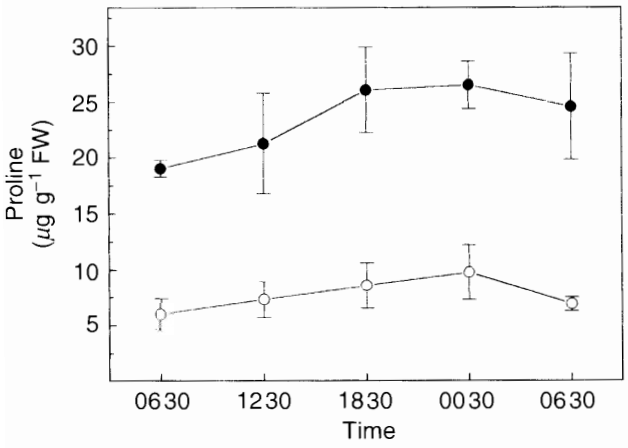

Figure 3. Proline content in leaves of *P. tarapacana* during a 24 h cycle in the wet warm (●) and dry cold (○) seasons.

capacity, to survive less extreme night-time conditions in the Venezuelan high mountains (Rada *et al.* 1985; Goldstein *et al.* 1994). On the other hand, our results support results of Squeo *et al.* (1991, 1996) which suggest that plants growing in the most extreme environments need to survive through frost tolerance, in comparison with those in less extreme conditions, which rely on avoidance mechanisms.

Although *P. tarapacana* may be considered a frost-tolerant species, differences in supercooling capacity between seasons also suggest differences in resistance mechanisms between seasons. Temperatures during the dry cold season may be well below −10 °C on any night of the year. During this season, avoidance mechanisms through supercooling would not be sufficient to protect against these low night-time temperatures. Therefore, freezing occurs in leaf and stem tissues at comparatively high temperatures (−3 to −5 °C) and injury occurs at temperatures below −20 °C. On the other hand, during the wet warm season, temperatures seldom reach below −8 °C. During this season, supercooling capacity increases significantly in the range of temperatures between −7 and −10 °C so that for most of the nights during this season, tissue freezing does not occur. This may be a great advantage for this species enabling it to initiate early daytime metabolic activity during summer, compared to the winter season when frozen tissues may take much longer to thaw before metabolic activity can begin.

A significant increase in content of total soluble sugars and proline between seasons contributes to differences in supercooling capacity through changes in amounts of osmotically active solutes. A decrease in starch content from the dry cold to the wet warm season also supports the idea that simple carbohydrates are not converted to starch during the latter, more favourable season. An alternative possibility is that during periods when temperatures are not extremely low the existing starch is converted to soluble carbohydrates, thus increasing the content of osmotically active solutes with resulting enhancement of freezing avoidance.

Even though we have suggested in the previous paragraph that this increase in osmotically active solutes is a strategy by which to avoid freezing temperatures, it has to be made clear that this should not be considered as a mechanism, as it is most likely a consequence of higher metabolic activity during the favourable, wet warm season. Large soluble sugar contents are not necessarily there to be used as cryoprotectants to avoid freezing injury, as demonstrated for afroalpine genera such as *Lobelia*, *Senecio* and *Alchemilla* (Beck *et al.* 1982; Beck 1988), but rather may be used in metabolism. The observed differences in starch and soluble sugar contents in *P. tarapacana* during the favourable season may be an indicator of higher metabolic and growth rates. In a preliminary study, Cardenas (2000) describes growth rates of 6 mm per month in branches during the favourable season (January–March), decreasing to 1 mm per month from April to May, with no additional growth in the following unfavourable months. At the same time, an increase in proline content during the favourable, wet warm season suggests an increase in the amino acids pool needed for biosynthetic activity.

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