

Freezing tolerance in grasses along an altitudinal gradient in the Venezuelan Andes

Edjuly J. Márquez · Fermín Rada · Mario R. Fariñas

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Abstract The tropical high Andes experience greater daily temperature oscillations compared to seasonal ones as well as a high frequency of night frost occurrence year round. Survival of organisms, under such environmental conditions, has been determined by selective forces which have evolved into adaptations including avoidance or tolerance to freezing. These adaptations have been studied in different species of trees, shrubs and perennial herbs in páramo ecosystems, while they have not been considered in grasses, an important family of the páramo. In order to understand survival of Poaceae, resistance mechanisms were determined. The study was performed along an altitudinal gradient (2,500–4,200 m a.s.l.) in the páramo. Supercooling capacity and frost injury temperature were determined in nine species in order to establish cold resistance mechanisms. Grasses registered a very low supercooling capacity along the altitudinal gradient, with ice formation between -6 and -3°C . On the other hand, frost injury temperature oscillated between -18 and -7°C . Our results suggest that grasses exhibit freezing tolerance as their main cold resistance mecha-

nism. Since grasses grow at ground level, where greatest heat loss takes place, tolerance may be related to this life form as reported for other small life forms.

Keywords Injury temperature · Poaceae · Supercooling capacity · Tropical high Andes

Introduction

The tropical high Andes are characterized by a high frequency of frost occurrence which can take place any night of the year (Sakai and Larcher 1987; Rundel 1994). As a result, plant species are conditioned to sustain freezing resistance mechanisms all year round as an evolutive response to this environment (Sarmiento 1986). However, plants have different ways of responding to low temperatures and partially depend on microclimatic environmental conditions around the plant (Azócar et al. 1988), giving rise to avoidance or tolerance to extracellular freezing (Levitt 1972).

Avoidance mechanisms have been favored in tropical high mountains when night temperatures do not drop far below 0°C , remaining there for short periods of time (Sakai and Larcher 1987; Larcher 1995). Supercooling capacity, a decrease in tissue temperature well below the equilibrium freezing point without the occurrence of extracellular freezing of water (Beck 1994; Körner 1999), has been frequently reported in páramo plant species: giant rosettes of the genus *Espeletia* (Asteraceae) (Goldstein et al. 1985; Rada et al. 1985a; Rada et al. 1987), *Polylepis sericea* (Rosaceae), a tree (Rada et al. 1985b), *Hinterhubera lanuginosa* (Asteraceae), a small shrub, and *Hypericum laricifolium* (Clusiaceae), a shrub (Squeo et al. 1991).

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E. J. Márquez · F. Rada · M. R. Fariñas
Instituto de Ciencias Ambientales y Ecológicas (ICAE),
Facultad de Ciencias, Universidad de Los Andes,
Mérida 5101, Venezuela

E. J. Márquez (✉)
Universidad Nacional Experimental Francisco de Miranda,
Centro de Investigación en Ecología y Zonas Áridas
(CIEZA), Calle Mapararí, entre callejón las Flores y Cristal,
Coro 4101-A, Edo. Falcón, Venezuela
e-mail: edjuly@gmail.com
e-mail: julio@ula.ve

On the other hand, tolerance consists of plant survival under extracellular frost conditions (Levitt 1972; Körner 1999). In tropical environments, tolerance has been selected where night temperatures drop far below 0°C remaining there for several hours (Rada et al. 1985b; Sakai and Larcher 1987). Freezing tolerance was first reported for the tropical Andes in *Draba chionophila* (Brassicaceae), a small rosette which grows under extreme climatic conditions at 4,800 m, and sustains tissue freezing at −5°C and injury at −14°C (Azócar et al. 1988). There seems to be a close relationship between plant height and life form: taller plants, including rosettes, display predominantly avoidance mechanisms, while smaller ones exhibit tolerance (Squeo et al. 1991, 1996). However, Afroalpine (Beck et al. 1982, 1984) and Hawaiian giant rosettes (Melcher et al. 1994), present freezing tolerance.

In contrast to the tropics, several studies have been carried out on physiological responses to low temperatures in grasses of temperate regions, which have considered tolerance to ice formation (Andrews 1996), photosynthetic response to low temperatures (Long 1983; Pittermann and Sage 2001; Sage 2002; Kubien et al. 2003) and ecophysiological perspectives on global change (Sage and Kubien 2003). Schwarz and Redmann (1987) and Sage and Sage (2002) suggest further studies for a better understanding of resistance mechanisms of grass species distributed in low temperature environments. Pearcy (2001) and Márquez (2002) also highlight the importance of understanding freezing in plants as a distributional pattern constraint. In the current investigation, in order to study the survival of Poaceae to frequent nighttime freezing temperatures of the tropical Andes, resistance mechanisms were determined in some páramo grass species, evaluating possible differences in mechanisms along an altitudinal gradient.

Materials and methods

The study was performed along an altitudinal gradient (2,500–4,200 m a.s.l.) in the Sierra de La Culata, Mérida, Venezuela (8°51′04N, 70°43′10W and 8°51′09N, 70°49′25W). Nine grass species were selected at different altitudes taking into account their altitudinal distribution (Márquez et al. 2004) (Table 1).

Whole plants were transported on August 2001 to the laboratory for the determination of both equilibrium freezing temperature and injury temperature.

Determination of supercooling capacity

Harvested leaves from five different individuals per species were placed into sealed glass tubes to avoid water loss during the experiments. Copper-constantan thermocouples were inserted inside each tube to record temperature continuously. Glass tubes were introduced into a bath of freezing alcohol (NESLAB RTE-IV, USA), and the bath temperature was decreased from 5 to −18°C at an approximate rate of 0.2°C/min. Sample temperatures were recorded on a strip chart recorder (model 8373-30, Cole Parmer Instrument; USA). The sudden rise in sample temperature (exotherm) produced by the heat released during the extracellular freezing process was recorded. The exotherm indicated the beginning of freezing and therefore, the supercooling capacity (Larcher 1995).

Determination of injury temperature

Leaves were introduced into sealed glass tubes which were placed in an alcohol bath. Similarly to the supercooling capacity determination, the temperature was lowered from 5 to −18°C at 5°C intervals (5, 0, −5, −10, −15 and then at −18°C), and held constant for 5 min at each temperature after which three replicates

Table 1 Grass species evaluated in this study. Altitudinal distributions for the Venezuelan Andes; phenological and morphological characteristics are indicated

Species	Altitudinal range (m a.s.l.)	Phenology	Morphological aspect
<i>Aegopogon cenchroides</i> Humb. & Bompl. ex Willd	800–4,200	Perennial	Erect
<i>Agrostis breviculmis</i> Hitch	3,300–4,650	Perennial	Miniature bunch grass
<i>Agrostis trichoides</i> (Kunth) Roem. & Schult	3,400–4,000	Perennial	Miniature bunch grass
<i>Danthonia secundiflora</i> J. Presl	2,900–4,200	Perennial	Bunch grass
<i>Muhlenbergia ligularis</i> (Hackel) Hitchcock	3,350–4,200	Perennial	Cespituous
<i>Muhlenbergia venezuelae</i> Luces	3,000–3,600	Perennial	Bunch grass
<i>Nassella linearifolia</i> (Fourn.) R. Pohl	2,900–3,850	–	Bunch grass
<i>Paspalum pilgerianum</i> Chase	3,100–3,600	Perennial	Rhizomatous
<i>Paspalum pygmaeum</i> Hackel	3,000–3,600	Annual	Erect

from three different individuals were removed. The triphenil tetrazolium chloride method (TTC) (Steponkus and Lanphear 1967) was used to determine injury. After incubation at 12°C for 24 h the TTC was added to the treated samples, extracted from the samples 48 h later and absorbance read at 530 nm (Spectronic 20; Bausch and Lomb, USA). Injury temperature was defined as the temperature at which a 50% absorbance decrease occurred in relation to the absorbance of the sample at 5°C.

In order to evaluate if a species was able to tolerate or avoid extracellular freezing of water, differences between equilibrium freezing temperature and injury temperature were considered. ANOVA and Student *t*-tests were applied for the statistical analysis. Tukey tests were used for post-hoc analysis.

Results

Initiation of the freezing process, i.e. supercooling capacity, in páramo grasses occurred at temperatures between −6.2 and −2.9°C with no differences between altitudes for any of the species ($P < 0.05$), suggesting no variations along the gradient (Table 2). No patterns between equilibrium freezing temperatures and altitude were found ($r^2 = 0.01$; $P = 0.97$). *Muhlenbergia venezuelana* was the only species which did not experience an exotherm, and it also had the most xeromor-

phic leaves with respect to the others. Conversely, all other species exhibited one exotherm with the exception of *Paspalum pygmaeum*, which showed a second one (Table 2).

Injury temperatures were lower compared to freezing temperatures (between −18.0 and −7.1°C). Most species showed no differences in injury temperature with respect to altitude; however, wider altitudinal ranges must be studied to confirm altitudinal patterns. *P. pilgerianum* displayed the lowest injury temperature, −18°C at 3,500 m a.s.l.

Differences between equilibrium freezing and injury temperature varied between 4.8 and 12.1°C, showing statistical differences for all species (Table 2), therefore indicating tolerance as the resistance mechanism. In the case of those species without an injury temperature shown in Table 2, it is reasonable to assume they are also tolerant when minimum site temperatures are considered. Alvizu (2004) has recorded minimum temperatures of approximately −12°C between 3,900 and 4,200 m for our studied gradient. In his 130-day study, Alvizu (2004) registered 36% of events with night temperatures below −6°C for these altitudes and 11% below −10°C, all recorded during the dry season. In the same manner, minimum absolute temperatures of −8°C were recorded for Mucubají (3,550 m a.s.l.) (Azócar and Monasterio 1980). A second exotherm has been related to injury temperature (Levitt 1972; Squeo et al. 1991). Taking this into account, a cold

Table 2 Relationship between supercooling capacity (SCC) and freezing injury temperature (FIT) of grasses (± 1 SD)^a

Species	Altitude (m a.s.l.)	SCC (°C) $n = 5$	FIT (°C) $n = 3$	Thermal differences	CRM
<i>Aegopogon cenchroides</i>	2,500	−5.2 \pm 0.8	–	–	Tolerance
	3,100	−5.8 \pm 1.6	−16.7	10.9*	
	3,550	−4.1 \pm 0.5	−11.3 \pm 2.9	7.2*	
<i>Agrostis breviculmis</i>	4,000	−6.2 \pm 2.8	–	–	Tolerance ^b
<i>Agrostis trichoides</i>	3,100	−2.9 \pm 0.2	–	–	Tolerance
	3,700	−4.3 \pm 2.3	−14.6	10.3*	
	4,000	−3.3 \pm 0.5	–	–	
<i>Danthonia secundiflora</i>	3,200	−4.5 \pm 0.4	−10.9 \pm 1.2	6.4*	Tolerance
<i>Muhlenbergia ligularis</i>	3,100	−5.6 \pm 1.8	−15.0 \pm 0.9	10.1*	Tolerance
	3,600	−5.4 \pm 2.3	−16.7 \pm 0.3	11.2*	
	3,500	–	−12.8 \pm 4.0	–	
<i>Muhlenbergia venezuelana</i>	3,500	–	–	–	Tolerance
<i>Nassella linearifolia</i>	3,250	−4.8 \pm 0.3	–	–	Tolerance ^b
	3,600	−5.0 \pm 0.7	–	–	
	3,600	–	–	–	
<i>Paspalum pilgerianum</i>	3,250	−4.8 \pm 1.2	−9.6 \pm 2.0	4.8*	Tolerance
	3,600	−5.9 \pm 2.6	−18.0	12.1**	
	3,600	–	–	–	
<i>Paspalum pygmaeum</i>	3,400	−3.0 \pm 1.3	–	–	Tolerance ^b
	3,400	−8.5 \pm 1.3 ^c	–	–	

** $P < 0.05$, * $P < 0.01$ (statistical differences between SCC and FIT)

^a If the SD is not indicated then $n = 1$

^b See text for explanation of tolerance mechanism

^c Appearance of second exotherm

injury temperature of -8.5°C would be expected for *P. pygmaeum*, which also supports the idea of tolerance as a mechanism.

Discussion

Grasses in the páramo grow under more unfavorable ground level thermal conditions due to nocturnal surface heat loss (Azócar and Monasterio 1980; Azócar et al. 1988; Earnshaw et al. 1990; Squeo et al. 1991), compared to the species of *Espeletia* (Goldstein et al. 1985; Rada et al. 1987) and *Polylepis sericea* (Rada et al. 1985b), which grow well above ground. Under such environmental conditions, supercooling itself does not seem to be an effective mechanism to resist night freezing temperatures. Freezing after a marked supercooling is far more likely to be fatal than the gradual freezing that occurs when there is no supercooling (Levitt 1972), favoring tolerance in grasses as a mechanism. As a consequence, avoidance of supercooling seems to be a prerequisite to prevent plasma dehydration (Beck et al. 1982).

There are adaptations allowing protection of growing structures in giant Andean, Afroalpine and Hawaiian rosettes (Beck et al. 1982, 1984; Goldstein et al. 1985; Rada et al. 1985a; Melcher et al. 1994), but in many other life forms, including grasses, tolerance arises as the convenient strategy to confront the thermal constraint present on tropical high mountains. This opposes Hedberg's (1964) statement that both the bunch arrangement of almost all páramo grasses and the aboveground dead leaves serve as cold isolation morphological strategies, a clear avoidance mechanism.

Plant resistance to nighttime páramo conditions may be a response to a combination of structural strategies (plant morphology, leaf anatomy, leaf position in the plant) and functional ones (supercooling capacity, low leaf injury temperatures, freezing tolerance, etc.). Phenological studies may be an important tool in understanding resistance mechanisms in páramo grasses through synchrony between reproductive cycles and favorable time of the year. Loss of aboveground biomass may be another extreme response to environmental stress. This seems to occur in *P. pygmaeum*, which was found during the rainy but not the dry season. However, tolerance permits its resistance to occasional frost temperatures occurring any night during the rainy season. Additionally, it is possible to think of cold resistance mechanisms as a function of plant water responses. Species of *Espeletia* (Goldstein et al. 1985) and *P. sericea* (Rada et al. 1985b) which show strong avoidance mechanisms to water stress also show freez-

ing avoidance. A close relationship between water potential and the ability to avoid freezing through supercooling has been reported (Goldstein et al. 1985; Rada et al. 1987). No pattern between supercooling capacity and altitude was observed. This may be explained by the fact that all studied grasses were frost tolerant and as stated previously, prevention of supercooling is a prerequisite for frost-tolerant plants. Our results support the hypothesis proposed by Squeo et al. (1991) which relates plant height to a low temperature resistance mechanism, and suggests that species which rely on freezing tolerance are successful under more extreme páramo conditions. Nevertheless, freezing temperature mechanisms in grasses do not provide enough information to predict distributional patterns, but a combination of thermal information with pollen and isotopic records may serve as a powerful tool to understand the present and past distribution of grasses.

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