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Effect of cold stress at cellular and foliar level and regrowth capacity of three *Cenchrus ciliaris* L. cultivars: Americana, Biloela and Texas 4464

Efecto del estrés por frío a nivel celular, foliar y capacidad de rebrote en tres cultivares de *Cenchrus ciliaris* L.: Americana, Biloela y Texas 4464

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Abstract

Buffelgrass (*Cenchrus ciliaris* L.) a native pasture species of Africa is widely used in arid regions because of its forage productivity and quality. It could become serious invasive species, not only for crops but also in natural vegetation. Buffelgrass is highly affected by low temperatures at all stages of its life cycle. The objective was to determine the behavior of three buffelgrass cultivars (Americana, Biloela, Texas 4464) under cold stress and define the critical temperatures. To determine the effect of cold stress on the plasma membrane, foliage damage and survival (regrowth), it was evaluated relative electrical conductivity (ECr) of leaves, percentage of foliar damage and the ability of regrowth after exposure to 2 temperatures (-5°C and -10°C) with 2 exposure times (4 and 7 hours). The results showed a higher percentage of foliar damage and greater ECr as temperature decreased and hours of exposure increased. A similar response was observed when survival (regrowth) was evaluated. There were no significant differences among cultivars in both temperature treatments and time when the regrowth was evaluated.

Keywords
Buffelgrass • abiotic stress • electrical conductivity • cold damage.

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RESUMEN

El buffelgrass (*Cenchrus ciliaris* L.) es una especie forrajera nativa de África ampliamente utilizada en las regiones áridas debido a su productividad forrajera y calidad. En muchos casos puede convertirse en una especie invasora, no solo para los cultivos, sino también para la vegetación natural. El buffelgrass es afectado por las bajas temperaturas en todas las etapas del ciclo de vida. El objetivo del presente trabajo fue determinar el comportamiento de tres variedades de buffelgrass (Americana, Biloela, Texas 4464) ante condiciones de frío y definir las temperaturas críticas. Para determinar el efecto de estrés por bajas temperaturas sobre la membrana plasmática, daños de follaje y la supervivencia (rebrote), se evaluó la conductividad relativa (ECr) de hojas, porcentaje de daño foliar y la capacidad de rebrote después de la exposición de las plantas y plántulas a 2 temperaturas (-5°C y -10°C) durante 2 períodos (4 y 7 horas). Los resultados mostraron un mayor porcentaje de daño foliar y mayor conductividad relativa. Medida que disminuía la temperatura y aumentaba las horas de exposición. Una respuesta similar se observó cuando se evaluó la supervivencia (rebrote). No hubo diferencias significativas entre cultivares en ambos tratamientos de temperatura y tiempo cuando se evaluó el rebrote.

Palabras claves
Buffelgrass • estrés abiótico • conductividad eléctrica • daño por frío

INTRODUCTION

Buffelgrass (*Cenchrus ciliaris L.*) also known as African foxtail or Ajangrass, is a perennial C4 grass, native of tropical and subtropical Africa and is distributed in arid and warm regions of the world (13). It has been introduced in America and Australia (21) to improve livestock production because of the high forage value (9).

In arid and semi-arid environments buffelgrass is considered a good pasture species and their naturalization is highly desirable. It may also be considered a weed, where its range of expansion puts natural ecosystems at considerable risk (10). In natural areas, this species tends to form pastures that exclude native vegetation, decreasing the biodiversity, altering the processes of succession (29), reducing the biomass production and primary productivity (9). The behavior of buffelgrass can vary widely and depends on the soil, climate, position in the landscape, rain, fire and grazing regimes (10). It is fundamental to know the physiology and the behavior of the plant under different environmental conditions as a tool to predict its performance.

Since plants do not have the capacity to move in response to environmental changes, they are exposed to different types of stress and must adapt to them (14). Temperature is one of the most important abiotic factors that cause stress in plants, limiting plants productivity and growth (20, 27). Temperature also modifies the geographic distribution of crops (1, 20) and wild species (25).

Extreme temperatures play a decisive role in seedling survival and its establishment in arid areas, limiting its distribution to certain regions or microsites (23). The process of freezing of tissues includes stress by dehydration of the cells in the simplast (19). The formation of ice on plants begins in the apoplastic spaces since they have a lower concentration of solutes. Unfrozen cytoplasmic water migrates through the gradient from the cytosol of the cell to the apoplast, which contributes to an increase in the ice which leads to the rupture of the cell (26), and dehydration (19).

The plasma membrane is the first cell site affected by low temperature (8), resulting in a change of phase in lipids of the plasma membrane and destabilizing the structure of the lipid bilayer (25), in addition to the denaturing of proteins (28). This damage is due in large part to severe dehydration associated with cold stress (25, 26). The cellular dehydration results in multiple lesions on the membrane including the expansion that induces the cell lysis (28), generates a loss of permeability (3) and compartmentalization, which is detected as a loss of electrolytes (3, 25, 26). This loss of ions can be measured in terms of electrical conductivity (3).

The integrity of the intracellular organelles is also disrupted, leading to the loss of cell function and affecting the metabolic processes in general (26).

The symptoms of lesion induced by cold stress in plants are shown in 48 to 72 hours (26). The duration of exposure to stress and the severity of temperature are key factors in the damage (5). The phenotypic response to cold stress symptoms can be a reduction of the expansion of the leaf, necrosis, leaf wilt, chlorosis in leaves, tips burns on leaves and stems, defoliation and damage to buds and fruits (3).

It is essential to know the physiology and behavior of the species introduced to different environmental conditions. These skills are a key tool to predict their behavior and be used in the eradication or the voluntary or involuntary introduction of species management programs.

Buffelgrass is sensitive to prolonged frost conditions (32) and most cultivars do not support several days of below-freezing temperatures (2). The minimum temperature that *Cenchrus ciliaris* needs for significant growth is 10°C and the optimum growth temperature is 38°C (16). There are few data related to identify the critical temperatures of *Cenchrus ciliaris*. The aims of the present work determines the effect of cold temperature on plasma membrane permeability, through the test of conductivity in leaves and evaluate the foliar damage after exposure to low temperatures and subsequent recovery (survival) in three *Cenchrus ciliaris* cultivars. The results of this work will allow selection of buffelgrass cultivars for livestock production and determine the critical temperatures that would prevent expansion.

Materials and methods

Seeds of three genotypes of *Cenchrus ciliaris*: Americana, Biloela and Texas 4464 were grown in a greenhouse, in a plastic container (1.300 cm^3) filled with compost and sand (2:1) under natural light conditions, 25/15°C day/night temperature and irrigated two times a week with nutrient solution. Plants reaching the reproductive stage were used for the cold treatments.

Cold Treatments

The plants were transferred from the greenhouse to a growth chamber set at -5 and -10°C (50% RH) for 4 and 7 hours respectively. The control plants were maintained in a growth chamber at 25°C and 50% RH. It was selected those temperatures and ranges to simulate the agroecological conditions of the arid areas of the west of Argentina, a potential area to expand buffelgrass implantation.

Relative electric conductivity

Membrane injury was determined by measuring the electrical conductivity according to Barranco and Ruiz (2005) with modifications. After the cold treatments, the plants were maintained 12 hours at laboratory temperature (25/27°C). The leaves were washed twice with deionized water to eliminate impurities from the surface. They were sectioned into squares 1x1cm until reaching a weight of 0.120 g (18) and rinsed again with deionized water. The leaf samples were separated into adult (>20 cm of length) and young leaves.

Leaf sections (0,120 g) were placed in glass tubes (80 ml) with 30 ml of deionized water, incubated during 24 h at 22°C and agitated (200 rpm) for 2 hours, prior to measuring the leachate conductivity. Initial (ECi) electric conductivity (μS.cm) of each sample was measured using an immersion conductivity meter (Waterproof OAKTON). After the lecture, the tubes were placed in a water bath at 100°C for 2 h to completely destroy the tissue and agitated during 2 h (200 RPM) to measure the total electric conductivity (ECt). Relative electric conductivity ECr = (ECi/ECt) x 100 was calculated for both data sets.

Foliar damage

After 48 hours of cold treatments, an assessment of canopy damage was carried out. A ranking of leaf damage was developed (1 = 0% damage and 9 = 100% damage) (table 1). The leaves of the canopy were considered damaged if spots of water appeared, if they were brown/ yellowish, dry or rolled. The ranking was carried out by visual estimate on the proportion of leaves damaged with regard to the entire canopy (19).

Survival capacity

After the cold treatments, the plants were transferred to a greenhouse and clipped after 5 days. The regrowth was registered every week for a month to calculate the rate of survival with regard to the total of plants treated (20). The plants, which had survived freezing and had begun to develop were clearly distinguishable from those which had died. The percentage of plants able to grow after freezing is given as the survival capacity.

Statistical analysis

The experimental design was three factor factorial (3 × 2 × 2) arranged in a completely randomized design; with nine replications. The first factor was cultivar (Americana, Biloela and Texas 4464), the second, temperature (-5 and -10°C) and the third was time (4 and 7 hours). Percentage data were subjected to arcsine transformation before analysis to improve homogeneity of variance. A three-way ANOVA was used to examine effects of cultivar (Cv), temperature (T) and hours (H) of cold treatment.

The means were compared using the LSD Fisher test. All data were analyzed by using the statistical software InfoStat (6).

Table 1. Ranking of foliar damage to the canopy in plants using the percentage of foliar damage and the percentage of green leaves in the canopy.

Tabla 1. Ranking del daño foliar en plantas considerando el porcentaje de daño foliar y del porcentaje de hojas verdes en la canopia.

Foliar damage	**Spots of water or/and brown/ yellowish; dry or rolled leaves**	**Green leaves**
Ranking	%	%
1	0	100
2	12	88
3	25	75
4	38	62
5	50	50
6	62	38
7	75	25
8	88	12
9	100	0

RESULTS

Relative electric conductivity (ECr)

The ECr showed significant differences in the interaction Cultivar (Cv) x Temperature (T) x Hours (H) in both young and adult leaves. Thus, it was analyzed by each temperature the effects of Cv and H factors (table 2).

In young leaves, at -5°C it was found a significant difference in the interaction Cv x H, as the hours of cold treatment increased the relative conductivity electric increased (figure 1, page 34).

At 4 hours of exposure the ECr of Texas 4464 (58.47 µS.cm) was almost twice the values of Americana y Biloela (21.9 µS. cm y 27.5 µS. cm) respectively.

At 7 hours of cold treatment there was no significant difference in the electric leachate in the three cultivars. At -10°C there was not a significant difference in the interaction (C x H) among cultivars and the ECr was significantly higher in plants exposed to 7 hours compared with plants exposed to 4 hours (figure 2, page 34).

Table 2. Effects of cultivar (Cv), temperature (T) and hours (H) of cold treatment on the relative electrical conductivity (ECr) of young and adult leaves of *Cenchrus ciliaris* cultivars.

Tabla 2. Efecto del cultivar (Cv), temperatura (T) y horas (H) de tratamiento de frío sobre la conductividad eléctrica relativa (ECr) de hojas jóvenes y adultas de los cultivares de *Cenchrus ciliaris* evaluados.

	p-value	
Variable	**Young leaves**	**Adult leaves**
Cultivar (Cv)	0.0027	0.0935
Temperature (T)	<0.0001	<0.0001
Hours (H)	<0.0001	0.0001
Cv x T	<0.0001	0.0517
Cv x H	0.0304	0.0233
T x H	<0.0001	<0.0001
Cv x T x H	0.0496	0.0496

Foliar damage

The symptoms for all three cultivars (Americana, Biloela and Texas 4464) exposed to -5°C consisted of wilt, chlorosis and necrosis of leaf, followed by burned tips and twisting after thawing in the sun. The symptoms for all three cultivars exposed to -10°C were more severe, showing a complete drying of leaf, stem and inflorescence and leaves with brown tips after the thaw in full sun (figure 3, page 35).

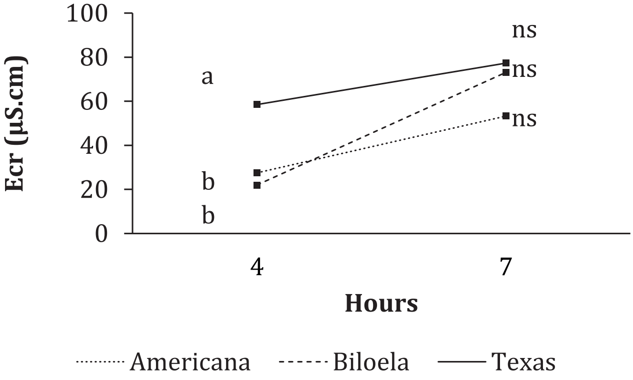
In the analysis of foliar damage (ranking analysis) there was a significant difference only in temperature (T) and time of cold treatment (H) (table 3).

Americana and Biloela presented an average of 6.3 ranking (equivalent to a percentage of foliar damage of 65.1%) and Texas 4464 presented an average of 6.9 (equivalent to 71.5% of foliar damage). There was a significant interaction between temperature and time of cold treatment.

Table 3. Effects of cultivar (Cv); temperature (T) and time of cold treatment (H) on foliar damage; calculated as a ranking damage; in the three *Cenchrus ciliaris* cultivars (Americana, Biloela and Texas 4464).

Tabla 3. Efecto del cultivar (Cv), temperatura (T) y el tiempo de exposición al frío (H) sobre la canopia en los tres cultivares de *Cenchrus ciliaris* (Americana, Biloela y Texas 4464).

Variable	**p-value**
Cv	0.8575
T	<0.0001
H	<0.0001
CV*T	0.1787
CV*H	0.9793
T*H	<0.0001
CV*T*H	0.9392

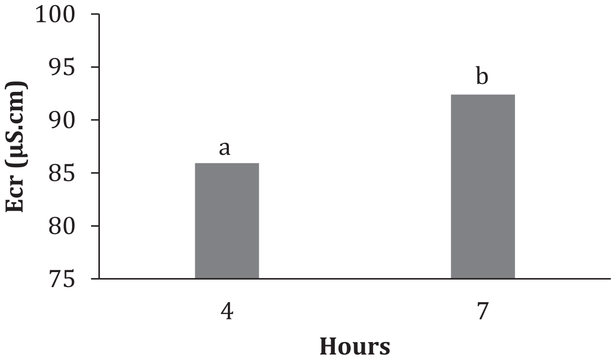


Different letters in each hour of exposure indicate significant differences; ns: indicates no significant difference according to LSD Fisher Test ($p < 0.05$).

Letras diferentes en cada hora de exposición indican diferencias significativas; ns: indica que no hubo diferencias significativas según prueba LSD de Fisher ($p < 0,05$).

Figure 1. Relative electrical conductivity (ECr) of young leaves of Americana, Biloela and Texas 4464 cultivars exposed to -5°C during 4 and 7 hours.

Figura 1. Conductividad eléctrica relativa (ECr) de hojas jóvenes de los cultivares mericana, Biloela y Texas 4464 expuestos a -5°C durante 4 a 7 horas.



Different letters in each hour of exposure indicate significant differences according to LSD Fisher Test ($p < 0.05$).

Letras diferentes en cada hora de exposición indican diferencias significativas según prueba LSD de Fisher ($p < 0,05$).

Figure 2. Relative electrical conductivity (ECr) of young leaves of the three cultivars (Americana, Biloela and Texas 4464) exposed to -10°C during 4 and 7 hours.

Figura 2. Conductividad eléctrica relativa (ECr) de hojas jóvenes de los tres cultivares (Americana; Biloela y Texas 4464) expuestas a -10°C durante 4 a 7 horas.

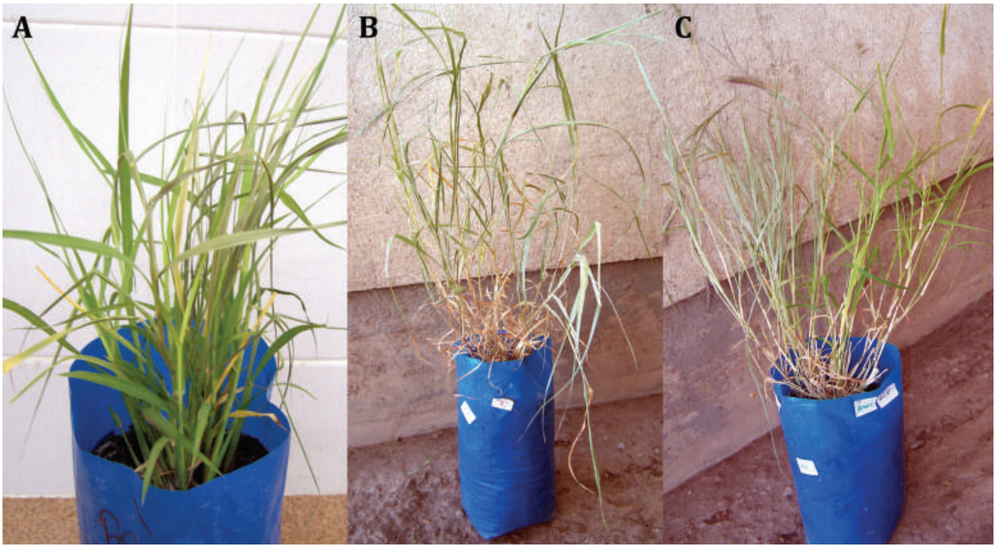


Figure 3. Leaf damage in plants of *Cenchrus ciliaris* at: -5°C ; 4 hours of treatment (A); -5°C ; 7 hours of treatment (B) and -10°C; 4 hours of treatment (C).

Figura 3. Daño en hojas en plantas de *Cenchrus ciliaris* sometidas a: -5°C; 4 horas de tratamiento (A); -5°C; 7 horas de tratamiento (B) y -10°C; 4 horas de tratamiento (C).

Lower temperatures caused greater foliar damage (figure 3).

At -5°C, as the hours of exposure increases, the damage on leaf increases significantly in the three cultivars (figure 4, page 36).

The plants after 4 hours at 5°C have a 25% of average foliar damage, while after 7 hours of exposure the average of foliar damage increase to 53%.

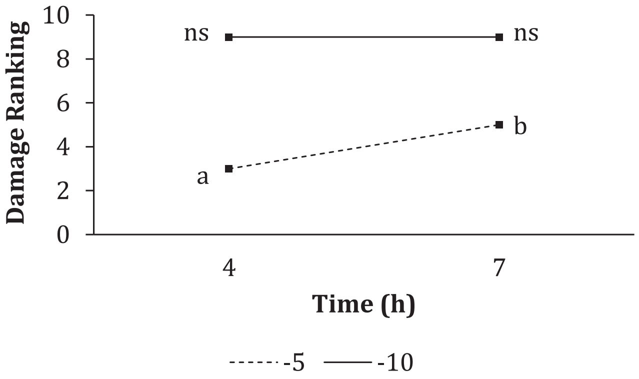
Survival capacity

When it was analyzed the capacity of regrowth after cold treatments (-5°C, -10°C) and control (25°C), there were a significant interaction (table 4, page 37). There were no significant differences among cultivars, where Americana reached an average of 67% survival, Biloela 69% and Texas 4464 80%.

As temperature decreases, the percentage of regrowth (survival capacity) decreases for the three cultivars. At 25°C (control) 100% of the plant regrowth after cutting, at -5°C, the percentage of regrowth was 81% after 4 hours of treatment and at 7 hours 78%, while when they were exposed 4 hours to -10°C the survival was significant higher (63%) compared to plants exposed 7 hours (7%) (figure 5, page 36).

DISCUSSION

In this experiment, it was evaluated the capacity of three *Cenchrus ciliaris* cultivars (Americana, Biloela and Texas 4464) to tolerate stress caused by low temperatures, through two direct methods (foliar damage at the level of the canopy and regrowth percentage) and an indirect method (relative electrical conductivity of the leaves).

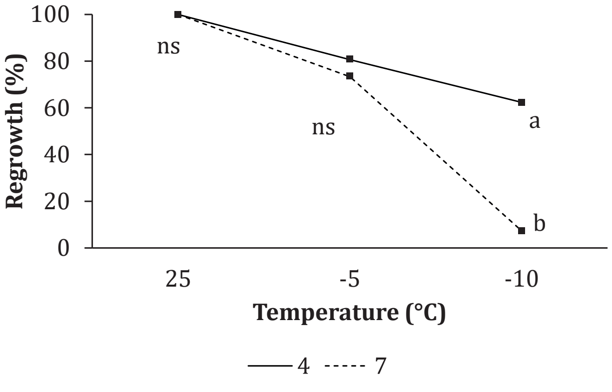


Different letters indicate significant differences; ns: indicates no significant difference in each hour; according to the Fisher LSD Test ($p < 0.05$).

Letras diferentes indican diferencias significativas; ns: indica que no hubo diferencias significativas en cada hora, según la prueba LSD de Fisher ($p < 0,05$).

Figure 4. Ranking of foliar damage at the canopy level of the cv Americana, Biloela and Texas 4446; exposed to -5°C and -10°C during 4 and 7 hours.

Figura 4. Clasificación de daño foliar a nivel de canopia de los cultivares Americana, Biloela y Texas 4446 expuestos a -5°C y -10°C durante 4 a 7 horas.



Different letters indicate significant differences; ns: not significant differences according to the Fisher LSD test ($p < 0.05$).

En cada tratamiento de temperatura, letras diferentes indican diferencias significativas; NS: diferencias no significativas según la prueba LSD de Fisher ($p < 0,05$).

Figure 5. Percentage of regrowth (%) of *Cenchrus ciliaris* (cv Americana; Biloela and Texas 4464) under two cold stress conditions (-5°C and -10°C) exposed during 4-7 hours. In each treatment of temperature.

Figura 5. Porcentaje de rebrote (%) de *Cenchrus ciliaris* (cv Americana, Biloela y Texas 4464) bajo dos condiciones de estrés frío (-5°C y -10°C) durante 4-7 horas.

Tabla 4. Efecto del cultivar (Cv), temperatura (T) y el tiempo de exposición al frío (H) en la capacidad de supervivencia; en los tres cultivares de *Cenchrus ciliaris* (Americana; Biloela y Texas 4464).

Table 4. Effects of cultivar (Cv); temperature (T) and time of cold treatment (H) on survival capacity; in the three *Cenchrus ciliaris* cultivars (Americana; Biloela and Texas 4464).

Variable	p-value
Cv	0.1987
T	<0.0001
H	<0.0001
Cv*T	0.4225
Cv*H	0.6919
T*H	<0.0001
Cv*T*H	0.9705

The results confirmed the susceptibility of tropical grasses to cold stress, where lower temperatures and longer times of exposure caused greater foliar damage (8, 11, 15, 19).

In the two types of leaves (young and adult), and in the three cultivars, as temperature decreases there is a significant increase in the leachate electrical conductivity. Similar results were reported by others authors with different species (23, 30, 31). The increase in time of exposure to -5°C generates an increase in the relative electrical conductivity which is accompanied by an increase in the foliar damage. Americana and Biloela presented similar lower ECr values (21.9 and 27.5 µS.cm) compared to Texas 4464 (58.4 µS.cm). This could be explained by the difference in the structure of the canopy between cultivars (17) and puts in to evidence the low resistance and great susceptibility of young leaves of Texas 4464.

Leaf damage by low temperatures was significantly higher at -10°C compared to -5°C producing more than 95% damage to the leaves. These results are similar to those reported by Jacque (2006) when he assessed the effects of low temperatures in several lines of Bahiagrass (*Paspalum notatum*) and Liu and Osborne (2008) when evaluating the percentage of leaf death in *Eragrostis minor*.

Plants regrowth is heavily influenced by low temperatures (24). At -5°C the rate of regrowth was similar in the three cultivars evaluated, Texas 4464 (83%), Biloela (72%) and Americana (78%), without observing significant differences in exposure times. At -10°C it was observed differences in the time of the cold stress, where after 4 hours the regrowth exceeded 60% in the three cultivars, but after 7 hours the percentage was reduced to only 7%.

The only cultivate that re-sprouted in such conditions was Texas 4464. This cultivar probably develops its crown prior to exposure to the stress. Plants that achieve a better development of the crown, are in a better position to deal with low temperatures and thus be able to regenerate the roots and leaves in spring (8).

The ability to re-sprout when the leaf damage exceeded 90% would be explained by the ability of this species of accumulating carbohydrates at the base of the stems as a reserve thus allowing a high rate of survival under conditions of stress such as drought or fire (4, 7). The ability of buds from the stems to survive and re-sprout after exposure to freezing temperatures is crucial for surviving the winter successfully and a subsequent growth in the spring (12).

In the three *Cenchrus ciliaris* cultivars (Americana, Biloela and Texas 4464) assessed, slight foliar damage was observed after 4 hours of exposition to -5°C, leaving enough green foliage and with 100% plants survival. Whereas, if the plant is exposed for 7 hours leaf damage is greater, markedly decreasing the available green foliage the survival was still 100%. When the temperature was -10°C after 4 hours of exposure, the foliage damage was very severe, but allowed a very good recovery (63%). However when the time was 7 hours, result in a complete death of the plant.

CONCLUSIONS

The results allow conclude a great resistance to the low temperatures from this species a time already established.

Considering that the stress of temperature was shock and not having a previous acclimatization, the likelihood of resistance to low temperatures would be much higher still. It would be of great interest a future research putting this species to acclimation to be able to predict their behavior under field conditions.

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