

Cold tolerance of forage plant species

Tolerância ao frio em espécies de forrageiras

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Abstract

The occurrence of frost in southern and southeastern Brazil affects pasture quality and limits the use of forage species with high yield potential. Therefore, elucidating the cold tolerance of individual forage species could facilitate the selection of species that will optimize production and animal feeding throughout the year. Accordingly, the aim of the present study was to evaluate the cold tolerance of forage species to low temperatures, based on cell membrane stability and photoinhibition. Alfalfa (*Medicago sativa*), sorghum (*Sorghum bicolor*), black oat (*Avena strigosa*), marandu grass (*Urochloa brizantha*), pearl millet (*Pennisetum americanum*), mombaça grass (*Megathyrsus maximus*), and bermuda grass 'Tifton 85' (*Cynodon* spp) plants were subjected to temperatures of 0.2, -0.9, -1.8, -2.7, -4.1, -4.6, and -6.2 °C for 1 h in a growth chamber. Cell membrane stability and photoinhibition were based on the electrical conductivity of leaf section solutions and chlorophyll fluorescence, respectively. Initial cold damage corresponded to a sudden increase in leaf solution conductivity and decrease in fluorescence. Millet and sorghum were able to tolerate exposure to temperatures as low as -2.7 °C, whereas black oat, marandu grass, alfalfa, and mombaça grass were able to tolerate exposure to -4.1 °C, and bermuda grass 'Tifton 85' was able to withstand temperatures below -6.2 °C.

Key words: Frost. Fluorescence. Ion lixiviation. Conductivity test. Low temperature.

Resumo

A ocorrência de geadas no Sudeste e Sul do Brasil afeta a qualidade das pastagens e limita o uso de espécies com elevado potencial produtivo. O conhecimento da tolerância das forrageiras ao estresse por baixa temperatura contribui para a seleção das melhores espécies a serem utilizadas, levando à otimização de sua produção e garantindo o pastejo dos animais o ano inteiro. Assim, o objetivo deste trabalho foi avaliar a tolerância de espécies forrageiras às temperaturas baixas pela estabilidade da membrana celular e fotoinibição após o estresse por frio. Foram analisadas as forrageiras: alfafa (*Medicago sativa*), sorgo (*Sorghum bicolor*), aveia-preta (*Avena strigosa*), capim-marandu (*Urochloa brizantha*), milheto (*Pennisetum americanum*), capim-mombaça (*Megathyrsus maximus*) e Tifton 85 (*Cynodon* spp). As plantas foram submetidas às temperaturas de 0,2; -0,9; -1,8; -2,7; -4,1; -4,6 e -6,2 °C, durante uma hora, no interior de câmara de crescimento com condições de luminosidade e temperatura controladas, e avaliadas por meio do teste de condutividade elétrica da solução de seções foliares e fluorescência. O início dos danos correspondeu a um aumento súbito na condutividade elétrica da solução e diminuição na fluorescência. A tolerância das espécies forrageiras ao frio foi até a temperatura

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-2,7°C para milho e sorgo; -4,1°C para alfafa, aveia-preta, Marandu e Mombaça, e inferior a -6,2°C para o Tifton 85.

Palavras-chave: Geada. Fluorescência. Lixiviação de íons. Condutividade elétrica. Temperatura baixa.

Introduction

Pastures are the main source of livestock feed in Brazil, owing to their low cost, when compared to other sources of nutrition. However, the productivity of such pastures is generally low, partly because little is known about the behavior of forage plants under different environmental conditions (MOREIRA et al., 2015). In many parts of southern Brazil, the climatic conditions of the cold season are severe enough to constrain animal production, since frosts interrupt the growth of forage plants (CANTO et al., 2010). Therefore, the selection of species that are tolerant to regional frost severity would improve the planning of animal feeding and, thereby, reduce the seasonality of feed production, and to achieve this, it is necessary to understand the cold tolerance of each species and cultivar (COSTA et al., 2008).

When plants are subject to stress, the stability of the whole organism is disrupted, including the alteration of metabolic routes and production of excess electrons, which increase intracellular levels of reactive oxygen species (ROS) that can damage cell structures. As part of the process, the plasma membrane is one of the first cellular structures to be affected by ROS, which destroy the lipid layer and impair its ability to interact with the cell's external environment (GILL; TUTEJA, 2010). This loss of membrane integrity includes the increase of membrane permeability and subsequent leaching of electrolytes, which reduces photosynthesis and mitochondrial activity (LIN et al., 1985). Therefore, evaluating plasma membranes can provide insight into overall plant health.

Among the tests used to evaluate membrane degradation, measuring electrical conductivity is relatively quick, cheap, and suitable for the analysis of large numbers of samples. This technique is also considered very versatile and highly sensitive for

evaluating the tolerance of plants to stress (ARVIN; DONNELLY, 2008; MOSHTAGHI et al., 2009). Electrical conductivity has been used to quantify cell membrane damage after exposure to a variety of abiotic stressors, including cold, and has been used in a variety of crop species, including alfalfa - *Medicago sativa* L. (SULC et al., 1991), olive - *Olea europaea* L. (BARRANCO et al., 2005), potato - *Solanum tuberosum* L. (ARVIN; DONNELLY, 2008), wheat - *Triticum aestivum* L. (HABIBI et al., 2011), and various eucalyptus species - *Eucalyptus* spp. (FLORIANI et al., 2013).

Low temperature stress can also affect photosynthesis by reducing the export of photosynthates from the chloroplasts (KRATSCH; WISE, 2000). The process has even been reported to occur in certain cold-sensitive plants, mainly owing to the alteration or reorganization of thylakoid components, such as the loss of membrane polyps (BERTAMINI et al., 2007), which reduces electron transport and phosphorylation (RAPACZ, 2007).

Meanwhile, chlorophyll fluorescence provides insight into the state and energy use of photosystem 2 – PSII (MAXWELL; JOHNSON, 2000). Chlorophyll fluorescence has been used to evaluate the effect of environmental factors on photosynthetic metabolism and to measure plant stress tolerance (PERBONI et al., 2015). Several studies have shown that cold stress reduces the ratio variable fluorescence to maximum fluorescence (Fv/Fm), which indicates that PSII is sensitive to low temperatures (JATIMLIANSKY et al., 2004; RAPACZ, 2007; PERBONI et al., 2015), since Fv/Fm ratio is an indicator of the energy efficiency of the PSII reaction center (PAPAGEORGIOU; GOVINDJEE, 2011). The typical Fv/Fm values of unstressed plants range from 0.75 to 0.85, and lower values are generally observed when plants are

exposed to stress (BOLHÀR-NORDENKAMPF; ÖQUIST, 1993; KALAJI; GUO, 2008).

The objective of the present study was to evaluate the cold tolerance of forage plant species by assessing the effects of low temperature on cell membrane stability and photoinhibition.

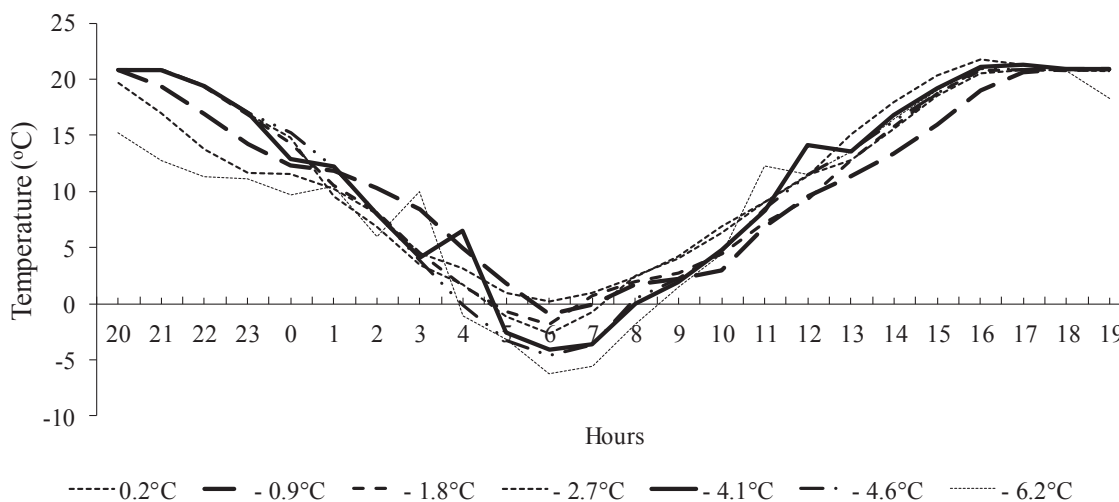
Material and Methods

The experiment was conducted at Instituto Agrônômico do Paraná (IAPAR) experimental station in Londrina, Paraná, Brazil, and included black oat (*Avena strigosa*), marandu grass (*Urochloa brizantha*), bermuda grass 'Tifton 85' (*Cynodon* sp.), alfafa (*Medicago sativa*), mombaça grass (*Megathyrsus maximus*), millet (*Pennisetum*

americanum), and sorghum (*Sorghum bicolor*). A completely randomized experimental design was used, with six replicates per forage species. Bermuda grass 'Tifton 85' was propagated from ~20-cm branches, whereas the other species were propagated by sowing seeds in 1000-mL pots that contained a 2:1 mixture of soil and manure, as well as 1 kg 4-30-10 (N-P₂O₅-K₂O) fertilizer per m³.

The plants were grown in a greenhouse for two months and then exposed to low temperatures inside a growth chamber (S. S. Cientific, Londrina-PR) that was programmed to reach minimum temperatures of 0.2, -0.9, -1.8, -2.7, -4.1, -4.6, or -6.2 °C for one hour before returning to ambient temperature (Figure 1).

Figure 1. Temperature curves of cold-temperature treatments used in the present study (the sudden increases and decreases correspond to the times when the chamber's cooling system automatically switched on and off, respectively, throughout each treatment).



Chlorophyll fluorescence and the electrical conductivity of leaf sections solutions were performed before and after treatment, in order to assess the damage caused by low-temperature exposure.

Chlorophyll fluorescence was measured using a Multi-mode Chlorophyll Fluorometer (Optiscience

model OS5). Duplicate readings were obtained from the adaxial epidermis of the same fully expanded leaf, before and after cold stress, and mean initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence (F_v), and quantum efficiency of PS II - F_v/F_m (BOLHÀR-NORDENKAMPF; ÖQUIST, 1993) values were calculated.

Electrical conductivity, which is used to measure the degree of cell membrane damage caused by stress, is based on the leaching of leaf cell electrolytes (ARVIN; DONNELLY, 2008). Before and after low-temperature exposure, leaves from each plant were cut into pieces (~1 cm²), and 10 sections from each plant were immersed in 20 ml deionized water. After 24 h at 25 °C, the electrical conductivity (µS cm⁻¹) of the solutions was measured.

Analysis of variance was used to assess the effects of the cold-stress treatments, and when significant differences were detected, Tukey's test

($P \leq 0.05$) was used to compare means.

Results and Discussion

Cold treatment affected the chlorophyll fluorescence of each species, except for bermuda grass 'Tifton 85' (Table 1). According to Bolh ar-Nordenkampf and  quist (1993) and Kalaji and Guo (2008), the typical value of the quantum efficiency (Fv/Fm) of a plant without stress ranges from 0.75 to 0.85, so values lower than 0.70 were considered to indicate damage to photosystem II (PSII).

Table 1. Maximum photochemical efficiency of photosystem II (Fv/Fm - Variable fluorescence/Maximum fluorescence) of forage plants exposed to cold stress.

Temperature (°C)	Forage species						
	Alfalfa	Black oat	Marandu	Pear millet	Mombaça	Sorghum	'Tifton 85'
Initial	0.780 a*	0.756 a	0.783 a	0.749 a	0.772 a	0.742 a	0.750 a
0.2	0.799 a	0.758 a	0.732 a	0.750 a	0.763 a	0.738 a	0.754 a
-0.9	0.778 a	0.755 a	0.784 a	0.742 a	0.764 a	0.746 a	0.734 a
-1.8	0.767 a	0.761 a	0.778 a	0.741 a	0.772 a	0.747 a	0.748 a
-2.7	0.752 a	0.741 a	0.759 a	0.612 ab	0.721 a	0.690 a	0.729 a
-4.1	0.677 a	0.477 ab	0.560 a	0.404 b	0.553 ab	0.371 b	0.734 a
-4.6	0.618 a	0.598 a	0.587 a	0.155 c	0.338 bc	0.150 b	0.734 a
-6.2	0.024 b	0.272 b	0.194 b	0.056 c	0.076 c	0.139 b	0.696 a
CV%	18.3	25.0	25.2	22.4	26.4	24.3	5.6
LSD	0.22	0.30	0.30	0.22	0.29	0.24	0.08

*Different lowercase letters indicate that means within columns are significantly different, according to Tukey's test ($P \leq 0.05$). CV = Coefficient of variation. LSD = Least Significant Difference.

Millet and sorghum first exhibited cold damage upon exposure to -2.7 °C, whereas black oat, marandu grass, alfalfa, and mombaça grass first exhibited cold damage at -4.1 °C, and bermuda grass 'Tifton 85' first exhibited cold damage at -6.2 °C.

The reduction of chlorophyll fluorescence with decreasing minimum temperature indicates that the plants were affected by the cold because of damage to photosystems I and II, that (BERTAMINI et al., 2007; EQUIZA; FRANCKO, 2010). Thus, even if Fv/Fm is only reduced slightly, the photosynthetic

electron transport of cold-sensitive plants can be inhibited (HENDRICKSON et al., 2004).

Using an approach that was similar to that of the present study, Bertamini et al. (2007) found that cold exposure reduced the Fv/Fm of cold-sensitive grapevine cultivars but did not affect that of more tolerant cultivars, thereby indicating that an important part of the PSII reaction center was damaged by cold exposure.

Zulini et al. (2010) concluded that the Fv/Fm ratio was a good criterion for identifying the beginning of

damage in 'Pinot noir' buds and proposed a critical value of 0.40. Reductions in PSII activity have also been reported for cold-stressed wheat - *Triticum aestivum* L. (BRESTIC et al., 2012), Guatemalan grass - *Tripsacum dactyloides* (JATIMLIANSKY et al., 2004), and young jatropha plants - *Jatropha curcas* (PLOSCHUK et al., 2014). Rapacz (2007) also reported that the measurement of chlorophyll a fluorescence was a rapid and objective method for

evaluating cold damage in wheat plants.

Cold treatment affected the electrical conductivity of each species, except for bermuda grass 'Tifton 85', which maintained similar values in all situations (Table 2). Therefore, low temperature did not damage the cell membranes of the bermuda grass 'Tifton 85', as previously reported for wheat 'Gaskoghen' (HABIBI et al., 2011) and olive 'Delghan' (MOSHTAGHI et al., 2009).

Table 2. Electrical conductivity ($\mu\text{S cm}^{-1}$) of leaf-section solutions from forage plants exposed to cold stress.

Temperature (°C)	Forage species						
	Alfalfa	Black oat	Marandu	Pearl millet	Mombaça	Sorghum	'Tifton 85'
Initial	6.44 b*	6.24 b	24.08 c	25.11 c	12.30 b	46.46 b	7.41 a
0.2	13.23 b	7.84 b	32.97 bc	12.86 c	14.77 b	23.44 b	5.88 a
-0.9	4.57 b	11.59 b	34.29 bc	18.35 c	19.38 b	35.68 b	5.79 a
-1.8	3.67 b	7.27 b	25.34 c	29.46 c	12.22 b	34.67 b	4.96 a
-2.7	12.63 b	7.03 b	73.71 bc	126.51 bc	21.18 b	75.74 b	4.74 a
-4.1	37.76 ab	102.50 a	149.60 b	75.37 bc	57.20 b	266.38 a	5.59 a
-4.6	34.10 ab	44.14 ab	137.41 bc	200.31 ab	80.62 b	291.03 a	7.98 a
-6.2	55.68 a	92.03 a	292.28 a	270.58 a	329.58 a	290.40 a	6.76 a
CV%	106.4	98.69	67.44	79.03	80.91	55.06	42.8
LSD	41.28	63.45	96.21	138.32	102.16	135.15	4.85

*Different lowercase letters indicate that means within columns are significantly different, according to Tukey's test ($P \leq 0.05$). CV = Coefficient of variation. LSD = Least Significant Difference.

The electric conductivity of pearl millet, marandu grass, and sorghum increased at -2.7°C , whereas the electrical conductivity of the other species, except for bermuda grass 'Tifton 85', increased at -4.1°C . Therefore, cold stress damaged the cell membranes of the cold-sensitive species and, thereby, caused electrolyte leaching (JATIMLIANSKY et al., 2004). According to Hasanuzzaman et al. (2013), this occurs because low temperatures cause fatty acids to become unsaturated and, thereby, alter the structure and fluidity of the membrane layer of lipids and proteins.

Similar stress-induced increases in electrolyte leaching have also been reported to occur in grapevine (BERTAMINI et al., 2007), wheat (HABIBI et al., 2011), maize, and Guatemala grass

(JATIMLIANSKY et al., 2004).

However, Jatimliansky et al. (2004) reported that the measurement of electrical conductivity was insufficiently sensitive for detecting differences in the cold tolerance of maize (*Zea mays*) genotypes. This was also observed for mombaça grass in the present study, where electrical conductivity only indicated damage at -6.2°C , even though fluorescence measurement indicated that PSII was already affected at -4.1°C . For the other species used in the present study, electrical conductivity was sufficiently sensitive, as reported for *Medicago truncatula* (THAPA et al., 2008).

The results of the present study demonstrate that it is possible to verify the cold tolerance of forage

plants based on their responses to cold exposure in controlled environments. The consistency of the results is due to the homogeneity of temperature during cold exposure, as previously demonstrated in white clover - *Trifolium repens* (ANNICCHIARICO et al., 2001) and peanuts - *Arachis hypogea* (BRESOLIN et al., 2008).

Conclusion

Millet and sorghum were able to tolerate exposure to temperatures as low as -2.7 °C, whereas black oat, marandu grass, alfafa, and mombaça grass were able to tolerate exposure to -4.1 °C, and bermuda grass 'Tifton 85' was able to withstand temperatures below -6.2 °C.

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