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RESEARCH LETTER

Tall fescue germplasm response to freezing temperatures

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Abstract

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] is a perennial cool-season grass in which improved freeze tolerance could result in greater winter-hardiness and persistence. Our objective was to evaluate survival and freeze-tolerance thresholds of 'Flecha' summer-dormant (SD) and 'Texoma Max QII' summer-active (SA) tall fescue at seedling (1–2 leaves) and tillered (5–6 leaves) growth stages using controlled environment freezing chambers. Target temperatures ranged from 0 to −9 °C (seedlings) and 0 to −12 °C (tillered plants). Predicted lethal temperature at which 50% mortality occurs (LT_{50}) was −5.7 and −5.5 °C for SA and SD seedlings, respectively, and −6.0 and −5.7 °C for SA and SD tillered plants, respectively. Based on these results, a controlled temperature chamber appears to be a useful tool for plant breeders when evaluating survival and freeze tolerance of SA and SD tall fescue germplasm at seedling and tillered growth stages.

1 | INTRODUCTION

Livestock grazing in the southern Great Plains of the United States is predominantly based on warm-season perennial grasses such as bermudagrass [*Cynodon dactylon* (L.) Pers.] and cool-season annual grasses such as wheat (*Triticum aestivum* L.). However, tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.], a perennial cool-season grass, is receiving more interest from producers who are looking to replace annual cool-season grasses with perennial forages that are less expensive and have fewer inputs.

In the United States, summer-active (SA), or continental, tall fescue is primarily grown in the transition zone between cool-temperate and subtropical zones (eastern Nebraska, Kansas, and Oklahoma to the East Coast at approximately the same latitude) (Sleper & West, 1996). Summer-dormant (SD), or Mediterranean, tall fescue is adapted to areas with hotter, drier summers (Dierking & Kallenbach, 2012), such as the western part of the U.S. southern Great Plains. In general, SA tall fescue is more freeze tolerant than SD tall fescue, whereas SD tall fescue is more heat and drought tolerant than SA tall fescue (Butler, Islam, & Muir, 2008). Summer-dormant tall fescue seedlings can be slower to germinate (Butler, Celen, Webb, Krstic, & Interrante, 2017) due to the lower temperature requirement, which suggests they should be planted later in autumn. Additionally, SD tall fescue seedlings appear to be more susceptible to winter mortality due to freezing temperatures when planting is delayed (unpublished data) as well as through frost heaving (Biswell et al., 1953).

Establishment and persistence of forage stands are primarily a function of climatic, pest, edaphic, and management stresses, and genetic variability for stress factors are generally observed within the plant species of interest (Smith & Kretschmer, 1989). Tolerance to climatic stresses such

Abbreviations: LT_{50}, lethal temperature at which 50% mortality occurs; SA, summer active; SD, summer dormant.

as freezing temperatures is a result of physiological, chemical, and physical mechanisms comprising many plant and cell traits (Săulescu & Braun, 2001). Improving freeze tolerance of plants with high agronomic value should result in greater establishment and persistence (Castonguay, Michaud, Nadeau, & Bertrand, 2009). In order to select germplasm that is resistant to specific stresses, it is necessary to impose those stresses under controlled environmental conditions (Săulescu & Braun, 2001).

The frost tolerance protocol developed by Fiebelkorn and Rahman (2016) consisted of 7-d acclimation of 2-wk-old canola (*Brassica napus* L.) seedlings at 4 °C followed by 16 h frost treatment at −4 °C. Landry and Wolyn (2012) developed an asparagus (*Asparagus officinalis* L.) seedling assay to select for winterhardiness by testing cold acclimation (10 °C day/5 °C night) and cold acclimation followed by 3 to −3°C temperatures in controlled environment chambers. Castonguay et al. (2009) developed a rapid, reliable, and reproducible alfalfa (*Medicago sativa* L.) phenotyping method for freeze tolerance, which helped to accelerate the breeding process.

The methods used in the aforementioned studies offer a reliable, low-cost tool for evaluating freeze tolerance of SA and SD tall fescue germplasm. This is beneficial during early assessments because temperature under natural conditions cannot be controlled, making in-field experiments more logistically difficult. Therefore, our objective was to use controlled-environment freezing chambers to identify survival and freeze tolerance thresholds for evaluating SD and SA tall fescue germplasm.

Core ideas

- LT_{50} was −5.7 °C for summer-active and −5.5 °C for summer-dormant tall fescue seedlings.
- All seedlings survived at or above −4.0 °C, while all seedlings died at or below −8.0 °C.
- Summer-active tillered plants had greater survival from −8.0 to −6.0 °C.
- LT_{50} was −6.0 and −5.7 °C for SA and SD tillered plants, respectively.

2 | MATERIALS AND METHODS

2.1 | Plant material preparation

Two types of tall fescue, represented by the cultivars 'Texoma Max QII' (SA) and 'Flecha' (SD), were evaluated for freeze tolerance. Two seeds were planted into each cell (5 cm × 5 cm) of a seedling tray (26.5 cm × 59 cm; Speedling Inc.) and were grown in a greenhouse. Peat-based, soilless potting mix (MetroMix 360; Sun Gro Horticulture) was screened (5 mm mesh) for uniformity, and seedling trays were bottom-watered to improve moisture uniformity. Each tray was divided into zones (left, center, and right) to correspond with the freezer shelf zones described in "Freeze Chamber" section. There were 12 cells in each of the left and right zones and 16 cells in the center zone. Summer-active and SD tall fescue seeds were planted in alternating rows so that there were an equal number of each type in each zone (i.e., six cells of each tall fescue type in each of the left and right zones and eight cells of each type in the center zone). Seedlings were thinned to a single plant per cell, and any plants with more than two fully emerged leaves (approximately 30 d after emergence) were removed. Prior to temperature treatment initiation, seedling trays were moved to an acclimation chamber (3 °C) for 24 h.

Texoma Max QII and Flecha plants were also evaluated for freeze tolerance of one-tillered plants (five- to six-leaf stage; approximately 60 d after emergence). Plants were grown in the same seedling trays and with the same methods and zone arrangement as described for seedlings above.

2.2 | Freeze chamber

For both seedling and tillered plant experiments, two 0.65-m^3 manual-defrost freezers (model FFFU21M1QW; Frigidaire) with five shelves were used as freeze chambers. Each freezer was retrofitted with a Ranco single-stage digital temperature controller with sensor (ETC Supply) and 14 Cooltron AC axial high speed fans (80 × 80 × 25 mm, 230 V) (Cooltron) to circulate air and minimize temperature variation within the chamber. Spacers were installed on the shelves to ensure equal distance between the surface of the trays and the cooling coils. Temperature varied (±1 °C) horizontally along each shelf, so each shelf was partitioned into three zones (left, center, and right). In order to measure the actual temperatures in each zone on each shelf, 15 HOBO S-TMB 12-bit temperature sensors (one per zone, three per shelf) and one HOBO model U30 USB weather station data logger (Onset Computer Corp.) were installed in each freezer. Temperature sensors were mounted to record temperature approximately 2.5 cm above the seedling trays within each zone (left, center, and right). Each freezer was allowed to stabilize at the target temperature for 24 h prior to the plants being added.

2.3 | Freeze experiment

The freeze experiment was replicated four times (runs). The target temperatures were 0 to −9°C and 0 to −12°C for the seedling and tillered plant experiments, respectively. When the target temperature was reached, one tray of either seedlings or tillered plants was placed on each shelf, and trays remained in the freezer until the target temperature was achieved again (approximately 16 h). Plants were then moved back to the cold acclimation chamber for 24 h, after which they were moved to the greenhouse and allowed to recover at optimal growing conditions (25 °C) for 14 d. Plants were scored as either alive (1) or dead (0), and the total number of plants per zone per tray were counted to calculate percentage survival. The actual temperatures as measured by the temperature sensors above the tray within each shelf (1–5) and zone (left, center, and right) were used as continuous independent variables.

2.4 | Statistical analysis

Logistic regression analysis was used to determine survival probability of seedling and tillered plants for the tall fescue types as a function of temperature while controlling for each independent replication (seedling and tillered plant data were analyzed separately). Freezer, shelf, and zone were not included in the analyses as design or nuisance variables because temperature sensors were installed at each combination of freezer × shelf × zone combination, which captured any temperature variations within and across freezers.

Generalized linear mixed models using the GLIMMIX procedure (SAS Institute, 2011) were used to calculate survival probability. Survival of each plant was analyzed as a binary response. A logit link function was also specified. The effects of temperature (continuous variable), tall fescue type (categorical variable with two levels), and their interaction were modeled. Model fit was assessed using the generalized chi-square divided by degrees of freedom statistic. From these analyses, predicted lethal temperature at which 50% of plants died (LT_{50}) was determined for seedling and tillered plants of tall fescue types based on survival probability data.

3 | RESULTS AND DISCUSSION

The actual measured temperature range during the seedling trial ranged from −8.7 to 0.0 °C. Tall fescue seedling (n = 1,594) survival was affected by temperature ($F_{1,\ 1583}$ = 304.6, P < .001; Figure 1), but not by tall fescue type ($F_{1,\ 1583}$ = 0.04, P = .835) or their interaction ($F_{1,\ 1583}$ = 0.61, P = .435). The LT_{50} derived from Figure 1 was −5.7 and −5.5 °C for SA and SD seedlings, respectively. Although temperature differed, this narrow LT_{50} range may not be of biological importance, which is surprising due to field observations where SA tall fescue seedlings appear to tolerate colder temperatures than SD seedlings. The two tall fescue types had similar survival curves except the SA type had slightly greater survival from −7.0 to −4.5 °C, but

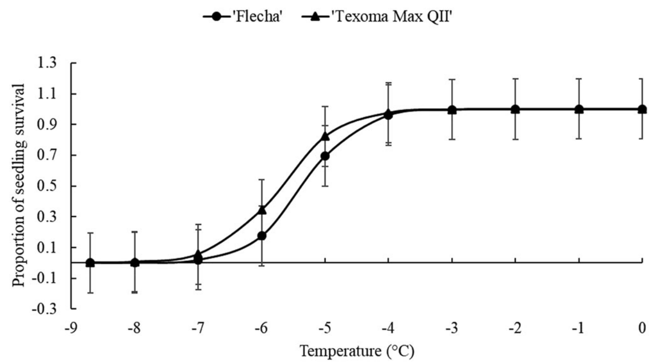

FIGURE 1 Survival probability of 'Flecha' summer-dormant and 'Texoma Max QII' summer-active tall fescue seedlings as affected by temperature (P < .001) in a freezer chamber experiment

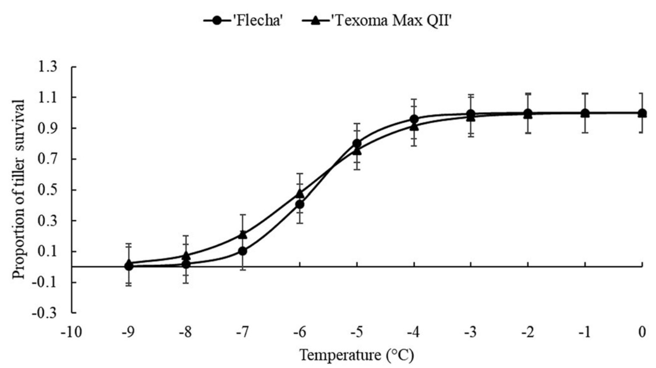

FIGURE 2 Survival probability of 'Flecha' summer-dormant and 'Texoma Max QII' summer-active tall fescue tillered plants as affected by temperature × tall fescue type interaction (P < .001) in a freezer chamber experiment

survival probabilities were the same across tall fescue types on either end of this range.

The actual measured range of temperatures during the tillered plant trial were −12.0 to −1.5 °C. Tillered plant (n = 1,845) survival was affected by temperature ($F_{1,1833}$ = 162.3, P < .001), tall fescue type ($F_{1,1833}$ = 12.8, P < .001), and their interaction ($F_{1,1833}$ = 18.4, P < .001); therefore the interaction is presented. Generally, the tall fescue types followed similar survival curves. The SA type had greater survival from −8.0 to −6.0 °C, but there was a change in survival from −5.0 to −3.5 °C in which the SD type had slightly greater survival (Figure 2). Survival between the tall fescue types was similar above −2.5 °C. The LT_{50} derived from Figure 2 was −6.0 and −5.7 °C for SA and SD tillered plants, respectively. In general, the LT_{50} of SA tall fescue seedlings and tillered plants occurred at slightly colder temperatures than for SD tall fescue.

Controlled temperature chamber experiments offer a reliable, useful, and inexpensive tool for plant breeders when evaluating SA and SD tall fescue germplasm for freeze tolerance. This method allows for temperature conditions to be repeatable and relatively simple to replicate. Differentiating among freeze-tolerant genotypes could contribute to the development of elite freeze-tolerant tall fescue populations. Superior selections could be used to develop SA and SD populations to be further evaluated or integrated through crossing into other elite tall fescue germplasm. Crosses could also be made between accessions with contrasting freeze tolerances to develop biparental mapping populations to map the freeze-tolerance trait for identifying freeze-tolerance genes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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