Comparative assessment of metabolic responses to cold acclimation and deacclimation in annual bluegrass and creeping bentgrass

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ABSTRACT

The level of freezing tolerance achieved by perennial grasses and their capacity to remain cold acclimated throughout winter is critical for their winter survival. Research is necessary to better understand the metabolic processes associated with cold acclimation and deacclimation. This study was conducted to assess the response of creeping bentgrass (*Agrostis stolonifera* L.) (CB) and two ecotypes of annual bluegrass (*Poa annua* L.), one freezing-tolerant (AB-T) and one freezing-sensitive (AB-S), to cold acclimation and deacclimation. Following cold acclimation, plants were exposed to 8 °C for 0.5, 1, 3, and 5 d to induce deacclimation. Plants were assessed for their freezing tolerance (lethal temperature resulting in 50% mortality, LT_{50}), their concentrations in soluble sugars and amino acids, and for changes in dehydrin-like proteins. Fully acclimated CB achieved higher level of LT_{50} (−21.5 °C) than AB-T (−19.8 °C), followed by AB-S (−15.3 °C). Total soluble sugars, mainly high molecular weight (HMW) fructans, accumulated in each species/ecotypes during cold acclimation with higher levels measured in CB. Dehydrin-like proteins were present in each species but they were mostly constitutive in AB while they were cold-inducible and linked with the LT_{50} in CB. Freezing tolerance decreased during deacclimation in each species. However, at each step of deacclimation, CB maintained higher freezing tolerance than AB in which the LT_{50} reached the low level of non-acclimated plants after five days at 8 °C. The depletion of HMW fructans observed during deacclimation followed a similar trend, with higher levels remaining at the end in CB, followed by AB-T and then by AB-S. The higher susceptibility of AB to winter injury is associated with both its lower cold acclimation capacity and deacclimation sensitivity.

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1. Introduction

Temperate perennial grasses represent an economically important group of plants adapted to northern climates with a variable capacity to withstand exposure to temperatures at or below freezing for prolonged periods of time. Creeping bentgrass (*Agrostis stolonifera* L.) (CB) is one of the most winter-hardy temperate perennial grasses that can survive prolonged exposure to subfreezing temperatures (Bertrand et al., 2013). Annual bluegrass (*Poa annua* L.) (AB) is a highly variable species with growth habits ranging from strictly annual to strongly perennial types (Johnson et al., 1993). Lower tolerance to subfreezing temperatures in perennial-types

of AB has been documented and could be a significant source of recurrent winter injury in swards invaded by unsown AB (Dionne et al., 1999; Tompkins et al., 2000, 2004).

Winter injury can result from a combination of factors including high crown hydration, extensive cell desiccation, infections by low temperature pathogens, anoxia, and insufficient tolerance to freezing temperatures. Among these, tolerance to freezing temperatures has been identified as the most important component of winterhardiness in many plant species (Fowler et al., 1981; Humphreys, 1989; Hayes et al., 1993). The accumulation of carbohydrates (Koster and Lynch, 1992; Ball et al., 2002), amino acids (Dörffling et al., 1997; Patton et al., 2007a) and changes in soluble protein amounts and composition (Houde et al., 1995; Patton et al., 2007b) has been documented during the acquisition of freezing tolerance. In some instances the cold induced accumulation of compounds such as polyfructose fructans (Valluru and Van den Ende, 2008; Dionne et al., 2010) or the amino acid proline (Patton et al., 2007a), have been shown to be positively related to the level of freezing tolerance of grass species.

Adaptation to cold is also determined by the ability of the plants to retain freezing tolerance during warm periods that can occur during winter and early spring (Arora et al., 2004; Pagter et al., 2011). Deacclimation typically occurs as air and soil temperatures gradually increase during mid- to late spring, which triggers metabolic and physiological changes that lead to the resumption of plant growth (Sasaki et al., 2001; Arora et al., 2004; In et al., 2005). Increased susceptibility of plants to sub-freezing temperatures after deacclimation is accompanied by a reduction of protective compounds (i.e. carbohydrates, amino acids, and proteins) that accumulated during cold acclimation (Svenning et al., 1997; Stupnikova et al., 2002; Arora et al., 2004; Pagter et al., 2011). Deacclimation has also been associated with a decrease in abundance of specific stress-related proteins including intrinsically disordered dehydrins (Arora et al., 2004).

Under future climate predictions (IPCC, 2001, 2007), longer growing seasons that prevent plants from achieving maximum hardiness (Thorsen and Höglind, 2010), untimely loss of freezing tolerance in response to freeze-thaw cycles, and less stable snow covers may cause more widespread winter injury (Bélanger et al., 2002).

AB ecotypes with contrasting tolerance to freezing temperatures have been previously identified (Dionne et al., 2001a). Recently, Dionne et al. (2010) documented a wide range of variability in freezing tolerance among AB ecotypes from diverse origins. Annual bluegrass ecotypes from regions with abundant snow covers were generally less freezing tolerant than ecotypes that evolved at sites with milder winter conditions but a less reliable snow protection. Differences in freezing tolerance between these two groups of AB ecotypes were attributed to variations in their capacity to undergo metabolic changes during the cold acclimation period. In addition to its impact on cold acclimation potential, previous studies have also found that a high degree of variability in deacclimation response exists depending on the site of origin (Rowland et al., 2005; Kalberer et al., 2006). Although few studies have examined deacclimation responses of AB, there is some indication of potential sensitivity to mid-winter or early spring freezes compared to CB (Tompkins et al., 2000, 2004; Hoffman et al., 2014) Therefore, the primary objective of this study was to make a comparative assessment of freezing tolerance and carbon and nitrogen metabolism in one CB cultivar and two AB ecotypes during cold acclimation and deacclimation to gain further insight on the traits associated with their differential winter hardiness.

2. Materials and methods

2.1. Plant material and growth conditions

Plant material consisted of one freezing-tolerant AB ecotype (AB-T) and one freezing-sensitive AB ecotype (AB-S) from Agriculture and Agri-Food Canada, Québec City, Québec. These ecotypes were selected based on previous research that determined their differential tolerance to freezing temperatures (Bertrand et al., 2011). For comparison, one CB cultivar ('L-93') was collected from the Joseph Troll Turf Research Center, University of Massachusetts-Amherst. Three to four tillers of CB, AB-T, and AB-S were propagated from plugs into pots (10 cm diameter, 10 cm depth) containing sand and were maintained in a greenhouse for 3 mo at 18/13 °C day/night temperatures. During the three-month establishment period, plants were trimmed once per week to a height of 2.5 cm, fertilized weekly with Hoagland solution (Hoagland and Arnon, 1950), and watered to prevent wilt stress. Following establishment, plants were transferred to a controlled-environment growth chamber (Conviron, Winnipeg, Canada) and maintained at 20/15 °C

day/night temperatures with a 10 h photoperiod and photosynthetic photon flux density (PPFD) of $300 \, \mu mol \, m^{-2} \, s^{-1}$.

2.2. Treatments

This study consisted of both cold acclimation and deacclimation treatments. Pots containing CB, AB-T, and AB-S were exposed to (i) 20 °C for 2 wk, followed by (ii) 2 °C for 2 wk, and then (iii) subzero acclimation at −2 °C for 2 wk. At 20 and 2 °C, plants were maintained under a 10 h photoperiod and PPFD of $300 \, \mu mol \, m^{-2} \, s^{-1}$ and plants subjected to subzero acclimation were maintained in the dark. This temperature regime was previously determined to induce cold acclimation in AB and other cool-season turfgrass species (Dionne et al., 2001a; Hoffman et al., 2010). Deacclimation was initiated after the completion of the 4-wk cold acclimation period and consisted of the following six treatments: −2 °C 2 wk (0 d), 8 °C 0.5 d (0.5 d), 8 °C 1 d (1 d), 8 °C 3 d (3 d), and 8 °C 5 d (5 d). During deacclimation, plants were maintained under a 10 h photoperiod and PPFD of $100 \, \mu mol \, m^{-2} \, s^{-1}$. The 0 d treatment served as baseline for comparison to all other deacclimation treatments. The temperature of 8 °C was previously shown to induce deacclimation of both AB and CB (Hoffman et al., 2014). In addition, the time sampling intervals (0–5 d) were selected to provide information on both early and more prolonged changes in response to exposure to 8 °C.

Plants were destructively harvested following each of the cold acclimation and deacclimation treatments. Plants subjected to −2 °C for 2 wk were thawed for 12 h at 2 °C to facilitate sampling as described by Dionne et al. (2001a). Whole plants were obtained for freezing tolerance determination and individual crowns were harvested (1 cm above and below the transition zone between shoot and root) for biochemical analyses. Crown material was immediately frozen in liquid nitrogen and then stored at −80 °C until all cold acclimation and deacclimation treatments were completed. Following the last deacclimation treatment, all crown material was ground with liquid nitrogen, freeze-dried, and stored in a desiccator until time of analysis.

2.3. Measurements

2.3.1. Freezing tolerance assessment

Freezing tolerance determination was based on whole-plant survival following controlled freezing tests according to the methods previously described by Ebdon et al. (2002). Briefly, ten individual plants per species/ecotype were wrapped in a pre-moistened paper towel to ensure ice nucleation, placed in freezer bags, and stored at 6 °C until sample preparation was complete. For each test temperature included in the freezing tests (see below), there were four replicates containing ten plants, for a total of 40 plants per test temperature per treatment. Following preparation, freezer bags containing plants were placed in a programmable freezing chamber (Tenney TC Series Cycling Test Chamber, SPX Thermal Product Solutions, White Deer, Pennsylvania) and subjected to a range of eight temperatures consisting of a non-frozen control (6 °C) and seven freeze-test temperatures: −6, −9, −12, −15, −18, −21, and −23 °C. The freezer was cooled in a stepwise fashion at a rate of $2 °C \, h^{-1}$ to the desired temperature and held at each test temperature for 1 h. After each target temperature was reached, plants were removed from the freezer and allowed to thaw for 12 h at 6 °C. After thawing, plants were replanted into cell trays (10 × 20 cm) filled with commercial potting media (Pro-Mix; Griffin Greenhouse and Nursery Supplies, Tewksbury MA) and then placed in a greenhouse at approximately 20 °C. Following a 3 wk regrowth period, whole plant survival (%) was calculated for each replicate as: (no. plants survived/total no. plants) × 100. The lethal temperature at which 50% of plants were killed (LT_{50}) was determined