Identifying Physiological and Metabolic Response Mechanisms Associated with Divergence in Chilling and Freezing Tolerance Between Upland and Lowland Switchgrass Cultivars.

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Project Goals

The goal of this project is to identify traits underlying divergence in cold tolerance (chilling and freezing) and flowering time between cold-tolerant (northern upland) and cold-sensitive (southern lowland) cultivars of switchgrass (*Panicum virgatum*). Accomplishing this goal will facilitate selection for high-yielding, cold-tolerant cultivars that can thrive despite adverse winter (freezing temperatures) or early spring (chilling events) conditions in the northern United States.

Abstract

Research on bioenergy crop production on marginal lands is important for environmentally sustainable production of biofuels and bioproducts. Switchgrass (*Panicum virgatum*), a potential lignocellulosic bioenergy crop species, is a native North American prairie grass with a range of genetic diversity, environmental adaptability, and high biomass yield.

Upland cultivars of switchgrass can survive cold winters in the northern U.S., but have lower biomass yield, in part because of their early flowering traits. In contrast, lowland cultivars accumulate more biomass, are more nutrient-use efficient, are more tolerant to drought and heat, and are more resistant to pathogens, but are less tolerant to cold environmental conditions.

In this study, we focused on the physiological and metabolic responses to a chilling event of two parental cultivars, VS16 (Nebraska-Summer-upland) and WBC3 (Texas-lowland), and their F1 hybrid (WBC3xVS16). For this purpose, plants were grown on a 14 h light – 10 h dark cycle with a light intensity of 1200 µmol/m^2/sec at 25°C during the day and 20°C at night (day 1, pre-chilling event). Plants were then submitted to a 24-hour chilling event, where the temperature was lowered to 10°C during the day and 5°C at night (day 2). On day 3 (post-chilling event), the temperature settings were identical to the initial condition (day 1).

For each cultivar, photosynthetic parameters including carbon assimilation (A), photosynthetic efficiency of photosystem II (F_v/F_m and $F_{v'}/F_{m'}$), stomatal conductance (g_s), internal CO_2 concentration (C_i) and intrinsic water use efficiency (iWUE) were measured. Pre-chilling event, the upland cultivar WBC3 and the F1 hybrid have the highest photosynthesis rate. Although all three cultivars are significantly affected by the chilling event (day 2), the WBC3 cultivar is more sensitive, with lasting damage to the photosynthetic electron transport chain (day 3). The cold-tolerant cultivar VS16 is distinct from the WBC3 cultivar and the F1 hybrid as it closes its stomata in response to the chilling event but continues to assimilate carbon at high rates. As a result, it

maintains good iWUE. This feature may explain how VS16 achieves cold-tolerance. The F1 hybrid, although affected by the chilling event, recovered in a similar manner to VS16. Overall, the F1 hybrid shows combined characteristics of both parental lines. To assess the metabolic responses of each cultivar to the chilling event, leaf samples were collected and their amino acid profiles were analyzed using LC-MS/MS.

In addition to chilling, we are currently conducting studies on these cultivars to understand the mechanisms of differential tolerance of upland and lowland rhizomes to freezing. Overall, the results of these studies will provide a new understanding of the divergence in cold tolerance between northern upland and southern lowland cultivars, which will facilitate the development of high yielding switchgrass cultivars that can survive northern winter conditions.

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