Heat Stress in Creeping Bentgrass and Analysis of Pathways Responsible for Protein Degradation

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Introduction:

Heat stress is a major abiotic stress for cool-season turfgrasses that leads to damage during summer months. In Georgia, creeping bentgrasses used on high value golf course greens are particularly sensitive to damage due to their intensive management, low mowing heights, and the high night-time temperatures and humid conditions that Georgia summers entail. The development of creeping bentgrasses with improved heat tolerance is required for greens to continue to provide excellent playing surfaces despite summer stress and be resilient against challenging environmentally conditions.

During heat stress one of the major symptoms in plants is decline in protein content. Proteins, mainly in the form of enzymes, are the primary driver of cellular metabolism. Damage to proteins ultimately can cause declines in growth and photosynthesis, prevent repair of cellular components, and ultimately result in plant death. Understanding the pathway of degradation, as well as the specific proteins which are being damaged and targeted for degradation at greater rates is an essential piece of the puzzle. By identifying the details of protein degradation we can begin to select for plants that are able to maintain these critical functions, or select against plants that have sensitive versions of these proteins that are being damaged.

Objectives:

1. Screen a collection of creeping bentgrass germplasm for heat tolerance.
2. Determine differences in protein degradation among lines.
3. Identify proteolytic pathways responsible for increased protein degradation.

Results:

Screening a group of commercial cultivars and experimental breeding lines identified a wide range of heat tolerance (Fig. 1). After 5 weeks of heat stress (38/33 C day/night temperatures) in growth chambers, lines ranged from severely damaged with little green tissue remaining, to still having acceptable quality, albeit not free from damage. The two most thermotolerant lines were S11 729-10 and BTC032, both experimental breeding lines. This indicates that there is potential to improve heat tolerance beyond cultivars that are currently commercially available. Other breeding lines along with commercial cultivars such as AU Victory were ranked in the middle for heat tolerance, while Crenshaw was found to be relatively heat sensitive. Crenshaw in the past has been described as moderately heat tolerant but has also been found to be heat sensitive depending on the specific study. This indicates a further need for field screening in diverse environments and interactions with multiple stressors. Regarding mechanisms of tolerance, greater oxidative damage and declines in photosynthetic efficiency were associated with reduced heat tolerance. Greater declines in protein content and great activity of protein degradation pathways were also observed in more heat sensitive lines that had greater reductions in quality (Fig. 2). These pathways include degradation via compartmentalized proteases, as well as the ubiquitin-proteasome system (UPS), which tags protein for degradation with polyubiquitin chains. In addition to increased activity of the ubiquitin-proteasome system, in more heat sensitive lines a greater number of proteins were tagged with polyubiquitin chains (Fig. 3). This indicates that more proteins are being damaged and needing to be recycled to prevent the build up of toxic aggregates. These proteins which are being damaged and degraded at higher rates are likely a large sink of metabolic energy due to the need to recycle and rebuild these proteins, ultimately representing a weak link in heat tolerance for these plants. A preliminary analysis of proteins altered under heat stress identified several proteins associated with photosynthesis, antioxidant metabolism, and protein metabolism were associated with greater heat tolerance in S11 729-10. These included several proteins associated Photosystem II, a main protein-pigment complex associated with light harvesting for photosynthesis; Catalase and Superoxide dismutase, two important antioxidant enzymes that detoxify reactive oxygen species; as well as HSP70, a heat shock protein that acts as a chaperone for damaged proteins. Perhaps unsurprisingly these changes in protein metabolism align with the indicators of physiological damage also measured (e.g. light harvesting efficiency, oxidative damages, and protein degradation). However, the greater level of details we are able to achieve in regard to specific protein changes will allow us to not only better understand the changes in plants under heat stress, but also identify specific targets for improvement while working towards improved heat tolerance.

Conclusions:

Understanding the pathways and specific proteins that are being damaged and destroyed under heat stress will be critical for developing plants with great heat stress tolerance. Differences in heat tolerance have been observed among creeping bentgrass lines, with several experimental breeding lines showing exceptional stress tolerance beyond commercial cultivars. These heat tolerant lines are better able to maintain protein contents during heat stress (and continue to carry out normal metabolic functions). Associated with the decline in protein content of more heat sensitive lines was also an increase in activity of protein degradation pathways, with more proteins being tag via polyubiquitin for degradation. This indicates that these proteins are being damaged and degraded at higher rates in these sensitive lines. Additional work is needed to identify the specific proteins and regulation of their degradation. Preliminary experiments have identified several important pathways that may be used for better understanding heat tolerance in creeping bentgrass, the specific physiological changes that are associated with damages, and ultimately improve selection for tolerant cultivars. The data from this projects data collection has been used as preliminary data for a USDA-AFRI grant, to hopefully expand the scope of this project and continue the next critical step of the project. If successful the USDA funded project would allow a more detailed and robust quantification of proteins that are altered under heat stress in creeping bentgrass, and ultimately the key proteins regulating summer performance. Graduate student Qianqian Fan is currently working on the experimental techniques needed to answer these questions. Preliminary results show promise for developing bentgrasses that will be able to maintain greater quality during challenging Southeastern Summers.

Figures:

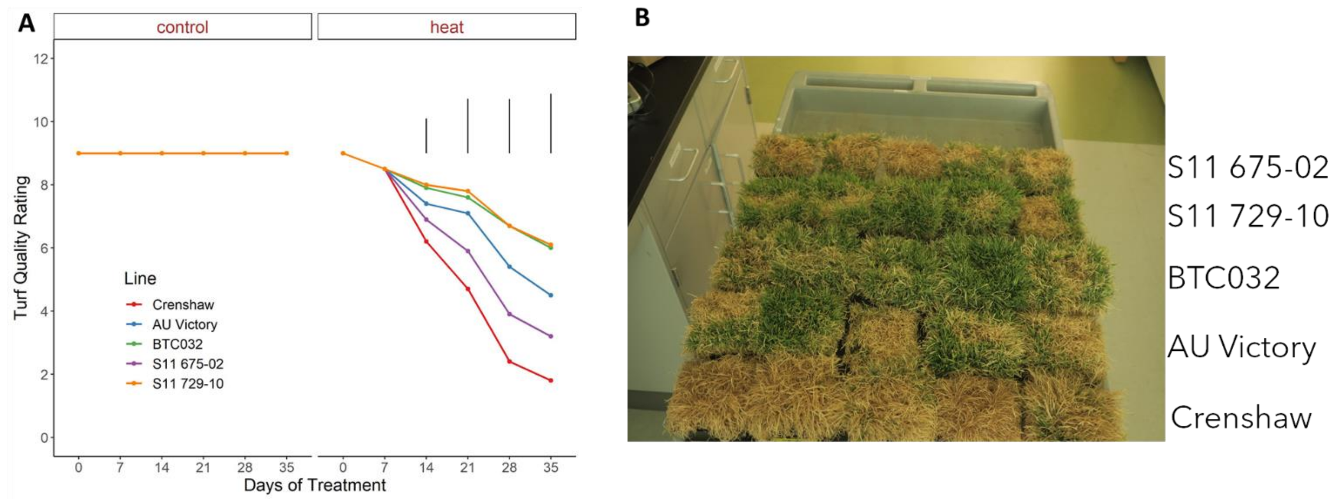

Figure 1: Visual turf quality ratings (A) under control and heat stress conditions, and an overview picture (B) taken at the end of heat stress, showing differences in heat tolerance among tested lines.

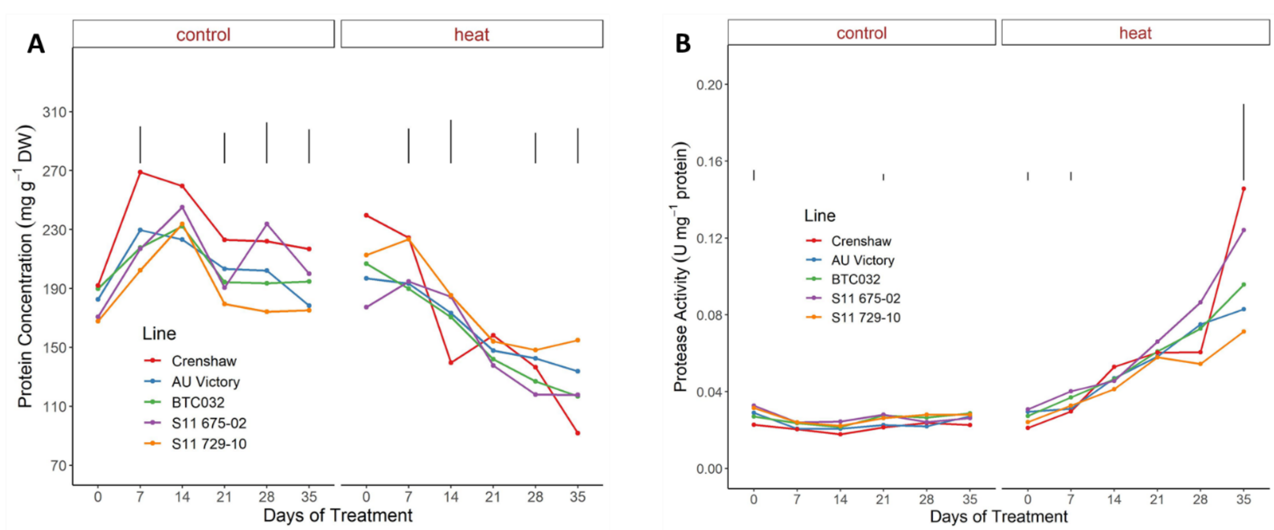

Figure 2:

Change over time in total protein content (A) and protease activity, responsible for protein degradation under control and heat stress conditions among tested bentgrass lines.

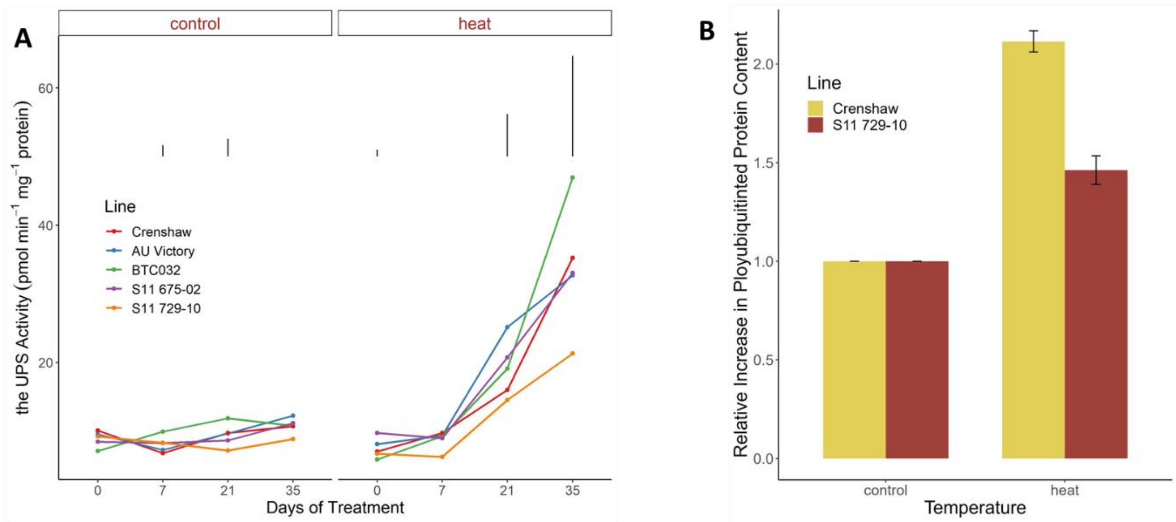

Figure 3: Change in activity of the ubiquitin-proteosome system (UPS) among bentgrass lines (A) and relative content to polyubiquitinated proteins (B) among lines differing in heat tolerance.