Optimizing Methods for Turfgrass Metabolomics

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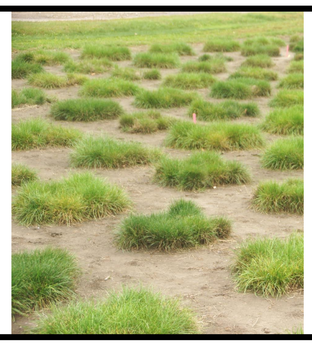
Why turfgrass metabolomics?

Identifying stress-resistant germplasm is vital for developing improved turfgrass cultivars, but common phenotypic screening methods for identifying this germplasm can be time consuming and cumbersome. Metabolomics – the comprehensive analysis of metabolites in a biological sample – is a promising tool for turfgrass breeding programs. By examining changes in metabolites associated with abiotic and biotic stress, information can be quickly provided on potential metabolic pathways that regulate stress tolerance responses.



Chemistry of cold-tolerance

A plant's tolerance to freezing is the result of various physiological and metabolic changes that occur during the cold acclimation process. Previous research has identified genes associated with cold acclimation in perennial ryegrass[1], and work in other grass species has identified amino acids, soluble sugars, fructans, phenylpropanoids, phytohormones, and cryoprotective proteins associated with increased membrane stability, slowed growth, and reduced freezing[2]. Improving the cold tolerance and the recovery ability of turfgrass after cold stress is a key component in improving germplasm for breeding programs.

Why perennial ryegrass?

Perennial ryegrass (*Lolium perenne*) is an economically important turfgrass and forage cool season grass. Although its rapid germination and quick establishment make it a valuable component of lawns, its use in northern climates is limited by its susceptibility to prolonged periods of extreme low temperatures. As part of the WinterTurf project, the range of cold tolerance is being explored in over 200 perennial ryegrass genotypes. Traditional screens for cold tolerance will be associated with metabolomic data to gain a deeper understanding of cold tolerance traits in perennial ryegrass.

1. OBJECTIVES

To guide the development of winter hardy perennial ryegrass germplasm and establish standardized procedures for turfgrass metabolomics, pilot projects are being conducted to:

- Examine the correlation between metabolite profiles of different tissue types to determine appropriate proxies for crown tissue.
- Determine when peak gene expression for cold tolerance occurs in perennial ryegrass to decide upon appropriate sampling timepoints that capture changes in the transcriptome.
- Associate freezing tolerance with various metabolites.
- Provide plant material for transcriptomic and proteomic analyses in tandem with metabolomic analyses.

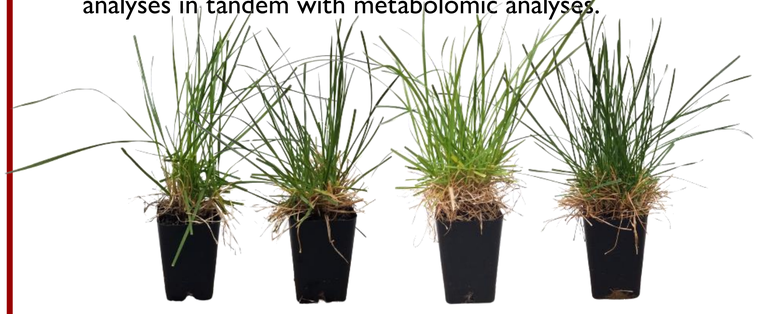


Figure 1. Perennial ryegrass genotypes (from left to right) SUS-1, SUS-2, TOL-1, and TOL-2. SUS genotypes are freezing susceptible and TOL genotypes are freezing tolerant.

2. EXPERIMENTAL DESIGN

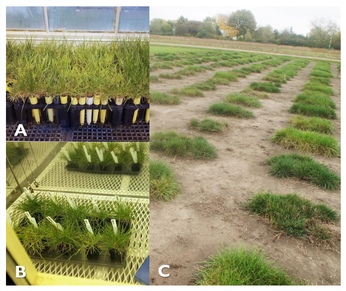


Figure 2. Perennial ryegrass growing in greenhouse (A), growth chamber (B), and field (C) conditions.

Metabolomics experiments must be designed to minimize variation that is not of experimental relevance. Different growing environments (Fig. 2) are subject to different sources of variation. Samples collected for the data presented were grown in a greenhouse with maximal temperature set points were 18/20 °C night/day and 16-hour photoperiod (0600–2200).

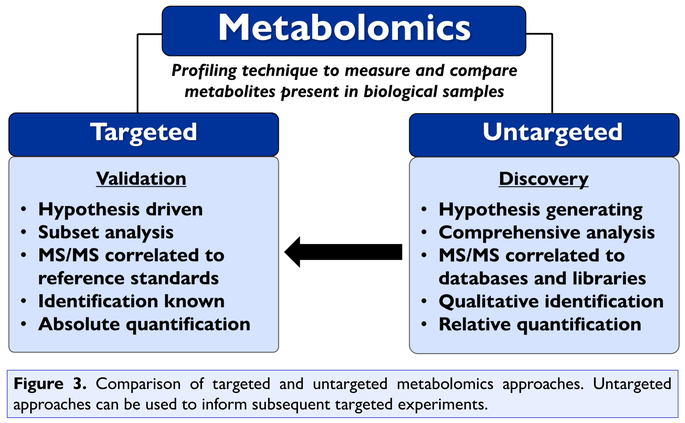


Figure 3. Comparison of targeted and untargeted metabolomics approaches. Untargeted approaches can be used to inform subsequent targeted experiments.

3. SAMPLING METHODS

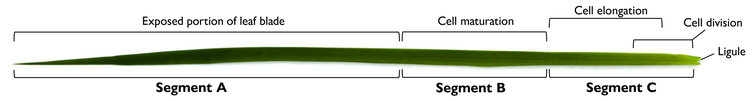


Figure 4. Approximate growth zones of elongating leaf blades.

Within leaf comparison

Eight replicates of SUS-2 and TOL-2 genotypes[3] were harvested by removing the first undamaged phytomer. Leaf segments are reflective of regions previously described in the literature in terms of zones of C and N deposition and physiological processes (Fig. 4): first 50% (A), the next 25 % (B), and the 25% closest to the ligule (C)[4,5,6].

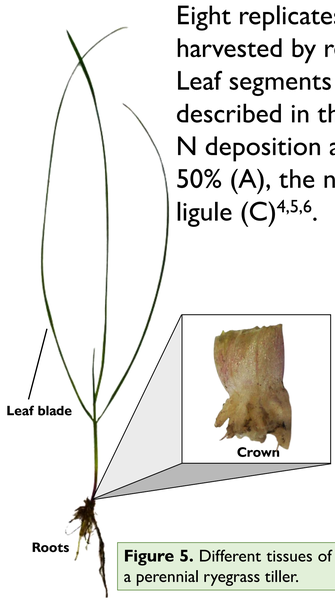


Figure 5. Different tissues of a perennial ryegrass tiller.

Tissue type comparison

Six perennial ryegrass genotypes were selected based on previous cold tolerance work[7]. Ten tillers per genotype were removed and separated by tissue type. All samples were extracted into 80% methanol at a rate of one milliliter per 200 milligrams of fresh frozen sample.

4. DATA COLLECTION

The choice of separation and detection instrumentation must reflect the chemical classes of greatest interest (Fig. 6). For pilot projects on perennial ryegrass, metabolomic profiles were obtained using C18 and HILIC liquid chromatography-mass spectrometry (LC-MS).

LC-MS parameters were optimized for untargeted metabolomics. A 15-minute gradient using mobile phases A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile was run according to the following gradient elution profile: initial 2% B, 1 min 2% B, 15 min 98% B, 0.5 min 98% B, 0.5 min 2% B.

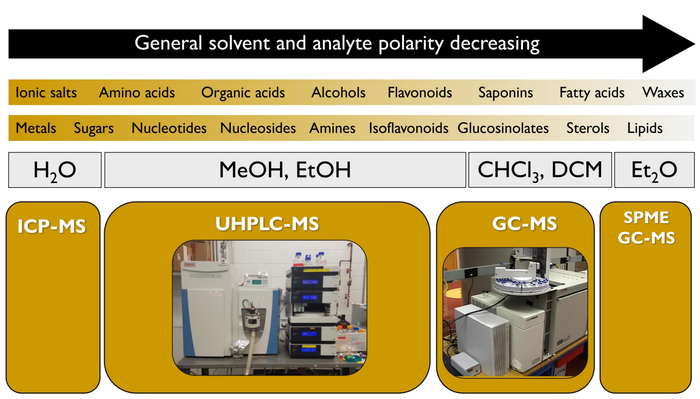


Figure 6. Summary of appropriate solvents and separation technologies for different classes of chemicals.

5. DATA ANALYSIS

MSConvertGUI4[8]and MZmine 2.53[9] were utilized for converting and processing LC-MS data. To examine differences in metabolomic profiles, multivariate statistical analyses by PCA, PLS-DA, and OPLS-DA were conducted using the ropls package in R[10,11].

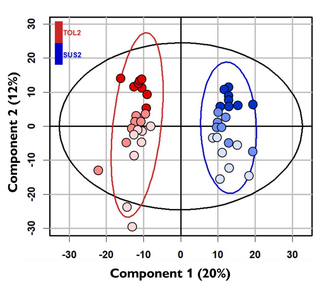


Figure 7. Score plot obtained from unsupervised principal components analysis (PCA) of clonal SUS-2 and TOL-2 perennial ryegrass genotypes based on 796 features. Color indicates genotype and shade indicates leaf segment (darkest shade is segment A, medium shade is segment B, and lightest shade is segment C).

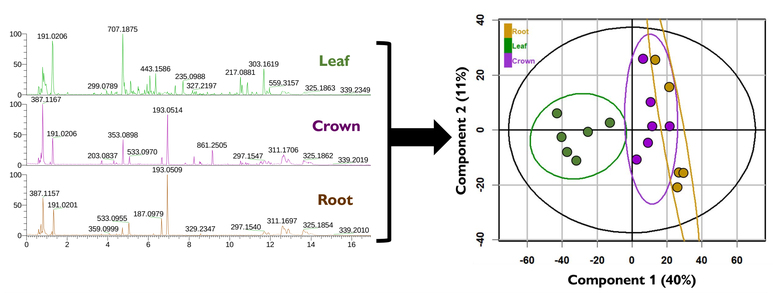


Figure 8. Chromatograms of tissue comparison in TOL-2 genotype and score plot obtained from unsupervised principal components analysis of six perennial ryegrass genotypes based on 1589 features.

6. CONCLUSIONS

Subtle differences in growing environment, harvesting methods, sample processing, and data analysis can lead to unintended variations in metabolomic data, so careful planning of metabolomic experiments is crucial.

- Different perennial ryegrass genotypes possess unique metabolomes.
- There is spatial variation in the metabolome within perennial ryegrass leaves, with patterns consistent across genotypes.
- Crown tissue is metabolically more like root tissue than leaf tissue.
- Ongoing experiments continue to optimize metabolomic methods prior to large-scale screening for winter hardiness (Fig. 9).

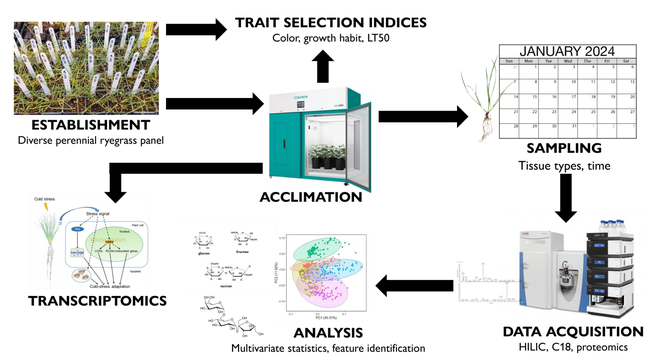


Figure 9. Workflow for ongoing WinterTurf projects on perennial ryegrass winter hardiness.

ACKNOWLEDGEMENTS

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