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A quantitative method for analyzing glycome profiles of plant cell walls

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ABSTRACT

Glycome profiling allows for the characterization of plant cell wall ultrastructure via sequential extractions and subsequent detection of specific epitopes with a suite of glycan-specific monoclonal antibodies (mAbs). The data are often viewed as the amount of materials recovered and coinciding colored heatmaps of mAb binding are generated. Interpretation of these data can be considered qualitative in nature as it depends on detecting subtle visual differences in antibody binding strength. Here, we report a mixed model-based quantitative approach for glycome profile analyses, which accounts for the amount of materials recovered and displays the normalized values in revised heatmaps and statistical heatmaps depicting significant differences. The utility of this methodology was demonstrated on a previously published dataset investigating the effects of moisture stress on the roots and needles of *Pinus taeda*. An annotated R script for the quantitative methodology is included to allow future studies to utilize the same approach.

1. Introduction

Plant cell walls are highly complex and heterogeneous structures critical to cellular form and function [1]. Considerable variation exists in the composition, integrity, and architecture of the macromolecular matrix of plant cell walls (hereafter collectively referred to as "ultrastructure") among different species, organs and developmental stages [2–4]. The cell wall is comprised largely of cellulose microfibers embedded in a macromolecular matrix of glycans [5]. It has been suggested that the plant cell wall ultrastructure contains a "glycomic code", from which critical biological information may some-day be deciphered [6,7]. The natural variation in plant cell walls and cell wall ultrastructure, a critical cell wall feature, creates challenges that require novel and robust tools for high throughput analyses. The development of glycome profiling, a comprehensive collection of glycan directed monoclonal antibodies (mAbs), capable of targeting major plant cell wall glycans, has enabled direct high-throughput analyses of the plant cell wall ultrastructure [8,9]. The broad application of glycome profiling has improved our understanding of plant biomass recalcitrance [10] and provided a way to assess responses of plant cell wall ultrastructure to genetic modifications [11], water stress [12], and microbial enzymes [13,14]. There is an increasing number of reports employing glycome profiling on plant cell walls to be used in diverse and multi-faceted applications [5,8]. Although the utilization of mAbs in glycome profiling is one of the best tools available for analyzing the structure of cell wall components, we argue that the complete value of glycome profiling has not yet been fully realized due to inadequate approaches to data analysis.

The contemporary approach to drawing inferences from glycome profiling of plant cell walls relies primarily on qualitative analyses based on the strength of the antibody binding which is determined by the intensity of the optical density of the response as quantified via an enzyme-linked immunosorbent assay (ELISA), and is displayed in a heatmap where the optical density binding strength, is a function of color [9,15]. The interpretation of colored

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heatmaps relies on subjective interpretation and usually lacks statistical inference. For example, large differences in composition and extractability of glycan components of cell walls between different species is typically apparent in heatmaps [5,10]. However, subtle differences are difficult to detect, particularly when results are focused on one species exposed to different environmental conditions that might impact cell wall ultrastructure [12]. Even more fundamental, the presentation and qualitative comparison of cell wall components derived from glycome profiling presents challenges when comparing across studies because the antibody strength data are not corrected based on the quantity of carbohydrate materials recovered in cell wall extracts. Rather than adjusting these values, reports simply state the quantity recovered and draw inference regarding comparisons from uncorrected values [10,16–18]. Thus, a comparison of an individual cell wall component could qualitatively appear different, not due to a meaningful difference in the actual ultrastructure, but rather a difference in what was recovered via the extraction procedure. Likewise, biologically meaningful differences could go undetected due to the differences in the quantity recovered. Perhaps the most concerning shortcoming of the qualitative approach is that lack of quantitative values for different cell wall components precludes the direct comparison of cell wall characteristics among species and treatments across different studies. This ultimately limits the utility of these data from being compiled across studies to investigate patterns and values that could provide benchmark ranges for different species and organs.

The lack of a quantitative approach to analyze glycome profiles of plant cell walls to date is largely due to the complexity of the techniques and analyses. For example, the antibody suite is epitope, not polymer, specific. For a given polymer, there are multiple mAbs detecting a diverse range of structural epitopes. Moreover, the mAbs themselves are diverse and may not necessarily be integrated into a neatly organized system to fully explain each polymer. The analytical technique, which requires cell wall preparation, extractions, dialysis, and sugar quantification, necessitates a significant time investment. Some cell wall materials can be readily extracted with the weak treatments, whereas others require strong base treatments before significant quantities of carbohydrate materials are obtained, so each extraction yields a different amount of recovered material. Finally, the method itself is expensive and very few laboratories are equipped to perform this analysis which has not expedited the development of a more quantitative approach.

To better understand and utilize glycome profiles of plant cell walls, a more quantitative methodology is needed. Because glycome profiles of plant cell walls often employ over 150 mAbs across six extractions, each experiment results in hundreds of reactions per sample and thousands of reactions per experiment. Therefore, flexible analytical tools increasingly need to be developed and employed to solve complex problems. In this study, we offer a quantitative approach to compare cell wall ultrastructure across species, treatments, and perhaps most importantly, across multiple studies. This framework, developed in open source R statistical software [19], will allow for specific epitopes to have published ranges, expected means values, and the level of variation between sample replicates available. Our objective is to promote a more rigorous framework to analyze and interpret data derived from glycome profiling of the plant cell wall. We focus primarily on correcting data according to the quantity of carbohydrate materials recovered by the sequential extracts and then subject these data to contemporary frequentist statistical approaches to test *a priori* hypotheses and determine differences between and among levels of experimental factors. To this end, we utilize a previously published dataset that was originally collected to examine the potential influence of moisture stress on cell wall ultrastructure of loblolly pine (*Pinus taeda* L.) organs [12] and to apply our suggested quantitative approach to determine if the inference derived from the initial qualitative analysis is mirrored through a more robust quantitative approach.

2. Method

2.1. Experimental design

To demonstrate the utility of a quantitative approach for assessing data derived from glycome profiling of plant cell walls, we utilize our published dataset [12] to determine if the inference derived from the initial qualitative analysis is maintained through a more robust quantitative approach. Briefly, the original study was conducted to investigate how an environmental stress (specifically, moisture stress) that impacts plant physiology and ultimately, growth, might influence cell wall ultrastructure of loblolly pine root and stem wood tissue. By employing the contemporary quantitative approach with glycome profiling values that were not corrected for the quantity recovered, we concluded that cell wall ultrastructure was influenced by moisture availability and that cell walls in the stem were more responsive than those in the root.

For the study, 114 saplings of a two-year-old loblolly pine (*Pinus taeda* L.) clone provided by ArborGen, Inc., Summerville, SC, USA, were planted in 4.3 L tree pots in a mixture of fritted clay and fine sand (3:1, v:v) in late March 2013. Saplings were grown in a greenhouse under air temperature that approximately matched ambient (Athens, GA, USA) for ~11 weeks prior to initiating soil moisture treatments. The saplings were randomly assigned to either a high (−0.3 MPa) or low (−1.5 MPa) soil moisture content, arranged in three blocks (n = 3) that consisted of one replicate plot of each treatment, with 19 saplings per plot. Following the 12-week period from June 15 to September 7, 2013, the saplings were harvested, partitioned into stems, roots, and needles, and dried to a constant mass.

2.2. Glycome profiling

The glycome profiles used in this study were generated in Pattathil, Ingwers, Victoriano, Kandemkavil, McGuire, Teskey and Aubrey [12] using the methods described in Pattathil, Avci, Miller and Hahn [9] and DeMartini, Pattathil, Avci, Szekalski, Mazumder, Hahn and Wyman [15]. Briefly, alcohol insoluble residues (AIR) were prepared from ground tissue. The resulting alcohol insoluble residues were subjected to a series of sequential extractions, of increasing harsh reagents, in order, from oxalate to carbonate, to 1M KOH, to 4M KOH, to chlorite, and finally to 4M KOHPC extracts. The resulting extracts were screened with a comprehensive suite of mAbs [29] targeting most major non-cellulosic cell wall glycans. Binding strength of each antibody that coincided with epitope abundance was determined via ELISA where we measured the optical density (OD). Further information about each of the mAbs used in this study can be found in Supplemental Table S1.

2.3. Quantitative analyses

To correct for the quantity recovered for each antibody by extraction combination, we used the amount of gravimetric material recovered rather than glucose equivalent because gravimetric amount accurately accounts for the entire spectrum of non-cellulosic glycan components in a reasonably unbiased manner. Glucose equivalents do not accurately reflect the actual amount of glycan components that are predominantly enriched with other types of monosaccharides, especially pentose residues like xylose enriched xylan polysaccharides or arabinose enriched glycans. For each extraction, the quantity (mg) recovered per gram AIR (QR) was