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Potato native and wound periderms are differently affected by down-regulation of FHT, a suberin feruloyl transferase

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ABSTRACT

Potato native and wound healing periderms contain an external multilayered phellem tissue (potato skin) consisting of dead cells whose cell walls are impregnated with suberin polymers. The phellem provides physical and chemical barriers to tuber dehydration, heat transfer, and pathogenic infection. Previous RNAi-mediated gene silencing studies in native periderm have demonstrated a role for a feruloyl transferase (FHT) in suberin biosynthesis and revealed how its down-regulation affects both chemical composition and physiology. To complement these prior analyses and to investigate the impact of FHT deficiency in wound periderms, a bottom-up methodology has been used to analyze soluble tissue extracts and solid polymers concurrently. Multivariate statistical analysis of LC-MS and GC-MS data, augmented by solid-state NMR and thioacidolysis, yields two types of new insights: the chemical compounds responsible for contrasting metabolic profiles of native and wound periderms, and the impact of FHT deficiency in each of these plant tissues. In the current report, we confirm a role for FHT in developing wound periderm and highlight its distinctive features as compared to the corresponding native potato periderm.

1. Introduction

Potato (*Solanum tuberosum* L.) ranks as the fifth largest staple crop consumed worldwide, fulfilling essential needs in human nutrition and health (FAO, 2015). The complex dermal structure or periderm that covers plant tubers is critical to the quality, storage, and shelf life of potatoes. Its outermost layer, designated as the phellem, consists of several strata of dead cells with suberized walls. The phellem forms the potato cork skin and provides the first line of constitutive defense for the tuber against dehydration, heat transfer, and pathogen infection, including both physical and chemical barriers. The chemical barrier consists of soluble phenolic compounds such as hydroxycinnamic acids and their amide derivatives, flavonoids, and glycoalkaloids, some of which display beneficial antioxidant properties (Akyol et al., 2016). The physical barrier comprises the phellem cellulose cell wall in which the complex polyaliphatic and polyphenolic (lignin-like) suberin materials are deposited (Bernards, 2002).

Suberin is a fatty polyester that upon transesterification releases mainly soluble C_{16}–C_{28} ω-hydroxyacids and α,ω-dicarboxylic acids as well as fatty acids (alkanoic acids), primary alcohols (1-alkanols), glycerol, and very small amounts of ferulic acid (Graça and Santos, 2007). It is deposited at the internal side of the cell wall facing the plasma membrane, forming an insoluble matrix within which are embedded a complex mixture of extractable lipids, collectively

Abbreviations: **4CL**, 4-Hydroxycinnamic acid; **PHT**, Putrescine hydroxycinnamoyltransferase; **FHT**, Fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyltransferase; **THT**, Tyramine N-Hydroxycinnamoyltransferase; **CYP**, Cytochrome P450 monooxygenase; **FAR**, Alcohol-forming fatty acyl-CoA reductase; **G3P**, Glycerol-3-phosphate; **GPAT**, Glycerol 3-phosphate acyltransferase. * saturated and unsaturated carbon chains.

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called wax and substantially similar to the released suberin monomers found in potato (Schreiber et al., 2005). The more recalcitrant lignin-like portion of the polymeric material, composed principally of *p*-hydroxycinnamates (e.g., ferulate, *p*-coumarate, and sinapate) and their derivatives, is deposited within the polysaccharide primary cell wall (Lapierre et al., 1995). The aliphatic and the phenolic polymeric structures are spatially separated (Yan and Stark, 1998; Gil et al., 1997), but they have been proposed to be linked covalently through ferulate ester bonds (Graça and Santos, 2007).

When the potato skin is broken, the tuber reacts rapidly to restore the barriers by forming a new periderm (wound periderm) that achieves impermeability and chemical defense for the newly exposed fleshy tissues. The healing process proceeds in two stages (Sabba and Lulai, 2002). First, cells adjacent to the wound surface exhibit an increase in polyamines and deposit suberin polymers prior to cell death, forming a wound closing layer within 1–3 days post-wounding. Secondly, a new periderm is formed internally by cell division during the next 5–12 days, where the time course is modulated by the environmental and physiological conditions under which the healing process takes place (Dean and Kolattukudy, 1976; Lulai and Corsini, 1998). Native and wound periderms have similar anatomical structures and undergo analogous maturation processes until they acquire resistance to excoriation (Sabba and Lulai, 2002). However, comparing mature wound and native periderms, the wound periderm is two orders of magnitude more permeable to water. At 21 days post-wounding, its released suberin and wax content is only 50–60% that of native periderm, but its wax fraction is proportionally enriched in alkyl ferulates (Schreiber et al., 2005). Comparison of native mature periderm and 7-day immature wound periderms by solid-state ^{13}C nuclear magnetic resonance (ssNMR) reveals that at this early-stage wound periderm has an enhanced hydrophilic–hydrophobic balance, lignin-like polymeric structures that are more resistant to degradation, and more flexible aliphatic chains, suggestive of a remodeled supramolecular organization (Serra et al., 2014). Achieving a better metabolic understanding of the wound compared with native periderm has the potential to improve the outcomes of crop management.

A fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase), FHT, is the enzyme responsible for the formation of alkyl ferulates in potato periderm, whereby fatty ω-hydroxyacids and fatty alcohols are esterified to feruloyl moieties (Serra et al., 2010). Down-regulation of ferulate ester synthesis by *FHT*-RNAi silencing leads to a reduction in alkyl ferulates in both the hydrolyzable aliphatic suberin and the extractable wax from native periderms (Graça, 2015; Serra et al., 2010). That is, native FHT-deficient periderms yield much lower quantities of ferulic acid (72% reduction), C18:1 ω-hydroxyacids (76% reduction), and most primary alcohols by suberin transesterification compared with wild-type (WT) native skins. However, because FHT-deficient tubers experience a concomitant increase in the periderm thickness, the total amount of hydrolyzable suberin, measured as $\mu g\text{-}cm^{-2}$, remains virtually unchanged. As regards the embedded wax fraction, ferulate and alkanes are greatly reduced (72% and 70%, respectively), but the overall amount of wax compounds is doubled due mainly to increases in fatty acids and 1-alkanols. Moreover in *FHT*-RNAi native tubers, transpiration via the tuber skin is enhanced 14-fold and the skin takes on a very russeted and brittle appearance, but notably, the typical lamellated ultrastructure of the cell wall remains intact (Serra et al., 2010). Furthermore, the macromolecular organization and mechanical properties of the *FHT*-RNAi native periderm are compromised. Solid-state ^{13}C NMR analysis reveals abundant aromatic constituents that resist transesterification whereas the aliphatic chains exhibit changes in flexibility that can be linked to both resistance to deformation and mechanical resiliency (Serra et al., 2014). On the other hand, although an intriguing activation of the *FHT* promoter has been observed in WT wounded periderm (Boher et al., 2013; Lulai and Neubauer, 2014), it is not yet known how FHT deficiency impacts the chemical composition or supramolecular organization of the wound tissues.

To gain a deeper understanding of native and wound periderms and to investigate the impact of FHT in healing tissue, the current work compares these two tissue types from *FHT*-RNAi and WT tubers in parallel using immature periderms from freshly-harvested tubers and healing disks. A bottom-up metabolomic approach, combining metabolite profiling with ssNMR, is used to compare the soluble metabolites and the insoluble cell-wall embedded structural moieties of the respective native and wound periderms. To add molecular insights for the lignin-like polymeric materials in suberized cell walls, the soluble phenolics obtained from a degradative thioacidolysis treatment are also compared. This 'holistic' analysis of native and wound-healing periderm both complements and augments the earlier investigations, yielding comprehensive and statistically robust information about the impact of knocking down the *FHT* gene in these protective plant tissues.

2. Results

Parenchyma-free phellem tissues, isolated by skinning freshly-harvested tubers and the wound tuber disks, were first extracted to obtain polar and non-polar soluble metabolites. The insoluble interfacial residue was then treated enzymatically to remove any unsuberized cell walls and remaining waxes before ssNMR analysis, as described in the Materials and Methods section. The following overview of our investigative strategy lays the groundwork for the specific findings presented in the subsequent sections.

Native and wound periderms contain diverse soluble metabolites of differing polarity, chemical class, and abundance. In this context, a broadly applicable biphasic extraction approach was used to concurrently separate the samples into soluble polar and non-polar extracts plus an insoluble solid suspension (Choi et al., 2004; Kim et al., 2010; Wolfram, 2006). For the soluble metabolites, multivariate statistics were used to conduct Principal Component Analysis (PCA) of liquid chromatography – mass spectrometry (LC-MS) and gas chromatography (GC-MS) data for the respective types of extracts. Then, to identify those metabolites that were unique or had notably increased or decreased relative abundance in a particular sample (potential biomarkers), an Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA (Worley and Powers, 2013),) was performed. By displaying the covariance and correlation from the OPLS-DA model as a scatter plot (S-plot), we visualized both the magnitude of the enhanced abundance (P [1]) and the reliability of the effect (P (corr)[1]). Points in the S-plot 'wings' were then linked to the corresponding chromatographic retention times, *m/z* values, and particular chemical compounds by comparison with reference databases or published MS data including molecular ions and fragmentation patterns (Wiklund, 2008). The insoluble solid suspension containing the interfacial residue was analyzed to deduce types and relative numbers of the major carbon-containing moieties by high-resolution ^{13}C ssNMR as well as examination of the ether-linked polyphenolic thioacidolysis breakdown products. First, we compared *FHT*-RNAi with WT for native and wound periderms (14 days post-wounding) with respect to their polar and non-polar soluble metabolites and the corresponding interfacial residues. Then, we compared native with wound periderm in each of the WT and *FHT*-RNAi tubers. Finally, to better understand the formation of the wound periderm, we analyzed extracts and solid suspensions