Postharvest Biology and Technology 90 (2014) 24–33

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Wound-induced suberization genes are differentially expressed, spatially and temporally, during closing layer and wound periderm formation

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ARTICLE INFO

Article history:
Received 6 June 2013
Accepted 30 November 2013

Keywords:
Closing layer
Potato tuber
Solanum tuberosum L.
Suberization
Wound-heal
Wound periderm

ABSTRACT

Potato tuber (*Solanum tuberosum* L.) wounds incurred at harvest and upon seed cutting require rapid suberization as a major part of the healing process to prevent infection and desiccation. However, little is known about the induction and expression of genes that are essential for these processes and in particular to the two major stages of wound-induced suberization, i.e. closing layer formation and wound periderm formation. The objectives of this research were to address these needs by determining the effects of wounding on the induction and expression profiles of specific genes involved in wound-induced suberization in potato tuber (*S. tuberosum* L.) during the initiation and completion of closing layer formation and wound periderm formation. Although both stages critically involve suberization, there are significant differences between the two processes. Closing layer development requires rapid suberization of existing parenchyma cells bordering the wound surface to provide the initial protective barrier for the wound. Wound periderm development occurs later, i.e. after completion of closing layer formation, and requires development of a wound phellogen layer which mediates the formation of highly organized files of suberized wound-phellem cells that provide a more durable protective barrier for the tuber. The processes delineating these two separate stages of wound-induced suberization are poorly understood. This research shows that, unlike some wound responding genes such as phenylalanine ammonia lyase (*StPAL-1*) and anionic peroxidase (*StPrx*), certain genes that are specifically involved in both of these processes do not remain uniformly up-regulated during the two stages of healing (i.e. *StTHT* encoding Hydroxycinnamoyl-CoA:tyramine N-(hydroxycinnamoyl)transferase, *StFHT* encoding a fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase, *StKCS6* encoding a 3-ketoacyl-CoA synthase, *StFAOH* encoding a fatty acid ω-hydroxylase and *StGPAT5* encoding a protein with acyl-CoA:glycerol-3-phosphate acyltransferase). Instead, they are up-regulated during closing layer formation; i.e. starting by ca. 1 d after wounding, but then slightly down-regulated or pause near completion of the closing layer (ca. 5–6 d) and then again up-regulated as wound periderm development is fully initiated (ca. 7 d) and down-regulated near completion (ca. 28 d after wounding). This differential in the expression profile, i.e. decrease between stages, was not anticipated and may be the first demonstration of measurable changes of any sort of biological flux as wound induced suberization transitions from closing layer to wound periderm development. Results were repeated using minitubers from two different crop years and demonstrate that these processes are separate, but coupled in some yet to be determined fashion. The biology of this differential expression is important because of the roles closing layer and wound periderm development play in protecting the tuber from disease and other challenges.

1. Introduction

Potato tuber (*Solanum tuberosum* L.) wounding at harvest and seed cutting induces critical suberization processes essential to post-injury survival (Lulai, 2007). These processes are active during the two different stages of wound-induced suberization; closing layer formation and wound periderm formation. Although these suberization processes constitute the major part of wound healing to the damaged area and provide an essential barrier to infection, the two stages of suberization are generally not distinguished in most wound-healing studies. Little is known about the biological integration of these two stages and the expression profiles of suberization genes induced during initiation and completion of closing layer formation versus that of wound periderm formation. The goal

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0925-5214/$ – see front matter. © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.postharvbio.2013.11.010

of this research was to develop and integrate details of wound-induced closing layer formation and wound periderm formation with the determined expression profiles of genes involved in suberization of the respective cell walls; all are of critical importance in blocking infection, defect development and desiccation in stored potatoes.

Wounding releases a cascade of temporal protective responses that help retard infection until the initial suberized barrier is constructed within the closing layer (Lulai and Corsini, 1998; Lulai, 2007). However, these temporal protective responses lack the robustness for broad and durable resistance to infection that is provided by a competent suberized barrier. Closing layer formation is also known as primary suberization and involves suberization of existing parenchyma cells neighboring the wound (Lulai, 2007). Wounding induces de novo synthesis of phenylalanine ammonia lyase (PAL) from a large family of related genes in potato (Joos and Hahlbrock, 1992). PAL catalyzes the first committed step in the phenylpropanoid pathway, producing trans-cinnamic acid which flows through a proposed route of biosynthesis forming precursors for assembly into suberin polyphenolic(s) (SPP) (Bernards, 2002). The SPP are assembled in a programed fashion into the walls of the parenchyma cells near the wound. As SPP accumulation is completed around the perimeter of these existing cells, suberin polyaliphatic(s) (SPA) precursors are synthesized by elongation, hydroxylation and omega oxidation of fatty acids, some of which are acylated to ferulic acid. These precursors are networked together via specific crosslinking or coupling mechanisms into a distinctly separate SPA domain (SPAD) which is biologically laminated onto the SPP domain (SPPD) (Lulai and Morgan, 1992; Lulai and Corsini, 1998; Bernards, 2002). Upon completion of the SPAD on the outer 1 or 2 parenchyma cell layers, closing layer formation concludes. At this point wound periderm formation, also known as secondary suberization, initiates from a newly created meristematic layer of mother cells, i.e. the wound phellogen/cork cambium, which gives rise to several layers of suberized phellem cells located under the closing layer, as well as generating a limited number of phelloderm cells located beneath the phellogen. The coupling of these two stages has not been studied within a framework that distinguishes the associated processes as physically separate while comparing the expression of wound-induced genes required for suberization in both stages. This distinction could be unique because suberization occurs sequentially on the walls of these different cell types, i.e. existing parenchyma vs. progenitor derived phellem cells during development of closing layer vs. wound periderm formation (Lulai, 2007). The suberization of different cell types in two different stages raises important questions. Do the same suberization genes act on both cell types? Are genes from large families that redundantly encode well-known enzymes involved in wound-healing and suberin biosynthesis, such as phenylalanine ammonia lyase (*StPAL-1*) and anionic peroxidase (*StPrx*), expressed uniformly throughout both stages? Do other genes that have been shown to be uniquely specific to suberin biosynthesis up-regulate throughout closing layer and wound periderm development; i.e. are there differences in expression when suberin biosynthesis occurs in parenchyma cells vs. phellogen progenitor cells? The answers to these questions are important as we strive to develop technologies to hasten suberization, particularly that of the closing layer, to reduce the huge losses from associated wound related infections, related physiological disorders such as the pink eye syndrome, and non-wound induced suberization associated with some slow advancing pathogens such as *Verticillium dahlia* (Brook, 1996; Lulai and Corsini, 1998; Lulai, 2001, 2005; Lulai et al., 2006).

A few genes have been identified that are specific to SPP and SPA biosynthesis as well as polymeric bridging to link the two suberin domains. Determination of the wound-induced expression profile of such genes during closing layer and wound periderm formation will help in answering the above questions. *StTHT* encodes Hydroxycinnamoyl-CoA:tyramine N-(hydroxycinnamoyl)transferase which catalyzes the formation of hydroxycinnamoyltyramines (Negrel et al., 1995; Schmidt et al., 1999); this enzyme is postulated to play an important role in channeling phenolics into SPP (Bernards, 2002). *StFHT* encodes a fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase which conjugates ferulic acid with ω-hydroxyacids and fatty alcohols (Gou et al., 2009; Molina et al., 2009; Serra et al., 2010; Boher et al., 2013). Through knockout and reverse genetic approaches, this gene has been shown to be involved in ferulate incorporation into suberin, control of water vapor loss and some aspects of suberin anatomy within potato tuber native periderm (Serra et al., 2010). *StKCS6* encodes a 3-ketoacyl-CoA synthase in potato tuber native periderm and through a reverse genetic approach has been shown to be involved in the formation of suberin aliphatics and waxes with chain lengths ≥ C28; this is supported by corresponding increases in water vapor loss and compositional changes in suberin "very-long-chain fatty acids" and waxes (Serra et al., 2009a). Serra et al. (2009b) isolated the coding sequence referred to as CYP86A33 from potato periderm and showed that it encodes a fatty acid ω-hydroxylase (*StFAOH*), a critical enzyme in suberin aliphatic biosynthesis in potato tuber native periderm. Application of RNAi techniques to this gene resulted in native periderm with decreased suberin aliphatics including decreased ω-hydroxyacids and α,ω-diacids as well as changes in suberin ultrastructure and increased periderm permeability (Serra et al., 2009b). *StGPAT5* encodes a protein with acyl-CoA:glycerol-3-phosphate acyltransferase activity which catalyzes critical glycerol based bridging between SPPD and SPAD. GPAT5 mutants of *Arabidopsis thaliana* had decreased aliphatic suberin loads in roots and seed coats accompanied by increased seed coat permeability, "but no changes in membrane or storage glycerolipids or surface waxes" indicating specificity to suberin biosynthesis (Beisson et al., 2007).

We have determined quantitative expression profiles of these genes within a detailed tuber wound-healing time course that documents initiation and completion of closing layer formation and wound periderm formation. Results reveal the differential association of these suberization specific genes within parenchyma and phellogen cells, including closely associated nascent progenitor derived phellem cells, during initiation and completion of closing layer and wound periderm formation.

2. Materials and methods

2.1. Plant material, storage conditions and wound model system

Pre-nuclear certified seed mini-tubers (18–23 g tuber^{-1}), cv. Russet Burbank (*S. tuberosum* L.), provided by Valley Tissue Culture, Halstad, MN, USA, were used in this research. All experiments were repeated using non-dormant mini-tubers obtained from greenhouse crops grown in different years. Results from these crops were qualitatively similar. Representative results from the two crops are presented. All analyses within an experiment were conducted using three or more biological replicates; each replicate was analyzed in triplicate. Unless indicated otherwise, the data presented represents the mean, plus or minus the standard error (±SE), obtained from the analysis of three or more biological replicates.

After harvest from the greenhouses, the mini-tubers were held for 14 d at 20 °C and 95% relative humidity (RH) to facilitate suberization/curing. Following the postharvest suberization/curing period, the tubers were stored in a controlled environment chamber in the dark at 3 °C and 95% RH to inhibit deterioration and sprouting. Three days before use, the tubers were gently hand