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DEFINING THE SUSCEPTIBILITY TO KERNEL COLOR DARKENING

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PROJECT OBJECTIVES:

1. To understand varietal differences in the accumulation of precursors to the brown pigment formed during walnut pellicle darkening (PD):
2. Determine the developmental and varietal differences in PD pigment precursor content (through targeted metabolomics).
3. Establish a simple, efficient, and easy to detect assay for PD pigment precursor content and darkening potential.
4. Characterize the varietal differences in composition of PD pigments.

BACKGROUND

Darkening of the walnut pellicle (syn. seed coat), which occurs both pre- and post-harvest, presents one of the major challenges to the walnut industry due to a strong consumer preference for light colored kernels. While modern varieties have been bred to produce fewer darkened kernels and light kernels that retain their color well during storage, a general decline in kernel visual quality has been noted in recent years. Increasingly harsh and unpredictable environmental conditions during production paired with lengthening storage times for kernels (due to increasing annual production) are likely responsible for this problem. Addressing this problem of pellicle darkening (PD) requires an understanding of what is occurring at the molecular level, but there are gaps in our knowledge concerning the biochemistry at play and the genetic elements that regulate those processes. To address these gaps, we initiated an investigation of these underlying mechanisms to understand them and to define potential therapeutic targets for further research and eventual development of remedial strategies for the walnut industry.

KEY FINDINGS

In order to determine the underlying processes of PD, we isolated pellicles from light and dark ‘Vina’ kernels and biochemically profiled (“fingerprinted”) them. Comparison of these groups revealed the molecular hallmarks of increased cellular stress and reduced free flavanols (a group of phenolic

compounds) coinciding with PD. These findings, within the greater context of PD and plant pigmentation, suggested the brown pigment was a condensed tannin (CT), large polymers of the flavanols that are brown in color. Preliminary analysis of this pigment extracted from darkened pellicle, along with other converging lines of evidence, indicates the hallmarks of a CT polymer composed of multiple different kinds of flavanols. Biochemically, CT polymers are produced when flavanols react with each other, becoming irreversibly connected to one another as if they had been superglued together. This “gluing” process uses up the available flavanols while yielding the polymer pigment, explaining our biochemical results. Importantly, this same process is instigated by the molecular signals for stress, which are reactive forms of oxygen, providing an explanation why PD incidence increases when growing in adverse field conditions and why modified atmospheric packaging helps preserve color during storage by limiting oxygen. Our current view is that PD is like starting a fire. Fuel, the flavanols, and a match, the stress signals, are needed to start the fire, with the amount of fuel likely changing how much fire you can potentially have. In favorable conditions (i.e., no match), no fuel is spent and so no fire is made, regardless of the amount of fuel available. But under unfavorable conditions (i.e., a match), the fuel is spent to make the fire, and the amount of fire may be different based on the amount of fuel. This framework begs the question: are varieties with lower PD incidence simply deficient in flavanol fuel? And if so, how are they genetically hardwired to achieve this? A re-analysis of pellicle data obtained in a previous CWB funded study, while limited in its scope, did indeed reveal significant varietal differences in the accumulation of flavanols during pellicle developmental maturation: with the PD-sensitive ‘Vina’ having higher free flavanols than the more PD-resistant ‘Chandler’. With this clarified view of PD in relation to the flavanols, we biochemically profiled the developing pellicle in five cultivars varying in darkening tendency and color retention. Comparative analysis of these biochemical “fingerprints” will definitively reveal whether flavanol content is variable across walnut varieties, correlates with PD tendency, and is a suitable biomarker for PD sensitivity for use in treatment development and varietal improvement. By identifying the hallmarks of proneness to PD, the groundwork is laid for novel solutions for the industry. These include screening methods for predicting kernel PD sensitivity, production-centered treatments, like suppressing flavanol production or reducing the instigating effects of environmental stress, as well as storage treatments for minimizing the effects of oxygen on the pre-made pellicle flavanols. From a genetics perspective, linking PD sensitivity to the genes controlling flavanol accumulation makes for a useful breeding marker, in addition to enabling precision bioengineering approaches for “fixing” existing industry-favored varieties.