Almond Bud Failure Genetic Disorder

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Project Summary/Abstract

Briefly describe the long-term objectives for achieving the stated goals of the project.

Almond Bud-Failure (BF) is a genetic aging disorder unique to California almond production practices, where a gene essential for normal vegetative bud development fails. The disorder does not appear to be caused by a change in the controlling DNA sequence (i.e. genetic mutation) but rather by epigenetic mechanisms which irreversibly switch a required gene off [6]. The epigenetic rather than genetic (i.e. DNA) basis makes it difficult to study using modern genomic techniques such as DNA sequencing and genetic fingerprinting. Based on the Kester BF paradormancy model [6, 9, 10], BF has been managed through the selection of low-BF foundation propagation-stock followed by appropriate foundation-stock maintenance and vegetative progeny monitoring. Low-BF foundation stock originates either directly from the initial seedling tree from which the variety was introduced or, if no longer available, from basal epicormic buds from trees originally planted near the time of initial variety release. This is because the genetic aging disorder increases irreversibly with the chronological advancements associated with normal tree growth or normal successive nursery propagations [6, 9]. Because epicormic meristems, a type of nascent and poorly understood plant meristem, can remain inactive for decades, 'pushing' bud development and growth from old epicormic meristems can recover propagation wood expressing the BF-age level at the time of meristem establishment. For example, virtually all of the ~40,000,000 Nonpareil trees currently in commercial production [1] are derived from epicormic shoots pushed from ~100 year old trees (McEnespy, Jeffries, IR2, etc. sources) [4]. While effective, this strategy is tedious and reactive, requiring multiyear vegetative-progeny field-testing to identify the BF-potential of each source-clone used in propagation. A more direct proactive diagnosis would utilize molecular strategies to characterize epigenetic status as well as the mechanism or mode-of-action of BF-aging, so that the appropriate pathway and candidate gene can be effectively targeted. While epigenetic-molecular diagnostics are becoming available [5], the mechanism for BF-development remains unknown. This research proposes to pursue the causal mechanism through studies of the two defining BFdevelopment processes: the BF-expressed failure of axillary meristems, and the suppressed BF-aging
of epicormic meristems.

Scope of Work

Describe the goals and specific objectives of the proposed project and summarize the expected outcomes. If applicable, describe the overall strategy, methodology, and analyses to be used. Include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate. Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the goals and objectives.

This is an ongoing project from a prior year Agreement. The work that will be completed is within the timeframe of July 1, 2020-June 30, 2021:

A. Test the Kester-model for BF expression in California almonds. We will evaluate the level of almond summer vegetative bud dormancy by topping or decapitating 20 proleptic shoots every two weeks from June 1 to August 30. This will be done on Nonpareil trees in Winters, California grown with standard commercial irrigation as well as on Nonpareil trees grown under traditional rain-fed conditions without irrigation. In October 2020, we will evaluate all treated shoots and record proportion of shoots in each test group where axillary buds begin to push or develop into new shoots. The pushing of such axillary shoots will then be used as an indicator of non-dormant status, while non-pushing axillary shoots will be taken as an indicator of dormant shoots and meristems. We will then plot the data over time and compare development trends with those expected from the Kester BF model and use appropriate statistical methods to test whether results support or reject the Kester BF model.

B. Characterize the origin, structure, and development pattern of axillary and epicormic meristems. Origin and vascular structure for both epicormic and axillary buds will be determined through whole tissue sectioning of dormant as well as newly emerging buds, using biological stains if necessary, to determine whether the vascular channels feeding those buds originate in the outer bark cambium or inner pith. Results will be documented using standard photographic as well as photo microscopy methods. Meristem structure of both epicormic and axillary buds at full vegetative dormancy during the first week of January 2021 will be further characterized by the number and development state of Nonpareil leaf primordia larger than 1 mm as determined with microscopic inspection and documented by photo microscopy. Appropriate statistical methods will be utilized to determine whether significant differences exist between these two types of meristems at full winter dormancy. Finally, the development pattern of almond vegetative meristems during winter dormancy will be determined and mapped by dissecting vegetative buds at biweekly intervals starting on October 1, 2020 and finishing with vegetative bud-break and shoot pushing at about February 15, 2021. At least 10 buds each, for the varieties Nonpareil and adjacent plantings of Monterey will be tested at each time interval. Results will be plotted as a developmental curve and appropriate statistical methods will be utilized to determine whether development patterns for Nonpareil differ significantly from those for Monterey. A nonsignificant development pattern between these distinctly different varieties would be interpreted as supportive the hypothesis that the number of leaf primordia at different fall/winter calendar dates within dormant but still slowly developing almond vegetative buds could be used as general indicators of stage of bud development throughout the winter dormant period.

Project's Benefit to Nursery Industry

Clone deterioration is an inevitable consequence of propagation (including micropropagation) and aging [15]. Because BF is a serious problem for almond in California, it remains the best characterized of this vague classification of 'genetic disorders' [8]. An improved understanding of the almond BF disorder should similarly improve the ability to recognize and characterize clone deterioration in other California nursery crops.

An improved understanding of mechanism for almond BF would have immediate benefits for almond nursery practices. For example, the suppression of further aging in current low-BF FPS foundation stock is pursued through rigorous hedging of foundation trees [4]. Ideally, such hedging should target basal epicormic shoots but inevitably, also pushes lower (hidden) axillary buds that contribute to continued chronological aging. An improved ability to discriminate and selectively target epicormic rather than axillary buds would thus contribute to improved foundation stock stability.

The development of BF diagnostics utilizing emerging molecular epigenetic tools would require an understanding of BF mode-of-action in order to effectively target candidate genes and developmental pathways. The development of such diagnostics would allow the nursery industry to proactively characterize potential for BF development in new varieties or propagation sources, including the identification of propagation sources currently free from BF-symptoms but with a high probability of eventual expression in commercial trees propagated from that source.

In the longer term, (possibly as short as 10 years), the understanding of the BF mode-of action would greatly facilitate the identification of the affected gene. CRISPR-based BF remediation methods could theoretically then be developed that could rehabilitate varieties such as Carmel whose BF-potential at the time of variety release were already too high to make low-BF source clone identification and management completely successful [11].

Objectives:

A. Test the Kester-model for BF expression in California almonds.

B. Characterize the origin, structure and development pattern of epicormic meristems.

Workplans and Methods (with Literature Review):
The research being proposed is preliminary and exploratory in nature and so at relatively low budget. Its intent is to establish basic proofs of concept: (a) that the seasonal differences in axillary bud activity predicted by the Kester-model exist and can be measured, and (b) that epicormic meristems can be distinguished and differentiated from axillary meristems in tissue of origin, basic structure and seasonal development patterns. Graduate students as part of their research training, will carry out some of the 1st year studies. If no significant opportunities are identified in year 1, the project will be terminated or modified. If promising results are obtained in year 1 a more detailed and comprehensive analysis may be proposed for year 2.

A. Test the Kester-model for BF expression in California almonds.
A characteristic distinguishing BF from other non-genetic bud failures is the collapse of affected vegetative buds when entering normal winter-dormancy during the previous fall [6, 8]. According to the Kester BF model [9], the apparent cause of this failure is abnormal development during the previous summer growing season [10]. In its native habitat, almond typically goes into a type of moderate summer dormancy (paradormancy) to avoid midsummer heat and drought [7]. Under the California management system, exceptionally high yields are achieved by pushing tree growth throughout the summer by propagating onto vigorous rootstocks and supplying additional fertilizer and water. (This explains why BF was not previously observed in European low-input/low-return almond management practices. When grown under high-input California systems, even traditional varieties such as Marcona can show BF). Prof. Kester demonstrated this 'summer-dormancy' affect in dryland almonds by showing that axillary shoot buds from standard California orchards will tend to push throughout the summer if excised and so freed from apical dominance, while similar shoots from dryland almonds will show suppressed bud-pushing, typically starting in July [9, 10]. Kester's study was originally done with excised axillary buds placed in agar under semi-sterile conditions that confounded accurate data analysis because of mold contamination and desiccation. In this study, we will examined excised shoots propagated essentially as softwood-cuttings (but with the terminal buds cut back to remove apical dominance) and grown in a growth-chamber on artificial media with fungicide applications when necessary.

The 1st year of the study will be to optimize growth-chamber conditions and to test for differences in summer-paradormancy (i.e. differences in levels of axillary bud suppression after release from apical dominance) for Nonpareil when grown under high-input versus dryland conditions. (An 80-year-old dryland Nonpareil/Mission orchard in Winters, California will be used for dryland sample collection). The expected result would be a significant suppression of axillary bud-push in dryland compared to the high-input conditions. If confirmed, results would help narrow the candidate modes-of-action for BF by a) identifying possible (summer paradormancy) pathways involved, b) extrapolating mechanisms and controlling genes from other plant systems such as Arabidopsis where this type of paradormancy is more comprehensively studied, and c) provide currently available molecular analyses such as RNA-seq with a more precisely targeted developmental time for comparing high-BF with low-BF sources in order to identify the critical gene being affected.

The validation of the 'summer-dormancy' aspect of the Kester-model for BF would also provide a tool for a real time comparison of high-BF versus low-BF source-clones (Nonpareil, Carmel, etc.) to characterize both developmental/growth differences as well as possible molecular (RNA, methylation, etc.) differences which might provide a starting point for developing real-time BF diagnostics.

B. Characterize the origin, structure and development pattern of epicormic meristems.
In nature, epicormic shoots are induced from nascent, poorly defined meristems after severe damage such as major limb loss in a windstorm, etc. In horticulture, these are the shoots typically induced by early heading-cuts during orchard tree-structure establishment. Unlike shoot axillary buds that contain a preset number of preformed leaf primordia resulting in a standard, constrained pattern of subsequent shoot expansion/growth, epicormic shoots have relatively few preformed primordia and no growth constraints (in terms of preformed versus neo-formed growth) and will continue growth throughout the growing season as long as adequate water and fertilizer are provided [13]. Epicormic shoots pushed from basal buds of ~ 100-year-old Nonpareil trees are also the source of the all low-BF Nonpareil source-clones at Foundation Plant Services (FPS) [4]. Despite their economic importance to almond and other orchard crops, virtually nothing is known of their origin, basic structure, and development. My field observations suggest that epicormic meristems are somewhat like those in oaks, which have been more carefully studied because of their effect on propagation and lumber quality [2, 3, 12]. Both appear to originate deep in the wood towards the original pith (see figure 1). Both appear composed of traces or rays of meristematic tissue within the developing hardwood, forming transient rudimentary structured meristems towards the fall that rapidly degenerate if not induced into active shoot development through limb breakage, heading cuts, etc. (see fig. 2). Some of John Preece's earlier research showed that epicormic shoots in oak also show an aging/position affect in their ability to develop adventitious roots [2, 14]. However, they also seem to differ in several aspects. For example, the next generation of oak epicormic traces appear to originate from vascular traces feeding the bud-scales of the previous season's rudimentary bud, resulting in the whorled, burl appearance of oak [3, 12]. New traces in almond appear to originate parallel to the old trace from unknown origins resulting in a lateralization of new traces (see figure 2).

Figure 1. Interior structure of an oak log showing epicormic structures. Structures are identified as: a, knot of a sequential or regular branch; b, primary living epicormic bud with only minor trace expansion signifying that it has not sprouted; c, sprouted epicormic buds showing distinct bud traces and expansion of the bud trace at the time of sprouting as well as development across the cambium; d, primary epicormic sprout that sprouted 7 years after initiation and subsequently died. [from 12].

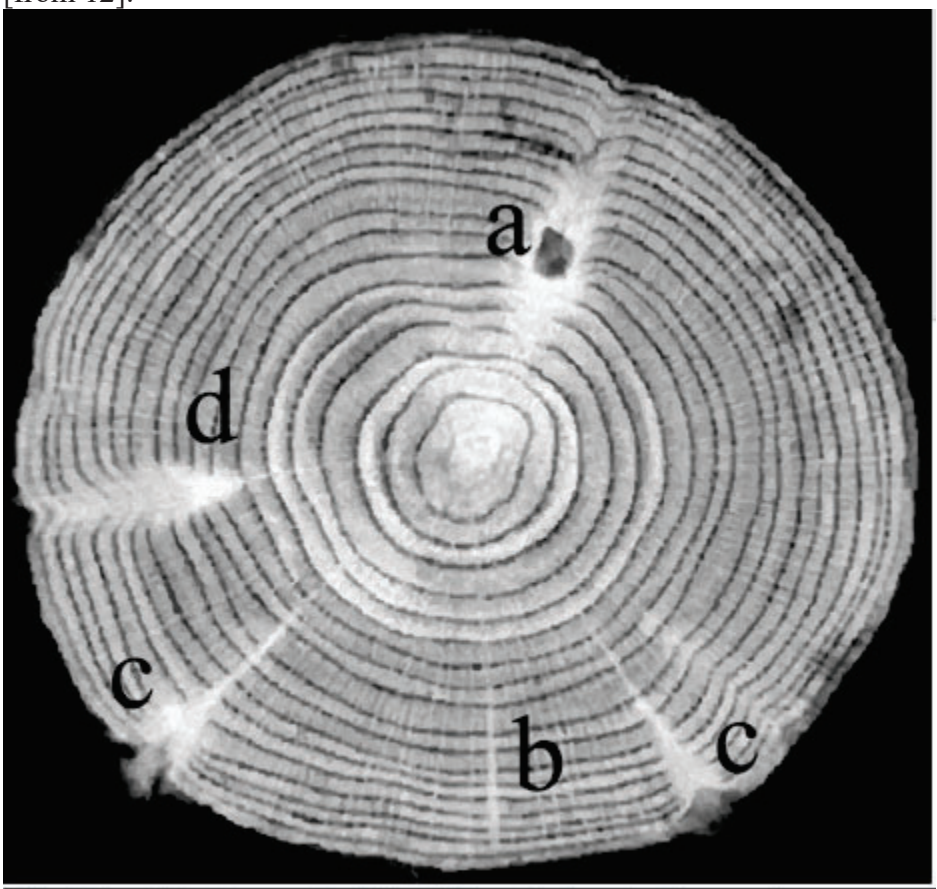

Figure 2. Previous season, degenerated epicormic bud
(center) bracketed by 2 current season epicormic buds on 4 year old Nonpareil almond wood.

This study will pursue a basic characterization of epicormic meristem development in almond using methods previously developed in oak [12]. The goals are: a) to characterize multiyear epicormic trace development by following patterns in branch cross-sections, (as in figure 1), as well as b) a seasonal characterization of external bud formation /development/degeneration (as in figure 2), identifying the general timing of bud initiation, duration of viability and subsequent timing of sprouting and/or degeneration.

A more accurate discrimination of epicormic versus axillary shoot growth (which can look very similar following aggressive pruning cuts) could then be used to develop recommendations for more effective FPS pruning/hedging of low-BF source clones to suppress BF-aging (i.e. encourage epicormic versus axillary shoot development).

An understanding of the origin and development of epicormic meristems/shoots is also necessary for appropriate tissue sampling for molecular analysis [5]. Leaves are currently the source-tissue of choice for molecular testing because they are readily accessible and methods for DNA/RNA, etc. extraction are well developed. However, leaves are problematic for two reasons. Leaves contain highly specialized tissue and this specialization is often programmed through normal epigenetic (methylation/demethylation its etc.) processes to fine-tune gene action. Consequently, a lot of the observed methylation and related epigenetic changes are representative of normal leaf development/differentiation, making it more difficult to distinguish them from BF-age related
changes. The 2nd and more basic problem is that leaves would probably not be the tissue where clonal-aging is logged within the individual tree or clone. Because the age-related differences can be most clearly traced back to (associated with) specific epigenetic origins (position on tree), the tissue (vascular ray, etc.) most closely associated with these meristem traces might be a more promising target for molecular analysis [6] searching for differential expression (high-BF vs. Low-BF) in candidate genes.

Finally, the nascent nature of epicormic meristems may make them more amenable than traditional strategies currently being pursued for CRISPR-type genetic remediation targeting BF rehabilitation (i.e. molecularly repairing the critical epigenetic switch), including the subsequent whole-plant regeneration required for commercialization.