AGENDA

California Avocado Commission
Production Research Committee Meeting

Meeting Information

Date: Tuesday, July 28, 2026
Time: 8:00 a.m.
Location: Hybrid Meeting

Physical Meeting Location:
Hilton Garden Inn Irvine/Orange County
2381 Morse Ave.
Irvine, CA, 92614

Web Conference URL:
https://californiaavocado.zoom.us/j/5375836823?omn=84993244142

Conference Call Number: (507) 473 4847
Meeting ID: 537 583 6823
Passcode: 348652

Meeting materials will be posted online at least 24 hours prior to the meeting at:
https://www.californiaavocadogrowers.com/commission/industry-calendar

Committee Member Attendance

As of Tuesday, July 21, 2026, the following individuals have advised the Commission they will participate in this meeting:

Danny Klittich, PRC Chair
Jason Cole
Jim Davis
Herman Els
Consuelo Fernandez
Leo McGuire
Daryn Miller

Time	Item
8:00 a.m.	**1. Call to Order** a. Roll Call/Quorum
	2. Opportunity for Public Comment Persons may address the Committee on subjects within the jurisdiction of the Committee.
	3. Approval of Production Research Committee Meeting Minutes of May 28, 2026
	4. Research Program Consultant's Report
	5. Consider research proposals to develop genetic testing to distinguish 'Hass' from 'Carmen/Mendez' a. Hass avocado variety authentication: A genetic diagnostic for supply chain integrity – Dr. Edwin Solares, ESB AI Lab Corporation b. Determining specific genetic markers for 'Carmen Hass/Mendez No1' avocado – Dr. Iñaki Hormaza, La Mayora Institute of Subtropical and Mediterranean Horticulture c. Genomic Identification and Molecular Marker Development for the Unique Identification of 'Hass' and 'Carmen' Avocado – Dr. Luis Herrera-Estrella, Texas Tech University d. Discovery of Carmen Hass-specific genomic variants through whole-genome resequencing – Dr. Noëlani van den Berg, University of Pretoria
	6. Research Priorities and 2026 Call for Proposals
10:00 a.m.	**7. Adjourn Meeting**

Disclosures

All meetings of the Commission are open to the public and subject to the Bagley-Keene Open Meeting Act. All agenda items are subject to discussion and possible action.

For information or a request regarding disability-related modification or accommodation for the meeting, please contact April Aymami at 949-341-1955 via email at aaymami@avocado.org. Such requests should be made at least 48 hours prior to the meeting.

This meeting schedule notice and agenda is available on the internet at https://www.californiaavocadogrowers.com/commission/meeting-agendas-minutes and http://it.cdfa.ca.gov/igov/postings/detail.aspx?type=Notices. Contact Tim Spann at tim@spannag.com or 423-609-3451 if you have any questions.

Summary Definition of Conflict of Interest

Committee members are responsible for determining whether they have a conflict of interest.

A member has a conflict of interest in a decision of the Committee if it is reasonably foreseeable that the decision will have a material effect, financial or otherwise, on the member or an immediate family member that is distinguishable from its effect on all persons subject to the Committee's jurisdiction.

No Committee member shall make, or participate in making, any decision in which they know or should know they have a conflict of interest.

No Committee member shall, in any way, use their position to influence any decision in which they know or should know they have a conflict of interest.

CALIFORNIA AVOCADO COMMISSION
PRODUCTION RESEARCH COMMITTEE
MEETING MINUTES

May 28, 2026

A meeting of the Production Research Committee (PRC) of the California Avocado Commission (CAC) was held on Thursday, May 28, 2026, with the following people participating:

MEMBERS PARTICIPATING:
Danny Klittich, Chair
Victor Araiza
Allisen Carmichael
Jim Davis
Herman Els
Matthew Fatino
Consuelo Fernandez
Daryn Miller
Rachael Laenen (*ex officio*)

CAC STAFF PARTICIPATING:
Ken Melban
April Aymami

OFFICIALLY PARTICIPATING:
Dr. Tim Spann, Spann Ag Research & Consulting

GUESTS PARTICIPATING:
Devinder Sandhu, USDA Salinity Lab
Jorge Ferreira, USDA Salinity Lab
Nathan Lurie

ITEM #1 CALL TO ORDER

Danny Klittich, Production Research Committee Chair, called the meeting to order at 10:03 a.m. with a quorum present.

ITEM #2 OPPORTUNITY FOR PUBLIC COMMENT

There were no public comments.

ITEM #3 APPROVAL OF MINUTES OF FEBRUARY 19, 2026 PRODUCTION RESEARCH COMMITTEE MEETING

MOTION
To approve the minutes of the February 19, 2026, Production Research Committee meeting.

(Miller/Fernandez) MSC unanimous

Motion 26-5-28-1

ITEM #4 RESEARCH PROGRAM CONSULTANT'S REPORT

Dr. Spann provided the Committee with an update on the IR-4 pesticide research for control of the avocado lace bug. Afidopyropen for use on avocados is moving forward with three of the four necessary field trials completed and harvested fruit received by the analytical lab for residue analysis. IR-4 estimates that a registration submission will be sent to the EPA in October 2028 if the trials continue to go according to schedule.

Dr. Spann let the Committee know that CAC is working with a researcher in Australia to develop a research proposal to determine if the GEM variety is a conditional non-host for the Queensland Fruit Fly. Due to the urgency of this project and timing constraints this proposal will be sent directly to the Board for their consideration, but the Committee will be kept up to date.

Lastly, Dr. Spann asked the Committee to please share any ideas they have for article topics for future issues of From the Grove.

ITEM #5 2026 REQUEST FOR PROPOSALS

Chair Klittich explained to the Committee that he would like to see CAC get back into the routine of producing an annual request for proposals based on the research priorities list the Committee has created. He requested that the Committee members review the priorities list and select their top two priorities from within each category and send them along with any new topics to add to the list to Dr. Spann by Friday, June 5.

ITEM #6 CONSIDER FUNDING REQUEST FOR "EVALUATING DIVERSE AVOCADO ROOTSTOCKS FOR SALINITY USING MORPHOLOGICAL, IONIC, AND PHYSIOLOGICAL PARAMETERS"

Dr. Devinder Sandhu, Research Geneticist USDA Salinity Laboratory, explained to the Committee that this small-scale project was started in 2025 after his and Dr. Jorge Ferreira's proposal for a broader trial was not funded by CAC. They were able to pull funding from other sources to get the project to its current point. Their original proposal included looking into the genetic basis for salinity tolerance/susceptibility but this project focuses on physiological parameters.

Discussion ensued, and the researchers were asked how the industry would be able to use the information this project is generating. The researchers explained they are using salinity levels higher than normally experienced in the field so this will help to develop

recommendations for which rootstocks to use for different salinity scenarios. A follow up question was asked about preliminary data and the researchers responded that they have data since late 2025 but they would like to continue data collection into the summer of 2026 to see how the rootstocks respond when the weather gets hot.

The Committee members noted that CAC has spent a lot of money on rootstock breeding and this is the first good field data that has been generated to help growers select rootstocks based on their field responses. Additionally, it was noted that all the rootstocks being used in this trial are commercially available as opposed to unreleased rootstocks that are not available to growers. One point of concern that was raised is that it appears the money has already been spent and now the researchers are requesting the funds as reimbursement.

MOTION
To recommend the Board approve the funding request for the USDA salinity project.

(Davis/Araiza) MSC 6 yea, 1 abstain

Motion 26-5-28-2

ITEM #7 CONSIDER FUNDING REQUEST FOR "ENHANCING INTEGRATED PEST MANAGEMENT FOR AVOCADO LACE BUG IN ORGANIC PRODUCTION"

There was a consensus from the Committee that the industry is developing a relatively effective toolbox for managing avocado lace bug in conventional groves, but organic solutions are still lacking, and some effort needs to be focused on organic production. Jim Davis indicated he would be willing to work with the researchers to develop the best list of products possible for testing. It was pointed out that the use of kaolin clay has been effective with the olive fly by effectively camouflaging the crop; however, this may not be practical for avocados since the industry is not setup to wash fruit.

MOTION
To recommend the Board fund the request for funding for organic solutions for the control of avocado lace bug if the researcher is willing to work with Jim Davis to better develop the list of products to be tested.

(Davis/Fernandez) MSC unanimous

Motion 26-5-28-3

ITEM #8 CONSIDER ADDITIONAL FUNDING REQUEST FOR MOWING NEEDS FOR PROJECT "CREATING A WEATHER STATION NETWORK TO GUIDE IRRIGATION DECISION OF AVOCADOS"

Chair Klittich provided some background on this funding request, reminding the Committee that CAC was already funding the weather station project. The University of California has a new field station in Ventura County and does not have a mower to maintain the 4-acre grass plot around the weather station, so funds are being requested to purchase a mower or hire a mowing service.

Discussion ensued and there was agreement among the Committee members that it would be in CAC's best interest to help ensure the mowing is done since CAC is invested in the project already. However, the Committee also agreed that both the mower purchase option and mowing service options were too expensive.

MOTION

Recommend that the Board provide additional funding not to exceed $10,000 to accomplish the mowing at the UC Hansen Agricultural Research and Extension Center in Camarillo.

(Carmichael/Miller) MSC unanimous

Motion 26-5-28-4

ITEM #9 CONSIDER ADDITIONAL FUNDING SUPPORT FOR PROJECT "DOES ARTIFICIAL POLLINATION IMPROVE YIELD OF 'HASS' AND 'GEM' AVOCADO?"

Chair Klittich provided some background information for the Committee about this funding request. He reminded the Committee that CAC agreed to fund this project for two years. However, one of the companies providing artificial pollination services did not participate in the first year. In addition, the researchers believe that with the natural variation in the test orchards, a third season of data would be beneficial.

Discussion ensued, with Committee members pointing out that many growers spend money on these pollination services with no independent data to substantiate their use. Members also agreed that the design of this project, including the use of paternity testing to confirm the fruit set are the result of artificial pollination, would definitively answer the question of whether these systems work. However, opinions were split as to whether the third year of data would really provide that much more information.

The Committee agreed to table a recommendation on this request until later in 2026 after they have had the opportunity to review the project's annual progress report and review the first two years' data.

ADJOURN MEETING

Danny Klittich, Production Research Committee Chair, adjourned the meeting at 11:41 a.m.

Respectfully submitted,

_____
Timothy Spann

EXHIBITS ATTACHED TO THE PERMANENT COPY OF THESE MINUTES

EXHIBIT A May 28, 2026, Production Research Committee AB 2720 Roll Call Vote Tally Summary

EXHIBIT B Proposal: Evaluating diverse avocado rootstocks for salinity using morphological, ionic, and physiological parameters

EXHIBIT C Proposal: Enhancing integrated pest management for avocado lace bug in organic production

EXHIBIT D Additional funding request for mowing needs for project, "Creating a weather station network to guide irrigation decision of avocados"

EXHIBIT E Additional funding support for project "Does artificial pollination improve yield of 'Hass' and 'GEM' avocado?"

CALIFORNIA AVOCADO COMMISSION

Production Research Committee

AB 2720 Roll Call Vote Tally Summary

To be attached to the Meeting Minutes

Meeting Name:	**Meeting Location:**	**Meeting Date:**
California Avocado Commission Production Research Committee Meeting	*Hybrid* *In-person – Ventura County Cooperative Extension, Camarillo, CA 93010 – Zoom*	*May 28, 2026*

Attendees Who Voted	***MOTION 26-5-28-1***	***MOTION 26-5-28-2***	***MOTION 26-5-28-3***	***MOTION 26-5-28-4***
Danny Klittich, Chair	Did not vote	Did not vote	Did not vote	Did not vote
Victor Araiza	Yea	Yea	Yea	Yea
Allisen Carmichael	Yea	Yea	Yea	Yea
Jim Davis	Yea	Yea	Yea	Yea
Herman Els	Yea	Yea	Yea	Yea
Matthew Fatino	Yea	Yea	Yea	Yea
Consuelo Fernandez	Yea	Abstain	Yea	Yea
Daryn Miller	Yea	Yea	Yea	Yea
Outcome	***Unanimous***	***6 yea, 1 abstain***	***Unanimous***	***Unanimous***

HASS AVOCADO VARIETY AUTHENTICATION

A Genetic Diagnostic for Supply Chain Integrity

Research Proposal and Statement of Work

Prepared for: California Avocado Commission
Prepared by: Dr. Edwin Solares, Principal Investigator
ESB AI Lab Corporation
June 2026

Total Program Estimate: $160,000

Timeline: 6 – 9 months | Two phases

Indirect cost rate: 10% of total direct costs

1. The Problem

Unauthorized avocado varieties that are morphologically indistinguishable from Hass are entering the U.S. market and being sold as authentic Hass. Visual inspection cannot reliably differentiate Hass from similar cultivars such as Carmen (Mendez), GEM, or Luna. No validated commercial diagnostic test currently exists. The consequence is downward price pressure on California growers, erosion of the Hass premium, and an enforcement gap that grows as import volumes increase.

2. What This Project Delivers

A validated genetic diagnostic panel that provides a definitive yes/no answer: is this avocado Hass, or is it Carmen, GEM, Luna, or another validated variety? The test is based on polymerase chain reaction (PCR), a standard laboratory technique that amplifies specific regions of DNA to detect the presence or absence of variety-specific genetic markers. The diagnostic will be transferable to any molecular biology laboratory and designed for eventual deployment at packing houses and ports of entry.

All computational analysis (marker discovery, primer design, data interpretation) is performed by the Principal Investigator (PI). All wet laboratory work (DNA extraction, PCR validation, sequencing confirmation) is outsourced to established core facilities or contract research organizations (CROs).

3. Statement of Work

Phase 1: Marker Discovery and Validation

Timeline: 8 – 12 weeks from execution of agreement

Budget: $60,000

Objective: Identify single nucleotide polymorphisms (SNPs, pronounced "snips"), which are single-letter differences in the DNA code, that are unique to validated varieties such as Carmen, GEM, etc and absent in Hass or vice-versa. Validate their uniqueness across all available avocado accessions using formal population-genetic methods and machine learning-based feature selection.

Approach: Using whole-genome resequencing data from the PI’s own published work (Solares et al. 2023, G3 Genes|Genomes|Genetics, 24 accessions) and publicly available genomic datasets (Rendón-Anaya et al. 2019, 13 accessions), the PI will align all 37 samples to the publicly available Hass reference genome (Nath et al. 2022, University of Queensland) and identify positions in the genome where varieties such as Carmen differs from Hass. SNP calls from short-read sequencing carry an approximately 10–15% error rate due to reads aligning to the wrong location in repetitive regions of the genome. The PI accounts for this through stringent computational filtering and machine learning-based ranking of candidate markers.

Key experimental design: The analysis exploits the full Hass pedigree chain available in the existing data: Lyon (putative Hass parent) → Hass → Thille (first-generation Hass offspring) → Gwen (second-generation offspring). Carmen’s genomic position along this gradient determines whether it is a clone or a genetically distinct seedling. Two independent Hass samples from Rendón-Anaya et al. serve as replicate controls, establishing the analysis pipeline’s baseline error rate before any Carmen comparison is made. Kinship analysis (KING software, which computes formal relatedness coefficients between all sample pairs) and principal component analysis (PCA, a

standard method for visualizing genetic relationships) will place varieties such as Carmen in their proper population-genetic context.

Wet lab work (outsourced): If candidate markers are identified, custom primers (short synthetic DNA sequences used to target specific regions for PCR amplification) will be ordered from Integrated DNA Technologies (IDT) and validated via outsourced PCR on authenticated samples collected from an authoritative germ plasm such as the germplasm from UCR. If Luna or other target varieties require sequencing, a short-read Illumina run will be contracted.

Deliverable: A catalog of variety-specific candidate markers with documented statistical confidence, a validated subset of these markers performed with PCR from young leaves demonstrating proof of concept, formal kinship analysis, population structure analysis, and documented evidence regarding whether Hass-like varieties (Carmen, GEM, Luna) carry markers distinguishable from Hass at short-read resolution. The deliverable is a technical report documenting the validated markers and their performance characteristics. The marker sequences, primer sequences, PCR protocols, and diagnostic methodology described in the report constitute proprietary intellectual property of ESB AI Lab Corporation. Commercial use, reproduction, deployment, or transfer of the markers or protocols described in the report requires a separate license from ESB AI Lab Corporation, as specified in Section 6 (Intellectual Property).

Known limitation: If any variety such as Carmen is a somatic mutant of Hass (a clone that diverged through mutations accumulated during vegetative propagation rather than through sexual reproduction), short-read-derived SNP markers may not distinguish them at conventional loci. However, somatic loss of heterozygosity (LOH, where one of the two parental copies of a genomic region is lost or replaced) and transposable element (TE, segments of DNA that can move to new positions within the genome) mediated structural rearrangements may still be detectable through windowed divergence analysis.

Phase 2: Diagnostic Development

Timeline: 4 – 6 months following successful Phase 2 completion

Budget: $100,000

Contingent on successful Phase 1. Funded separately upon CAC approval.

Objective: Develop validated markers into a deployable PCR-based diagnostic.

Scope: The PI will design PCR primers targeting the markers identified in Phase 1, outsource PCR optimization and validation on authenticated samples, benchmark performance across 50–100 samples from commercial fruit, nursery material, and imported product, and prepare a standard operating protocol (SOP) for use in a qualified molecular biology laboratory. If candidate markers produce nonspecific amplification or fail to discriminate at the required confidence threshold, the workflow iterates: additional sequencing to cover gaps, revalidation, and retesting until the panel meets performance standards. This phase includes validation of DNA extraction from avocado fruit tissue (mesocarp), which requires specialized protocols due to high oil, polysaccharide, and polyphenol content.

Deliverable: A laboratory-based diagnostic protocol with approximately 48-hour turnaround, operable by a trained technician. This is not a field tool. The deliverable is a technical report documenting the diagnostic protocol and its validated performance characteristics. The primer

sequences, amplification protocols, interpretation criteria, and standard operating procedures described in the report constitute proprietary intellectual property of ESB AI Lab Corporation. Commercial use, reproduction, deployment, sublicensing, or transfer of the diagnostic to third parties requires a separate license from ESB AI Lab Corporation, as specified in Section 6 (Intellectual Property). Delivery of the report does not constitute a grant of rights to use the intellectual property described therein.

Please Note: A field-deployable rapid test usable by inspectors at packing houses or ports of entry requires a separate research and development effort beyond the scope of this proposal. That work would be funded through federal research grants, not solely by CAC.

4. Budget

Indirect cost rate: 10% of total direct costs (TDC). This rate reflects ESB AI Lab Corporation's status as a newly formed nonprofit research organization.

Line Item	Phase 1	Phase 2
PI salary and fringe benefits	$30,000	$42,000
DNA extraction (UCI GRT Hub; leaf tissue)	$1,500	—
DNA extraction: leaf + fruit, 100 samples (UCI GRT Hub; leaf + fruit tissue, 50–100 samples)	—	$6,000
Fruit tissue extraction protocol optimization (outsourced)	—	$5,000
Custom primer synthesis (Integrated DNA Technologies)	$500	$400
PCR validation (UCI GRT Hub)	$3,250	$9,500
Sanger confirmation sequencing (outsourced)	$1,200	$2,000
New Illumina sequencing (Carmen ×3, GEM, Luna, Hass ×3; outsourced UCI)	$2,450	—
Sample procurement, shipping, handling	$2,500	$3,500
High-performance computing access	$800	$500
Consumables and reagents (provided to CRO)	$500	$3,000
Travel (sample collection, CAC meetings)	$1,200	$3,000
Contingency reserve (sequencing reruns, additional samples, iterative validation)	$5,545	$7,000
SOP development and documentation	—	$1,500
Provisional Patent Filings	$3,000	
Subtotal Direct Costs	**$52,445**	**$83,500**
Indirect costs (10% of TDC)	$5,244.50	$8,350
Rounding / reserve	$2,310.50	$8,150
PHASE TOTAL	**$60,000**	**$100,000**
COMBINED PROGRAM TOTAL		**$160,000**

Of every dollar in this budget, 90 cents goes directly to research. ESB AI Lab's 10% indirect cost rate reflects its commitment to delivering maximum research value per sponsor dollar. By comparison, the University of California system charges 55–60% in facilities and administrative costs on federally sponsored research.

Phase 2 funding is contingent on successful Phase 1 completion and separate CAC approval. CAC may elect to fund Phase 1 alone ($60,000) and evaluate results before committing to Phase 2.

Payment Terms

The full award amount for each phase shall be disbursed to ESB AI Lab Corporation within 30 days of execution of the applicable agreement and prior to the commencement of any research. This is standard practice in sponsored research and ensures that the scientific analysis is conducted with complete independence from the funding timeline.

In the event of early termination by either party with 90 days written notice, ESB AI Lab Corporation shall return any unexpended direct costs to the Commission within 60 days of the termination date. All completed work products and data generated to that point shall be delivered to CAC upon termination.

Phase	Payment Trigger	Amount
Phase 1	Execution of Phase 1 agreement	**$60,000**
Phase 2	Execution of Phase 2 agreement (contingent on Phase 1 success and CAC approval)	**$100,000**

5. Principal Investigator

Edwin Solares, PhD., is the author of the first published haplotype-resolved avocado reference genome (Solares et al. 2023, G3 Genes|Genomes|Genetics). That study resequenced 34 avocado accessions spanning all three botanical races (Mexican, Guatemalan, and West Indian), identified candidate genomic regions for heterodichogamy (the mechanism controlling A-type and B-type flowering) on chromosome 10, and reported 3.8% genic hemizygosity in the Gwen cultivar. The bioinformatic pipelines, reference assemblies, and variant calls generated in that study provide the direct data foundation for the marker discovery proposed here.

Dr. Solares holds a PhD in Computational Biology from UC Irvine and has published peer-reviewed methods for genome assembly optimization (HapSolo, BMC Bioinformatics 2021) and structural variant analysis in plant genomes (Genome Research 2023). He serves as Executive Director of ESB AI Lab Corporation, a California Nonprofit Public Benefit Corporation whose mission includes Food Security research, and teaches at the University of California San Diego in both the Halıcıoğlu Data Science Institute and the Computer Science and Engineering Department.

6. Intellectual Property

ESB AI Lab Corporation retains all patent rights and intellectual property arising from this sponsored research. Any invention, discovery, marker sequence, diagnostic protocol, or patentable idea conceived or reduced to practice in the course of this research belongs to ESB AI Lab Corporation. Funding by the California Avocado Commission (CAC) does not give rise to ownership of the resulting intellectual property.

ESB AI Lab Corporation will grant to the Commission a time-limited first right to negotiate an exclusive or nonexclusive royalty-bearing license, based upon the level of sponsor support. The option period shall not exceed 180 days from delivery of the applicable phase report. An outright license grant is not included in this research agreement. The scope, field of use, and financial terms of any such license will be negotiated separately upon exercise of the option.

This structure is consistent with the intellectual property policies of the University of California system, the Salk Institute for Biological Studies, and comparable nonprofit research institutions.

7. Scientific Independence

The full upfront disbursement of each phase’s award ensures that the PI conducts the analysis without financial dependency on the outcome. The analysis is data-driven. Results will be dictated by the data, not by the funding schedule. This structure is standard in sponsored research and is essential for maintaining the scientific integrity that gives the results credibility in trade enforcement, regulatory proceedings, and peer-reviewed publication.

8. Future Work

If Phase 1 determines that Carmen and Hass are indistinguishable at short-read resolution, the path forward involves higher-resolution long-read sequencing to resolve somatic structural variants. Recent advances in sequencing chemistry have substantially reduced the cost of such work. That

phase could be funded through federal research grants, with CAC participating as a co-investor. Phase 1 results provide the preliminary data necessary for a competitive federal application.

Contact

Dr. Edwin Solares, Executive Director & Principal Investigator

ESB AI Lab Corporation

esbailab.org

Determining specific genetic markers for ‘Carmen Hass/Mendez No1’ avocado

The availability of molecular tools to identify genetic differences among genotypes is essential for breeding programs and the management of genetic resources (Kämper et al., 2021), as these tools support both crop improvement and the conservation of genetic diversity. In avocado, after more than a century breeding programs (Lahav et al., 2024), a wide range of molecular markers have been used to identify accessions and clarify genetic relationships, including minisatellites, VNTRs, RAPDs, RFLPs, SSRs (Alcaraz & Hormaza, 2007), and SNPs (Kuhn et al., 2019; Talavera et al., 2019; Rubinstein et al., 2019; Rendón-Anaya et al., 2019; Solares et al. 2022; Berdugo-Cely et al., 2023; Cañas-Gutiérrez et al., 2025).

Despite these advances, global production remains highly dependent on a limited number of cultivars, with a single cultivar, ‘Hass’ accounting for approximately 90% of the global avocado market (Pérez et al., 2025; Talavera et al. 2019). In addition to most cultivars developed by selection from seedlings, occasional spontaneous somatic mutations (bud sports) have been reported in avocado (Lahav et al., 2024). In the case of ‘Hass’ examples include ‘Carmen Hass’/‘Mendez No 1’, ‘Jimenez-1’, ‘Jimenez-2’ and ‘Flor de Maria’ in Mexico, ‘Andes 3’ and ‘Andes 4’ in Chile or ‘Turner Hass’ in Australia. The most economically important of those mutants is ‘Carmen Hass’, which is grown in several countries, due to its ability to produce fruit outside the typical ‘Hass’ season, allowing growers to access premium market prices. This cultivar originated from a spontaneous sport identified by the observation of its early flowering compared to ‘Hass’ in Uruapan, Michoacan, Mexico. Because these bud sports generally result from single or very few mutations, distinguishing them at the molecular level remains challenging. Consequently, currently available fingerprinting methods based on molecular markers have not been able to unequivocally distinguish ‘Carmen Hass’ from ‘Hass’.

To address this challenge, we propose the use of whole-genome resequencing at 30x coverage to identify Single Nucleotide Polymorphisms (SNPs) specific to ‘Carmen Hass’ and validate them using complementary molecular approaches to determine the most suitable method for routine application. This approach should provide higher resolution and accuracy, particularly given the availability of recently published reference genomes (Nath et al. 2022; Solares et al, 2023), making the detection of these markers more feasible. This strategy is expected to enable the identification of validated molecular markers capable of reliably distinguishing ‘Carmen Hass’ from ‘Hass’ fruit throughout the supply chain.

- **Materials and Methods.**

Leaf tissue will be collected from a total of 10 avocado trees each of the ‘Carmen Hass’ and ‘Hass’ cultivars provided by The California Avocado Commission. Samples will be obtained from trees showing clear phenotypic differences in the field. Genomic DNA will be extracted from dried young leaves using the Qiagen DNeasy plant Mini kit. DNA quality will be determined using a NanoDrop spectrophotometer (ND-1000; Thermo Scientific, New York, USA), while DNA integrity and fragment size will be evaluated by 1% agarose gel electrophoresis.

Paired-end sequencing libraries will be prepared following the NovaSeq PE150 library preparation protocol and sequenced on the Illumina platform. Raw sequencing reads will be processed using Trimmomatic (0.39) (Bolger et al. 2014) and mapped to the reference genome (Nath et al. 2022) using BWA-mem (2.2.1) (Li, 2013). PCR duplicates will be identified and removed using GATK MarkDuplicates (Poplin et al. 2018). Variant discovery will be conducted using GATK Best Practices (Van der Auwera & O'Connor, 2020) using HaplotypeCaller. Variant files from all chromosomes will be concatenated and filtered to retain high-quality biallelic SNPs using GATK SelectVariants and VariantFiltration with hard-filtering criteria. VCFtools (0.1.14) (Danecek et al. 2011) will be used to remove missing data. Only variants passing all filtering criteria will be retained.

Genetic relationships between the samples will be analysed using Principal Components Analysis (PCA) and phylogenetic approaches using R. Putative private SNPs (specific to 'Carmen Hass') will be identified and validated using DNA extracted from fruit skin of 'Carmen Hass' using the DNA extraction kit indicated above. Different validation approaches, such as Kompetitive Allele-Specific PCR (KASP) (He et al. 2014), High-Resolution Melting (HRM) (Erali & Wittwer 2010) or Sanger sequencing (Hyten et al. 2010) will be evaluated to identify the most reliable and cost-effective method. For this purpose, specific primers will be designed, and the protocols will be optimized for each validation method.

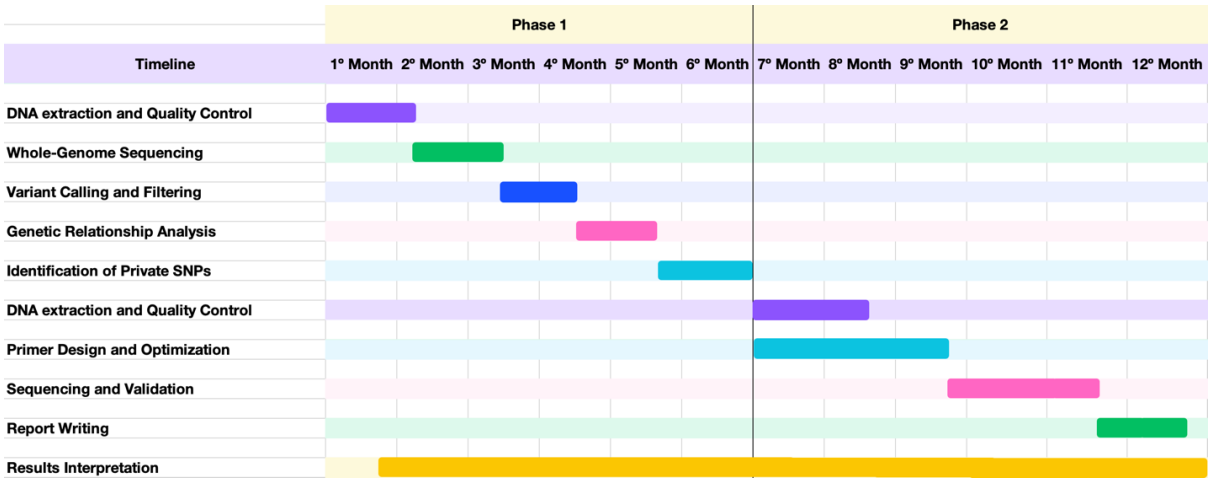
- **Contingency plan.**

While most bud sports in fruit trees result from DNA sequence mutations, some are driven by stable epigenetic modifications, including changes in DNA methylation or histone modifications, that alter gene expression without changing the underlying DNA sequence of the plant (Zhou et al., 2023). Therefore, it is possible that the phenotypic differences between 'Carmen Hass' and 'Hass' are epigenetic rather than genetic. If whole-genome resequencing does not identify robust cultivar-specific genetic markers, we propose to extend this work to investigate epigenetic differences between the two cultivars using appropriate epigenomic approaches.

- **Budget.**

	Price $
Phase 1	19,500
Phase 2	21,250
Indirect cost	9,556
Total	**50,306**

- **Timeline and milestones.**



- **Research Team and scientific contributions.**

This project will be executed by a group of three scientists from La Mayora Institute of Subtropical and Mediterranean Horticulture (IHSM La Mayora) with extensive experience in genetics, genomics and bioinformatic analyses in avocado and other subtropical fruit crops: Alicia Talavera (orcid: 0000-0002-1285-7891), Noé Fernández-Pozo (orcid: 0000-0002-6489-5566 and Iñaki Hormaza (orcid: 0000-0001-5449-7444). The group has considerable expertise in the identification and characterizing of avocado cultivars from different collections worldwide and has been involved in numerous avocado projects, as well as the development and maintenance of Avobase, a web portal dedicated to avocado genomics (https://www.avocado.uma.es/easy_gdb/index.php). Below we attach some of the publications of our group since 2019 on avocado research.

Alcaraz ML, Hormaza JI (2021). Fruit set in avocado: Pollen limitation, pollen load size, and selective fruit abortion. *Agronomy*, *11*(8), 1603.

Alcaraz ML, Hormaza JI (2024). Inadequate pollination is a key factor determining low fruit-to-flower ratios in avocado. *Horticulturae*, *10*(2), 140.

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PHASE 1 RESEARCH PROPOSAL
Genomic Identification and Molecular Marker Development
for the Unique Identification of 'Hass' and 'Carmen' Avocado

Submitted to: California Avocado Commission
Principal Investigator: Luis Herrera-Estrella
Co-Investigators: Dr. Gerardo Alejo Jacuinde
Project Duration: 18 months | Total Budget: $331,166

Executive Summary

The 'Hass' and 'Carmen' avocado varieties are morphologically indistinguishable by vegetative and fruit characteristics, differing principally in flowering time. This phenotypic similarity creates critical vulnerabilities across the supply chain, from nursery certification and orchard management to post-harvest variety verification and intellectual property protection, with direct economic consequences for California growers, packers, and retailers. Phase 1 of this two-phase research program will deliver a definitive set of validated genetic markers that unambiguously distinguish 'Hass' from 'Carmen' at the DNA level, grounded in haplotype-resolved, chromosome-level genome assemblies of both varieties. Our preliminary genomic analysis has already confirmed that 'Carmen' harbors distinct structural architectures and allelic variations that are entirely absent from the existing public 'Hass' reference genome, underscoring the absolute necessity of de novo assembly for both varieties before robust markers can be identified. By the end of Phase 1, we will deliver a validated, commercially deployable panel of 3–5 diagnostic molecular markers, establishing the scientific foundation for the field-ready testing procedures to be developed in Phase 2.

Problem Statement and Industry Relevance

California produces most of the avocados grown in the United States, with 'Hass' accounting for most commercial production. 'Carmen' (also known as 'Mendez') has emerged as a variety of growing commercial interest, valued particularly for its early-flowering phenology, which offers advantages for orchard management and market scheduling. However, the near-identical leaf morphology, tree architecture, and fruit characteristics of the two varieties mean that visual inspection cannot reliably distinguish them at any point in the supply chain.

This creates three categories of industry risk that a validated DNA-based marker system would directly address:

- **Nursery and orchard integrity:** Mislabeled planting material leads to unintended variety mixing, with consequences for orchard management, pollination strategy, and harvest scheduling that may not become apparent for years.
- **Post-harvest and supply chain verification:** Without a rapid, reliable testing method, packing houses and retailers cannot confirm the variety identity of incoming fruit, creating exposure to mislabeling claims and brand integrity issues.
- **Plant variety protection and grower investment:** Growers and breeders investing in 'Carmen' production require legally defensible tools for variety verification to protect their intellectual property and commercial interests.

Scientific Rationale and Intellectual Merit

The phenotypic difference between 'Hass' and 'Carmen', early flowering, is most likely driven by a low-frequency somatic mutation, a small structural variant (SV), or a transposable element (TE) insertion near a gene that modulates flowering time. Because the *Persea americana* genome is highly heterozygous (1.96–2.08% for 'Hass'; 1.61–1.92% for 'Carmen' based on our *k*-mer analyses), this early-flowering phenotype

may be driven by a mutation occurring on only one of the parental haplotypes, making it invisible to standard collapsed (haploid) assemblies.

Our preliminary *k*-mer spectral analysis using Merqury (**Figure 1**) against the publicly available 'Hass' reference genome (Nath et al., 2022) reveals a critical limitation: the current reference fails to capture a substantial pool of heterozygous *k*-mers shared by both lines. Crucially, the 'Carmen' ('Mendez') profile shows a broader, enriched read-only signature relative to 'Hass', demonstrating that 'Carmen' harbors distinct structural architectures and allelic variations entirely absent from the standard reference. Attempting to identify the mutations responsible for the early-flowering phenotype via standard reference mapping would fundamentally risk false negatives by ignoring this unrepresented allelic space. Although not strictly necessary for the main objective of this proposal, it would be highly desirable to have strong candidates for the causal mutation underlying the 'Carmen' flowering phenotype to enable more precise identification and future gene-editing-based breeding programs.

This empirical bottleneck underscores the necessity of our proposed haplotype-resolved, de novo assembly strategy for both varieties, which will capture the true genetic markers that distinguish 'Carmen' from 'Hass' with the fidelity required for commercial deployment.

Specific Objectives

1. Generate chromosome-level, haplotype-resolved de novo genome assemblies for both 'Hass' and 'Carmen'.
2. Identify genome-wide sequence polymorphisms, SNPs, indels, structural variants, and TE insertions that consistently and exclusively distinguish 'Carmen' from 'Hass'.
3. Validate a minimal panel of 3–5 diagnostic molecular markers across geographically diverse populations of both varieties.
4. Deposit all genomic assemblies and marker sequences in publicly accessible repositories as a permanent resource for the California avocado industry.

Methodology

Task 1: Sample Collection and DNA Extraction (Months 1–3)

Verified 'Hass' and 'Carmen' mother trees will be sourced from certified populations in California's principal avocado-producing counties (Ventura, San Diego, and Santa Barbara). For de novo genome assembly, a single high-quality representative individual per variety will be selected. For population-scale validation, leaf material will be collected from 20 verified 'Hass' and 20 verified 'Carmen' individuals from geographically distinct orchards.

High-molecular-weight (HMW) DNA will be extracted from young leaf tissue using an optimized CTAB and magnetic bead-based purification protocol validated for the high-lipid, high-polyphenol content of avocado leaf tissue, targeting fragment sizes in the 50–300+ kb range required for PacBio HiFi library preparation. Hi-C crosslinked nuclei will be prepared from fresh leaf tissue following the Arima-HiC 2.0 protocol.

Task 2: De Novo Genome Sequencing and Assembly (Months 2–6)

Each genome will be sequenced using a three-technology strategy designed to maximize both base-level accuracy and chromosomal contiguity, targeting the known ~920 Mb avocado genome:

- **PacBio HiFi sequencing** (50× coverage): Long, highly accurate reads essential for phasing heterozygous haplotypes and spanning the repeat-rich regions that dominate the avocado genome.
- **Hi-C chromatin conformation capture** (184 M PE reads; approximately 55 Gb): Proximity ligation data to scaffold assembled contigs into chromosome-level pseudomolecules.

- **Illumina paired-end sequencing** (PE-150 bp, 50× coverage): High-accuracy short reads for base-level polishing and SNV validation.

Phased assembly will be performed using Hifiasm, with Hi-C scaffolding into pseudochromosomes using HapHiC. Assembly completeness, continuity, and phasing precision will be rigorously evaluated using BUSCO completeness benchmarks and Merqury spectral analyses. Repetitive sequences will be characterized using RepeatModeler (de novo) combined with homology-based approaches using RepBase and PlantRep, followed by RepeatMasker annotation. Structural gene annotation will be performed using BRAKER3, integrating public *Persea americana* protein databases and available transcriptomic evidence.

Both assemblies will be generated using identical sequencing technologies and bioinformatics pipelines to eliminate technical artifacts arising from platform or methodological differences, ensuring that observed genomic differences between varieties reflect true biological variation.

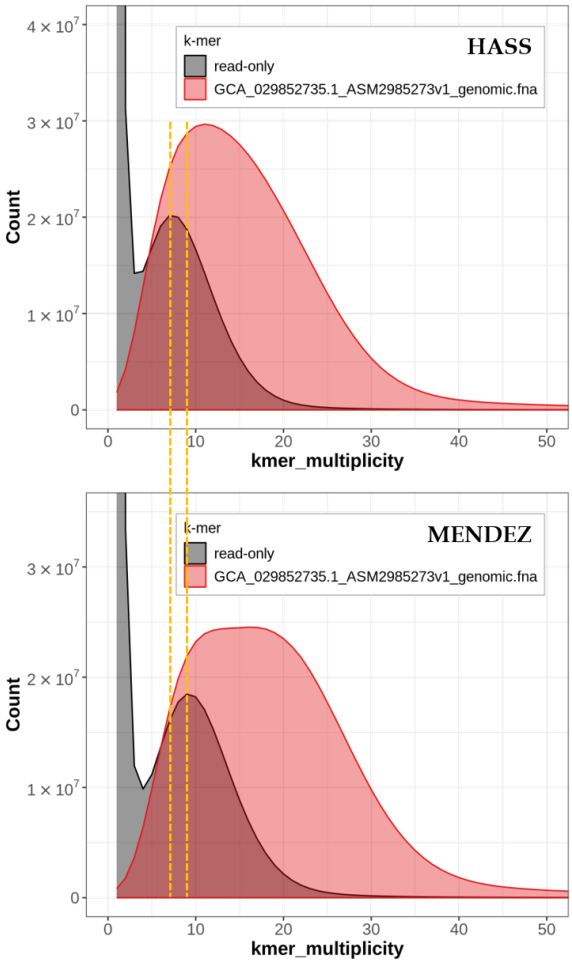


Figure 1. *k*-mer spectrum analysis (Merqury) of 'Hass' and 'Mendez' ('Carmen') genomes against the public haploid reference. Whole-genome Illumina reads from 'Hass' (top) and 'Mendez' (bottom) were tracked against the standard collapsed 'Hass' reference assembly. The prominent gray/brown peaks spanning the heterozygous multiplicity space (marked by yellow dashed lines) represent high-confidence read *k*-mers completely absent from the reference genome. The expanded read-only signature and altered assembly coverage profiles in 'Mendez' highlight significant, unrepresented genomic divergence between the two varieties.

Task 3: Comparative Genomics and Variant Discovery (Months 6–10)

A parallel bioinformatic discovery pipeline will be applied to separate true somatic mutations and variety-specific variants from technical assembly artifacts:

Genome-to-genome alignment

Direct whole-genome alignments between the finalized phased ‘Hass’ and ‘Carmen’ assemblies will be performed with minimap2, and large-scale insertions, deletions, inversions, and translocation breakpoints will be systematically extracted using paftools.js.

Read-based cross-validation

Short and long reads from both varieties will be reciprocally cross-mapped to both assemblies to establish a high-confidence baseline for variant calling. Short-read alignments (Bowtie2) will be used to run VarScan2 for SNV and micro-indel calling; long-read alignments (minimap2) will be used to run PBSV for structural variant detection. Only variants identified independently by both genome-alignment and read-mapping approaches will advance as high-confidence candidates.

Population-scale filtering

Candidate variants will be screened against an expanded cohort of 40 commercial populations (20 ‘Hass’, 20 ‘Carmen’) sequenced at 30× depth (Illumina). Short-read data from the 40-population cohort will be processed using the GATK HaplotypeCaller pipeline. Subsequently, custom Bcftools filters and PLINK association analyses will be applied to exclude intra-variety polymorphisms and isolate stable, high-

confidence variants that segregate perfectly with the 'Carmen' early-flowering phenotype. The software Paragraph wiil be used to assess candidate structural variants, while transposable element (TE) insertions will be profiled across the cohort using tools such as PopoolationTE2. Priority will be given to stable structural variants and TE insertions, which offer the most reliable and unambiguous diagnostic signatures.

Task 4: Molecular Marker Development and Validation (Months 10–18)

From the validated candidate locus pool, PCR primers will be designed flanking variety-diagnostic polymorphisms. Three marker formats will be developed in parallel to accommodate different deployment contexts and equipment levels:

- **Indel markers:** Size-polymorphic amplicons distinguished by agarose gel electrophoresis — lowest cost, no specialized equipment required, suitable for nurseries and field laboratories.
- **CAPS markers (Cleaved Amplified Polymorphic Sequences):** SNPs creating or abolishing restriction enzyme sites, distinguished by restriction digest of a PCR product — no specialized equipment required beyond standard PCR and gel electrophoresis.
- **KASP assays (Kompetitive Allele-Specific PCR):** High-throughput fluorescence-based SNP genotyping for large-scale certification programs and high-volume supply chain screening.

An initial panel of 20 candidate markers will be developed to identify a minimally validated set of 3–5 markers that provide complete, unambiguous discrimination of variety. Final validation will be performed by blind genotyping of 50 ‘Hass’ and 50 ‘Carmen’ individuals from independent orchards not used in variant discovery, plus 20 individuals of other commercially relevant California varieties (Lamb Hass, GEM, Maluma) to confirm specificity.

Phase 1 Deliverables

- **Two chromosome-level, haplotype-resolved genome assemblies** for ‘Hass’ and ‘Carmen’, fully annotated and deposited in NCBI, represent the first variety-resolution genomic resource for the California avocado industry and a permanent platform for future research.
- **A high-confidence catalog of variety-specific genetic variants** distinguishing ‘Carmen’ from ‘Hass’, validated across 40 geographically diverse populations.
- **A validated molecular marker panel** of 3–5 diagnostic loci capable of unambiguously distinguishing ‘Hass’ from ‘Carmen’ from any tissue — leaf, seed, or fruit — at any developmental stage, in three marker formats (indel, CAPS, KASP).
- **Peer-reviewed publications** reporting the genome assemblies and marker development, ensuring scientific credibility and long-term visibility for the Commission’s investment.
- **A genomic and bioinformatic framework** ready to support Phase 2 commercial testing protocol development without additional sequencing.

Significance and Connection to Phase 2

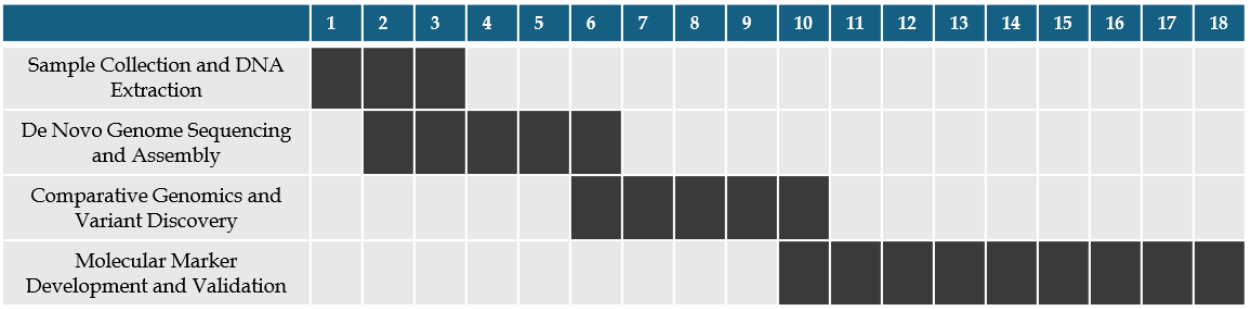
Phase 1 will resolve the fundamental scientific question of what makes ‘Carmen’ genetically distinct from ‘Hass’ and deliver validated genetic markers on which all subsequent commercial testing depends. The haplotype-resolved genome assemblies generated in Phase 1 will serve as permanent, publicly available resources that benefit the California avocado industry far beyond this project, providing a foundation for future research into avocado breeding, disease resistance, and climate adaptation.

The marker panel and genomic data from Phase 1 will directly and efficiently enable Phase 2, where the focus shifts from marker discovery to the development of commercially viable testing procedures deployable across the supply chain, from nursery certification to packing house verification, at the speed, cost, and simplicity required for routine industry adoption.

Research Team

The proposed project will be led by Dr. Luis Herrera-Estrella, a member of the United States National Academy of Sciences and the Royal Society of London, one of Latin America's most distinguished plant scientists with over 30 years of experience in plant biotechnology and genomics. Dr. Herrera-Estrella has direct and relevant expertise in large-scale plant genome sequencing, having led sequencing and assembly projects for avocado, chili pepper, and chia, among others, which makes him uniquely positioned to lead the de novo assembly of the 'Hass' and 'Carmen' genomes. He will be joined by Dr. Gerardo Alejo-Jacuinde, an expert in plant genomics with extensive experience in the sequencing and assembly of complex, highly heterozygous plant genomes, including multiple *Selaginella* species, chia, and most recently, the genome sequencing of five species of resurrection plants, organisms renowned for their genomic complexity and repeat content. Both investigators are faculty members of the Institute of Genomics for Crop Abiotic Stress Tolerance (IGCAST) within the Department of Plant and Soil Science at Texas Tech University, an institute purpose-built for the kind of large-scale, integrative genomics research proposed here. We are requesting funds for a MSc student, who will be trained to participate in the sample collection, data processing and analysis. Together, the team brings directly applicable expertise in every technical component of the proposed Phase 1 strategy, from high-molecular-weight DNA extraction and long-read sequencing to haplotype-resolved assembly, comparative genomics, and molecular marker development.

Project Timeline



Budget Summary

Category	Amount
Personnel	$179,824
Graduate tuition and fees	$13,696
Travel - Sample collection	$22,452
Sequencing and Genomics	$30,000
Bioinformatics and Computing	$4,000
Supplies and Other Direct Costs	$26,000
Subtotal (Direct Costs)	$275,972
Indirect Costs (indirect rate applied to direct costs)	$55,194
TOTAL PHASE 1 REQUEST	$331,166*

*Budget reflects estimated direct and indirect costs; final indirect cost rate to be confirmed by administering institution. The personnel costs above reflect estimated totals, including benefits.

Discovery of Carmen Hass-specific genomic variants through whole-genome resequencing

Robert Backer and Noëlani van den Berg

Hans Merensky Chair in Avocado Research

Forestry and Agricultural Biotechnology Institute

University of Pretoria

Key team members

Dr Robert Backer
Bioinformatics Researcher
Dr Backer has expertise in whole-genome resequencing, reference-based read alignment, variant discovery, comparative genomics and the development of reproducible bioinformatics workflows. He will lead the analysis of SNPs, indels, optional structural variant candidates, candidate-marker prioritisation and evaluation of marker specificity across avocado resequencing datasets.

Prof. Noelani van den Berg
Chair of the Hans Merensky Chair in Avocado Research
Prof. van den Berg has extensive experience in avocado research, plant pathology and the coordination of multidisciplinary research programmes. She will provide project leadership, ensure alignment with avocado-industry priorities and support access to authenticated plant material and relevant industry networks.

Dr Nicky Olivier
Senior Lecturer
Co-manager of the University of Pretoria Genomics Laboratory (UPGL)
Dr Olivier has expertise in genomics, molecular biology and high-throughput sequencing. As co-manager of the UPGL, he will provide oversight of DNA quality control, sequencing-library preparation and generation of high-quality whole-genome resequencing data.

Prof. Fourie Joubert
Director of the Bioinformatics and Computational Biology Facility, African Centre for Gene Technologies
Prof. Joubert has extensive expertise in bioinformatics, computational genomics and research infrastructure. He will provide senior bioinformatics oversight, support workflow optimisation and assist with computational resource planning, data management and quality assurance.

Executive summary

Hass is the dominant commercial cultivar of *Persea americana*, accounting for approximately 80% of global avocado consumption (Nath et al., 2022). Carmen Hass, originally described as Mendez No. 1, is widely accepted as a somatic sport of Hass identified through earlier flowering and fruit production, while retaining fruit highly similar to Hass in appearance and quality (Mendez Vega,

2000). This extends Hass-type fruit availability, but their close similarity creates a barrier to cultivar authentication and enforcement of plant breeders' rights.

Previous avocado genotyping studies have been unable to distinguish Hass and Carmen Hass confidently. A 384-SNP study placed Hass and Carmen accessions in the same near-identical genotype group, with only 15 differences among 8,671 genotype comparisons and no opposing homozygous calls (Kuhn et al., 2019). A ddRAD-seq study likewise identified 52 homozygous SNPs distinguishing nine other cultivars, but no private homozygous SNPs separating Hass from Carmen Hass (Kämper et al., 2021). These studies demonstrate their close relationship, but were restricted to reduced genomic fractions and focused mainly on homozygous variation, making them poorly suited to detecting rare heterozygous somatic mutations.

Recent low-coverage whole-genome resequencing (WGR) of 205 avocado trees identified more than 64 million SNPs at approximately 4.69x mean depth, but was designed for population structure rather than rare variant discovery between clonally related cultivars (Cañas-Gutiérrez et al., 2025). These data are useful as a specificity panel, but low coverage limits confidence in the absence of individual heterozygous variants. In contrast, preliminary GATK Mutect2 analysis by the Hans Merensky Chair in Avocado Research identified candidate heterozygous SNPs present in Carmen Hass but absent from Hass. Recovery against both the Nath et al. Hass genome and an independently assembled West-Indian genome reduces the likelihood of mapping artefacts and supports targeted whole-genome investigation, consistent with clonal-crop precedents (Calderón et al., 2021; Wang et al., 2024).

This Phase 1 project will resequence approximately four confirmed source trees of each cultivar using PCR-free, 150-bp paired-end Illumina sequencing at approximately 30x usable genome coverage. Closely related cultivars will support stringent filtration. Reads will be mapped to the chromosome-level Hass reference genome, and complementary variant-calling approaches will identify candidate variants distinguishing Carmen Hass from Hass. Variants will be retained only if reproducible across Carmen Hass samples, absent from Hass, close relatives and public datasets, supported by high-quality evidence and suitable for assay development.

The anticipated outcome is three to six independent, high-confidence Carmen Hass-specific genomic variants suitable for KASP, TaqMan or targeted amplicon assay development for cultivar authentication across the avocado supply chain.

Methodology

Discovery material and sampling

To identify diagnostic markers, WGR will be performed on a curated set of authenticated samples:

- **Target accessions**: four authenticated Carmen Hass trees (original/early-generation source material) and four authenticated Hass trees from verified clonal lines.
- **Comparative controls**: Representative sequencing (n = 2 each) of close relatives to ensure marker specificity, including Gwen, GEM, Lamb Hass, and Thille.
- **Background specificity panel:** A curated set of public avocado resequencing datasets with ≥ 10x mean genome coverage will be reprocessed against the same reference and used downstream to filter out common or non-Carmen variants.

DNA extraction and sample requirements

As Carmen Hass is a clonally propagated sport, distinguishing mutations may vary in allele frequency across tissues. Consequently, DNA will be obtained from both **young, recently expanded flush material** (for high-quality initial discovery) and **unripe fruit peel** (for supply-chain relevance).

Three options for sample provision are available:

1. **Provision of extracted DNA**: The California Avocado Commission may provide extracted DNA meeting the following specifications: $A_{260/280}$ ratio of 1.8-2.0; $A_{260/230}$ ratio ≈ 2.0 (minimum 1.6); fluorometric concentration (Qubit) > 20 ng/µl; and a minimum of 1 µg total DNA per sample (2 µg preferred).
2. **Fresh tissue provision**: Alternatively, young flush and unripe fruit peel may be sent to the Hans Merensky Chair in Avocado Research for extraction using an optimised in-house CTAB-based protocol.
3. **Fresh tissue acquisition**: Finally, fresh tissue may be acquired in South Africa, through local industry partners. This would, however, lead to an increased risk of IP complications. It is a viable route, nonetheless.

Strategic application: Initial variant discovery will leverage high-quality leaf DNA. However, all candidate variants must be confirmed in matched fruit peel DNA before assay development. Shortlisted variants will undergo targeted PCR-based validation and Sanger sequencing in peel samples; the final assay format (e.g. KASP/TaqMan for clear heterozygosity, or amplicon deep sequencing/ddPCR for variable allele frequencies) will be determined by the observed tissue-specific representation.

Sequencing strategy

A high-fidelity sequencing approach will be employed to maximise variant sensitivity:

- **Platform**: Illumina 150-bp paired-end, PCR-free libraries.
- **Coverage**: Target 30x usable mapped coverage (generating ~ 35-40x raw coverage) to account for trimming and duplication removal.
- **Consistency**: Identical library preparation and sequencing platforms will be used for Hass, Carmen Hass and close relatives to minimise technical bias.

Bioinformatics pipeline

Processing and alignment

Reads will be processed via **fastp** (QC/trimming) and **MultiQC**. Alignment will be performed against the *Nath et al.* Hass reference genome using **BWA-MEM2**. BAM files will be processed with **samtools**/**GATK MarkDuplicates**, and coverage verified using **mosdepth**. Samples failing quality thresholds (mean depth < 20x, excessive duplication, or evidence of contamination) will be excluded.

Variant discovery

A dual-analysis approach will be used, with joint cohort variant calling providing the primary genotype matrix and somatic-style calling providing an independent Carmen-versus-Hass candidate-locus set:

- **Joint cohort variant calling: GATK HaplotypeCaller** will be used in GVCF mode, followed by GenomicsDBImport and GenotypeGVCFs, to identify variants across the newly generated high-coverage Carmen Hass, Hass and close-relative samples.
- **Somatic-style calling: GATK Mutect2** will be used for Carmen-versus-Hass comparisons, treating Carmen Hass as the case genotype and authenticated Hass accessions as pseudo-normal controls, including a pooled Hass "panel-of-normals" with subsequent FilterMutectCalls refinement.
- **Structural variant screening**: SVs may be considered as a secondary candidate class, but the primary focus will be SNPs and small indels suitable for targeted assay development.

Candidate loci from the **joint cohort** and **somatic-style** analyses will be normalised and reconciled at the locus level. The joint cohort VCF will provide the primary genotype matrix across Carmen Hass, Hass and close-relative samples. Initial Carmen-private candidates will be defined as loci where the alternate allele is reproducibly present in Carmen Hass and absent from Hass and close relatives. To generate the highest-confidence candidate set, these loci will be intersected with the independent Carmen-versus-Hass Mutect2 candidate-locus set. If this strict intersection yields too few candidates for validation, secondary candidates may be considered where they show a clean Carmen-private pattern in the joint cohort VCF and strong read-level support, but these will be ranked below candidates supported by both analytical approaches. Candidates will then be screened against the curated public WGS background panel, which will be used as a downstream exclusion panel. Any candidate with alternate-allele support in non-Carmen public data will be removed.

Candidate marker selection and specificity

To ensure markers are robust for diagnostic use, candidates must meet the following criteria:

- **Distribution**: Primary candidates must be present in 100% of authenticated Carmen samples, absent from all Hass and close relatives, independently supported by Carmen-versus-Hass Mutect2 analysis, and absent from the curated public non-Carmen WGS background panel where locus-level coverage is informative.
- **Quality metrics**: Mapping quality ≥ 50; coverage ≥ 20 with at least 8-10 alternate reads; allele frequency ≥ 0.4 and ≤ 0.6.
- **Genomic context**: Located outside repetitive or low-mappability regions, surrounded by clean, assayable flanking sequences for downstream primer design.
- **Validation against public data**: Candidate markers must be absent from the curated ≥10x public WGS background panel. Any candidate with alternate-allele support in a non-Carmen public accession will be excluded, provided the locus has sufficient coverage and read quality for interpretation.

Targeted validation in fruit peel DNA

Shortlisted candidate loci will be validated by PCR amplification and bidirectional Sanger sequencing in matched fruit peel DNA from authenticated Carmen Hass and Hass samples. This

step will confirm that candidate markers discovered from leaf-derived WGS data are reproducibly detectable in fruit peel, the tissue most relevant for supply-chain authentication. At minimum, validation will include fruit peel DNA from the four Carmen Hass and four Hass accessions used in the discovery panel. Candidate loci showing clear Carmen-specific heterozygous signal in fruit peel and absence from Hass fruit peel will be prioritised for downstream KASP, TaqMan or targeted amplicon assay development. Where Sanger chromatograms indicate reduced or variable allele representation, candidates may be redirected to amplicon deep sequencing or ddPCR-based validation.

Budget

Cost category	***Description***	***Quantity basis***	***Estimated cost***
DNA extraction reagents and consumables	Extraction and QC of leaf and/or fruit peel DNA from authenticated Carmen Hass, Hass and control accessions.	16 leaf samples + 8 fruit peel samples = 24 samples; 32 samples including contingency	$470.59** (R8,000.00)
WGS Sample QC, library preparation, sequencing	TruSeq PCR-free libraries, NovaSeqX, 150 bp paired-end sequencing to achieve ~30x usable mapped coverage.	16 libraries	$ 4,281.39** (R 72,783.59)
Data delivery	Cloud	1 unit	$ 68.29 (R1161.00)
Candidate primer sets	Synthesis of primers for 20 shortlisted candidate loci.	20 primer sets	$ 705.88** (R12,000.00)
PCR reagents and consumables	PCR validation of candidate loci across Carmen Hass and Hass fruit peel DNA.	20 loci x 8 fruit peel DNA samples plus controls/repeats = 200 PCRs	$ 964.71** (R16,400.00)
Sanger sequencing	Bidirectional sequencing of amplified candidate loci.	Up to 160 amplicons x 2 reads = 320 Sanger reads	$ 2,020.35** (R34,346.00)
Student Bursary	One Masters student will be trained to assist in data analysis (alongside Dr R Backer) and academic article writeup.	2 years	$ 21,176.47 (R360,000.00)
UP overhead	Overhead applied to all funds entering University cost-centers.	15%	$ 4,453.15** (R75,703.56)
Total project cost			**$ 34,140.83 (R580,394.15)**

This budget reflects only the proposed sponsor/funder contribution and excludes the full economic cost of research, including facilities, staff time, student support, supervision, additional overheads, and indirect costs.

** Based on ZAR (R) to U.S. Dollar ($) exchange rate of $1.00 = R17.00*

*** If required, cost may be reduced by lowering the number of samples in the resequencing phase and/or reducing the number of candidate loci screened in fruit peels. However, the experimental design is, in our opinion, balanced in terms of cost-benefit ratio.*

Timeline and milestones

Activity	***Milestone***	***Time***

Sample confirmation and DNA preparation	Authenticated leaf and fruit peel DNA panel prepared and QC-verified.	2 months (DNA extraction)
Whole-genome resequencing	PCR-free WGS data generated for Carmen Hass, Hass and close relatives.	3 months
Bioinformatics processing	Pipeline development and QC-passed, reference-aligned BAM files generated.	2 weeks
Variant discovery	Preliminary Carmen Hass-associated SNP/indel candidate list produced.	1 month
Candidate filtering and specificity assessment	High-confidence candidate markers shortlisted after filtering against Hass, close relatives and public WGS data. Manual curation and verification.	2 months
Fruit peel validation	Shortlisted loci PCR/Sanger-validated in Carmen Hass and Hass fruit peel DNA.	4 months
Final marker recommendation	Ranked diagnostic marker list and assay-development recommendation delivered.	1 month

**Estimated time is based on prior experience along with safety margins. Overall, this project is not expected to take more than 18 months to complete.*

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California Avocado Commission

Production Research Priorities

California Avocado Commission
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CAC Production Research Priorities

Cultural Methods

High Priority Topics:

1. Pollen sprays for avocado orchards: Do they work?
    a. Dusting/spraying of pollen has been done in the avocado industry for decades. Some growers swear by it and others feel that it is not needed. Controlled replicated studies are needed. Methods could include embryo genotyping to verify cross-pollination. Application methods could also be assessed by comparing liquid (AvoSolutions) or dry applications.

    Funded: Mary Lu Arpaia (2024-25 — 2026-27 FY) Does artificial pollination improve yield of 'Hass' and 'GEM' avocado?
2. Updated Cost Studies;
    a. Farming is a business and costs vary depending on operation size and location. A project updating the 2011 Cost Study, providing reference formats for cost analysis, and general reference information to growers would be of value.
    b. Updated avocado cost studies - UC Davis, Riverside, Cal Poly SLO for northern growing regions. Possible senior Projects using 2011 Cost study updated with pricing
3. "Above Average Grower" Survey
    a. The annual California production average is between 5000-7000 pounds per acre however there are growers who consistently outperform this average with 10-year average yields exceeding 12,000 pounds per acre. A project that quantifies what practices and/or conditions allow for this level of success would provide a framework for growers to invest in their operations and build future yields.
4. Leaching Fractions with Modern Rootstocks and Water Sources
    a. Reclaimed water is becoming more available but differs chemically from well and surface water sources. Work assessing how management techniques may need to differ when using these water sources may be of interest.
5. Nitrogen planning and nitrate credits
    a. Nitrogen regulations may impact avocado growers in the future. Many wells and some reclaimed water may contain high nitrate levels, however this nitrogen source does not seem to be 100% plant usable. Work assessing the efficiency of nitrate in well water as a nitrogen source is needed. How does a fertilizer program need to be adjusted for these nitrates?
    b. Are current nitrogen recommendations correct for all clonal rootstocks and scion varieties? Some cultivars, such as GEM, can flower very heavily and defoliate. Would increased nitrogen be an effective strategy to mitigate behavior?
6. Investigate and Evaluate Current Research into Soil Health;
    a. USDA has conducted considerable research into soil health, somewhat at the cornerstone of sustainability. The objective is the building of organic matter. Quantitatively OM translates into higher crop yields. How does this research

relate to commercial avocado production in California, and if so the best approach to evaluate it and ultimately educate growers for their application.

Other Topics of Interest:

7. Rootstock trials for high carbonate and salinity conditions (Do we have enough areas with high carbonates issues that would make it worth establishing a trial?)
8. Tree stress monitoring tools review
9. Use of sulfur for soil acidification. Rates and timing by soil general type (injection of sulfuric acid vs. sulfur burner vs soil applied dry sulfur).
10. Does mulch have effects in frost areas? Does it make it colder or warmer?
11. Is pulse irrigation better than other methods? Do avocados like it or are their roots too saturated too often?
12. Can you apply too much fertilizer that it will harm the tree? Can you oversalt an avocado tree to the point of killing it with common fertilizer?
13. Precision Farming; Introduce the concepts of precision farming into avocado production in California

Irrigation

High Priority Topics:

14. Small farm automation cost analysis: many different systems exist that integrate soil moisture sensors and weather stations with automatic valves and computers to manage irrigation more efficiently and effectively. How does efficient irrigation improve grove performance (yield, phytophthora levels, pest pressure, fertilizer uptake efficiency, etc.). If automation doesn't improve these then does it ever make economic sense to invest in these systems? At what scale does it make economic sense for growers to invest in these systems?
15. Determine practical cultural practices to help mitigate chloride in groves and pursue promising technologies for this problem.
    a. Salinity in general, and chlorides specifically, are especially detrimental to avocado production. What cultural practices or promising technologies exist to practically manage salinity and chlorides in California avocado groves?
16. The CIMIS system provides valuable data to the agricultural community. Unfortunately, we have lost several stations in the avocado growing regions of California which has reduced the system's utility to avocado growers. Work is needed to place new stations or develop and test alternative systems (local stations, small on-farm stations, satellite-based modeling, etc.) support and advocacy needed
    Funded: Andre Biscaro (2025-26 — 2026-27 FY) Creating a Weather Station Network to Guide Irrigation Decision of Avocados
17. Update/code a simple irrigation calculator tailored for avocado growers
    Funded: Andre Biscaro (2024-25 FY) Adapting a User-friendly Online Irrigation Calculator for Avocados

Other Topics of Interest:

18. Irrigation Planning/Management tool including water budgeting tool
19. Small farm automated valves
20. Determine appropriate timing and duration of leaching irrigation for different levels of salinity in irrigation water, soil type, effects of winter rains, weather, etc. to maintain a healthy soil solution for avocado trees.

    Priorities 14, 15, 18 and 20 are all touched upon by Ali Montazar (2025-26 — 2027-28 FY) Assessing irrigation management tools and strategies on avocado fruit quality and yield impacts

Pest Research

High Priority Topics:

21. Avocado Lace Bug: Dr. Mark Hoddle recently completed detailed work on the biology of the avocado lace bug in California. Important follow-up work is now needed to develop management practices for this pest of increasing importance to avocado growers.
    a. Develop effective scouting methods to accurately assess lace bug populations in avocado groves.
    b. Determine treatment thresholds for lace bugs based on feeding damage and predicted damage based on the new biology and lifecycle data so that timely control measures can be implemented.
    c. Conduct pesticide efficacy trials, with particular emphasis on organic options, for the avocado lace bug.
22. Fruit flies: California has seen an unusually high number of fruit fly invasions in recent years. Hass avocados are recognized by the USDA as a non-host of fruit flies in their mature, hard green state on the tree, but this status cannot be extended to other varieties without data to support each variety's non-host status.
    a. Data are needed for GEM, Lamb Hass and other minor varieties to establish their non-hosts status. Ideally, these data will be collected in areas where fruit fly species of importance (Oriental fruit fly, Mediterranean fruit fly, Mexican fruit fly, Queensland fruit fly) are naturally occurring, in cooperation with local cooperators, to avoid having to establish colonies in quarantine facilities in California.
23. Avocado thrips degree day model: the data for the existing degree day model for avocado thrips were collected about 25 years ago. Compared to current techniques for this type of work, these data are very outdated and do not reflect changes in population biology or climate that have taken place over the past 25 years.
    a. Updated data, similar to what was recently completed for the North American bean thrips, for the degree day model of avocado thrips is needed to help avocado growers more effectively manage this pest.
24. The U.S. avocado market has grown exponentially over the past several decades. As a result, many avocado producing countries are trying to gain access to the U.S. market.

However, imported fruit poses a risk of introducing unknown pests and diseases that could be detrimental to the California avocado industry. Most known avocado pest risks are only known due to previous CAC-funded field surveys in potential export countries, but it has been more than a decade since any such work has been conducted.

a. Countries that are likely to gain access to the U.S. market in the near future need to be identified and field surveys of commercial groves and wild populations (if they exist) of avocados should be surveyed for pests and diseases. New pests and diseases will then need to be identified and their biology understood to ascertain the potential risk of invasion through commercial fruit export.

Other Topics of Interest:

25. Persea mite bio-control
26. How can we measure overall tree stress levels to observe how heavy pest presence impacts productivity/fruitfulness? Dendrometers? NDVI?
27. Find out the impact on leaf productivity of both Persea Mite and Avocado Brown Mite in order to develop a treatment threshold to prevent both damage to tree health and over-spraying of miticides.
28. Make field studies to determine naturally occurring biocontrol agents currently active for both Persea Mite and Avocado Thrips as possible candidates for insectary mass-rearing and release.
29. Elucidate the role of *Eusieus hibisci*, a common predatory mite on avocados throughout California, in controlling Avocado Thrips and if there is a link between numbers of *E. hibisci* found in an orchard and the severity of Avocado Thrips pressure.

Ag Chem Product Research

High Priority Topics:

30. Avocado thrips and persea mite management: Abamectin has been the primary tool for managing avocado thrips and persea mite for decades and concern about resistance is growing.
    - A survey of thrips and mite populations for abamectin resistance throughout California avocado groves would be helpful to pest control advisors for making informed decisions about abamectin use.
      Funded: Hamutahl Cohen (2024-25 — 2028-29 FY) A pesticide resistance monitoring program for avocado thrips
    - What insecticides are most effective against persea mite and avocado thrips that can be used in lieu of abamectin?
    - What are the best timing, rate, adjuvants to use in combination with pesticides for most effective control of mites and thrips? Are any products effective if applied through chemigation?
    - What new bee-safe products are available to replace neonics?
    - What products are effective for control in organic production systems?

31. Resistance management of chemical controls for Phytophthora. How best to rotate phosphites, Orondis and Ridomil for maximum effect and control of resistance management.
    - Are any phytophthora populations in California avocado groves showing resistance to currently registered products?
    - Do other active ingredients exist that could be added to the suite of products available for phytophthora management?
      Funded: Patricia Manosalva & Jim Adaskaveg (2025-26 —2027-28 FY) Improve Phytophthora cinnamomi management by monitoring field populations for changes in fungicide sensitivity and conducting efficacy field trials
32. *CDPR approval for Indaziflam (Alion)*
    - *Alion was included in the project that Peggy Mauk completed in 2022. At that time Bayer was unwilling to support registration due to issues they have seen in other shallow rooted species, although no such issues were observed in avocados.*
33. *CDPR approval for Rimsulfuron (Matrix).*
    - *Matrix is in the IR-4 program and residue trials began in 2023.*
34. Avocado Branch Canker (Dothiorella canker, Botryosphaeria canker): recent research projects have done a good job of identifying the suite of pathogens causing branch canker in California avocado groves.
    a. Research is needed to screen fungicides for control and work with manufacturers to get products registered (ex. Syngenta Topsin)
    b. Cultural management practices for branch canker control: pruning, when to pruner, how to prune, sanitation practices, tree removal/when to remove trees.
       Funded: Fatemeh Khodadadi (2025-26 — 2026-27 FY) Integrating Chemical and Cultural Practices for Bot Canker Control in Avocado

Other Topics of Interest:

35. *Herbicide Resistance Management; Survey industry for new herbicides with potential in avocados. (Funded with Peggy Mauk, concluded in 2023)*
36. Evaluate Microbes that convert or fix atmospheric nitrogen to plant utilizable nitrate nitrogen. Rationale: Source of nitrogen with the potential of being more economical than nitrogen produced via petroleum and natural gas, both now and in the future. (ex: Pivot Bio, Kula Bio, Azotic Technologies, Joyn Bio, Max Plans Institute for Terrestrial Microbiology, New Leaf Symbiotic, Intrinsyx Bio, Novozymes, Corteva Utrisha)
37. Evaluate Microbes that enhance the availability and utilization of phosphate by plants. Phosphate is very immobile within the soil, certain microbes can enhance uptake and reduce the quantities of phosphate applied. (ex: Novozymes, AgBiome, BioConsortia, Bayer, Stoller, Valent BioSciences, Verdesian, Lallemand Plant Care, Symborg)
38. Humic Acids; Humates are known to provide a carbon source for stimulating the soil microbiome, which in turn provides for enhanced uptake of macro, secondary and minor elements. Research into evaluating what microbes are stimulated (UC Santa Barbara Ph.d. project) and their efficacy in taking up nutrients by humic acid…objective is to reduce the quantities of fertilizer.

39. Deer Control; Deer cause significant damage to newly planted groves (and established) each year. Several candidate sprays are being advertised. Set up and evaluate deer repellants in randomized trials. Establish project to survey for potential repellants
40. Additional research into alternative weed control methods for new orchards.
41. Preemergent herbicide use on new orchards assessing impacts on tree establishment.