STATE MARKETING BOARDS
GRANT AGREEMENT
SIGNATURE PAGE

AGREEMENT NUMBER
QUI-23

1. This Agreement is entered into between the Grantor and the Recipient named below:

GRANTOR
CALIFORNIA CITRUS NURSERY RESEARCH AND EDUCATION BOARD

RECIPIENT'S NAME
The Regents of the University of California on Behalf of UC Riverside **(campus name or ANR)**

2. The term of this Agreement is: One Year

The project term as identified in Attachment 1, Scope of Work and Budget is: 1/1/23 to 12/31/23

To extend the project term, the Recipient ***must*** obtain written approval from the GRANTOR in accordance with the terms of this Agreement.

3. The maximum amount of this Agreement is $ 87,928

4. The parties agree to comply with the terms and conditions of the following exhibits which are by this reference made a part of the Agreement:

Exhibit A - Contacts/Notifications	1 Page
Exhibit B - Budget & Payment Provisions	2 Pages
Exhibit C – General Terms and Conditions	6 Pages
Attachment 1 – Scope of Work and Budget	

Name of Project: In Vitro Propagation of Citrus Rootstocks and Bioindexing Indicators

IN WITNESS WHEREOF, this Agreement has been executed by the parties hereto.

RECIPIENT

RECIPIENT'S NAME *(Organization's Name)* The Regents of the University of California

BY *(Authorized Signature)*	DATE SIGNED *(Do not type)*
Karen Garcia Karen Garcia (Jan 18, 2023 15:14 PST)	

PRINTED NAME AND TITLE OF PERSON SIGNING Karen Garcia, Senior Contract and Grant Officer

ADDRESS Sponsored Research Administration, 245 University Office Building, Riverside CA 92521-0217

STATE OF CALIFORNIA

STATE ENTITY'S NAME California Citrus Nursery Research and Education Board
(GRANTOR)

BY *(Authorized Signature)*	DATE SIGNED *(Do not type)*
Don Dillon Don Dillon (Jan 18, 2023 15:09 PST)	

PRINTED NAME AND TITLE OF PERSON SIGNING
Don Dillon, Board Chairman

ADDRESS
531-D North Alta Avenue, Dinuba CA 93618

EXHIBIT A

RECIPIENT AND PROJECT INFORMATION

1. The Grant Manager/Principal Investigator for this Agreement are:

FOR GRANTOR:	**FOR RECIPIENT:**
Name: John Gilstrap , Manager	Name: Paulina Quijia-Lamina
Section/Unit: Grants Management Office	Department/Program: Micro & Plant Path
Address: 531-D North Alta Avenue	Address: University of California
City/Zip: Dinuba CA 93618	City/Zip: Riverside CA 92521
Phone: 559-591-9005	Phone: 352-870-9483
Email Address: john@tabcomp.com	Email Address:paulinaq@ucr.edu

The ***Grant Specialist/Administrative Contact*** for this Agreement are:

FOR GRANTOR:	**FOR RECIPIENT:**
Name: Kim Sakamoto , Grant Administrator	Name: Karen Garcia, Senior Contract and Grant Officer
Board Name: California Citrus Nursery Research Board	Office: 245 University Office Building
Address: 531-D North Alta Avenue	Address: 245 University Office Building
City/Zip: Dinuba CA 93618	City/Zip: Riverside CA 92521-0217
Phone: 559-591-9005 Fax: 559-591-5744	Phone: 951-827-3692
Email Address: kim@tabcomp.com	Email Address: karen.garcia@ucr.edu

FISCAL CONTACT (If different from above):	
Name:	Rosaline Alonzo-Le, Manager
Office	Extramural Funding
Address:	900 University Blvd
City/Zip:	Riverside CA 92521-9800
Phone:	951-827-1954
Email Address:	rosaline.alonzo-le@ucr.edu

2. For a detailed Scope of Work and Budget, see Attachment 1.

EXHIBIT B

PAYMENT PROVISIONS AND BUDGET

1. Invoicing and Payment

A. The Recipient may not invoice for work performed prior to the commencement date or completed after the Project term date of this Agreement, unless prior approval from GRANTOR (Grant Manager) is obtained, and the Project term date is amended.

B. For activities satisfactorily rendered and performed according to the attached Scope of Work and Budget (Attachment 1), and upon receipt and approval of the invoices, the GRANTOR agrees to reimburse the Recipient for actual allowable expenditures incurred in accordance with this Agreement and stated herein, which is attached hereto and made a part of this Agreement. Approval of invoices shall not be withheld based on scientific differences between Recipient and GRANTOR in the interpretation of the research data and final conclusions.

C. Invoices shall be submitted to the Grant Manager within sixty (60) days after the end of each quarter or month in which work under this Agreement was performed. Should GRANTOR require additional documentation supporting an item of expense on RECIPIENT's invoice, Recipient shall provide the documentation within 15 days of receipt of GRANTOR's request.

D. A final invoice shall be submitted for payment no more than 60 calendar days following the expiration date of this Agreement, or 60 calendar days after the project is complete, whichever comes first. Recipient 's invoice should be clearly marked "Final Invoice," indicating that all payment obligations of the GRANTOR under this Agreement have ceased and no further payments are due or outstanding.

E. At least ten percent of the total amount awarded in this Agreement will be withheld until receipt, review and acceptance of the Final Performance Report for one-year projects, and the Annual Progress Report for multi-year projects.

2. Suspension of Payments

This Agreement may be subject to suspension of payments or termination, or both, and Recipient may be subject to debarment if the GRANTOR determines that:

A. Recipient or Subgrantees, as defined in Exhibit C, Article 11(F), have made a false certification, or
B. Recipient or Subgrantees violates the certification by failing to carry out the requirements noted in this Agreement.

3. Budget Contingency Clause

If funding for any fiscal year is reduced or deleted for purposes of this program, the GRANTOR will have the option to either terminate this Agreement in accordance with the Right to Terminate (Paragraph 16) or offer to amend the Agreement to reflect the reduced amount.

4. Prompt Payment Clause

Payment will be made in accordance with, and within the time specified in, California Government Code Title 1, Division 3.6, Part 3, Chapter 4.5, commencing with Section 927 – The California Prompt Payment Act.

5. Allowable Expenses/Fiscal Documentation

A. The GRANTOR will reimburse Recipient only for allowable expenses in accordance with the Scope of Work and Budget and applicable State and Federal laws.

B. GRANTOR shall have no obligation to reimburse Recipient under this Agreement until Recipient has satisfied the following condition (if applicable):

1. For the term of this Agreement, Recipient shall submit timely Progress Reports as specified in Attachment 1 – Scope of Work and Budget. If Progress Reports are not received per Attachment 1, the GRANTOR may invoke the Disputes Clause, Exhibit C, Paragraph 7. When Progress Report is received, payment will not be withheld. If Disputes Clause is not invoked by GRANTOR, timely payment will be made to Recipient.

C. The Recipient must maintain adequate documentation for expenditures of this Agreement to permit the determination of the allowability of costs under this Agreement. If the GRANTOR cannot determine expenditures are allowable under the terms of this Agreement because records are nonexistent or inadequate, the GRANTOR may disallow the expenditures. These records shall be kept for three (3) years after project completion, or final invoice, or resolution of a dispute or litigation, whichever comes last.

6. Budget

For a detailed Budget for all work to be performed under the Scope of Work, see Budget (Attachment 1).

7. Budget Flexibility

Budget revisions between identified budget categories in cost reimbursement agreements that are within the total contract amount are allowed as described below:

Up to 10% of each annual budget amount or $10,000, whichever is less, is allowed with approval of the Grant Manager.

Exceeding 10% or $10,000, whichever is less, of the last approved budget will require: the Grant Manager's prior approval; and a formal amendment to this Agreement. The Recipient shall submit a revised budget to the GRANTOR for approval.

Budget transfers that would cause any portion of the funds to be used for purposes other than those consistent with the original intent of this Agreement are not allowed. Notwithstanding the above provision, the GRANTOR may proceed with a formal amendment to this Agreement for budget revisions.

EXHIBIT C

GENERAL TERMS AND CONDITIONS

1. Approval

This Agreement has no force or effect until signed by both parties.

2. Governing Law

This Agreement is governed by and will be interpreted in accordance with all applicable California laws.

3. Recipient Commitments

Recipient accepts and agrees to comply with all terms, provisions, conditions and commitments of the Agreement, including all incorporated documents, and to fulfill all assurances, declarations, representations, and statements made by the Recipient in the application, documents, and amendments that were provided and authorized by the Administrative Contact in support of its request for funding.

4. Performances and Assurances

Recipient agrees to faithfully and expeditiously perform or cause to be performed all project work as described in Attachment 1 – Scope of Work and Budget, and to apply grant funds received only to allowable project costs.

5. Assignment

This Agreement is not assignable by the Recipient, either in whole or in part, without the written consent of the GRANTOR.

6. Liability/Indemnification

A. The Recipient agrees to indemnify, defend and save harmless the GRANTOR, its officers, agents and employees from any and all liability, loss, expense, attorneys' fees or claims for injury or damages in the performance of this Agreement, but only in proportion to and to the extent such liability, loss, expense, attorneys' fees, or claims for injury or damages are caused by or result from the negligent or intentional acts or omissions of the Recipient, its officers, agents, or employees.

The GRANTOR agrees to indemnify, defend and save harmless the Recipient, its officers, agents and employees from any and all liability, loss, expense, attorneys' fees or claims for injury of damages in the performance of this Agreement, but only in proportion to and to the extent such liability, loss, expense, attorneys' fees, or claims for injury or damages are caused by or result from the negligent or intentional acts or omissions of the GRANTOR, its officers, agents, or employees,

7. Disputes/Default

A. Dispute

The Recipient shall continue with the responsibilities under this Agreement during any dispute, except in the event of a substantial breach. In the event of a dispute, a "Notice of Dispute" is filed with the [GRANTOR/Recipient] within 30 calendar days of discovery of the problem. The Notice of Dispute must contain the Agreement number. Within 30 calendar days of receipt of the Notice of Dispute, GRANTOR and Recipient will meet for the purpose of resolving the dispute. In the event of a dispute, the language contained within this Agreement prevails.

B. Default Provisions

Subject to Article 10. Force Majeure, Recipient will be in default under this Agreement if any of the following occur:

1. Substantial breaches of this Agreement or any supplement or amendment to it.
2. Knowingly making any false representation, or statement with respect to this Agreement or the application filed to obtain this Agreement.
3. Failure to operate or maintain project in accordance with this Agreement.
4. Failure to make any remittance required by this Agreement.

Should an event of default occur, GRANTOR shall provide in writing a Notice of Default to the Recipient within 30 calendar days. Recipient shall have at least 30 calendar days to cure the default from the date the written notice is sent to the Recipient. If the Recipient fails to cure the default within the time prescribed by this Agreement, GRANTOR may terminate the Agreement according to the Right to Terminate (Paragraph 16).

C. Failure to Mutually Resolve Dispute

If both Parties cannot agree upon a resolution after following the processes described in this Agreement for Disputes or Default, both Parties retain the right to bring a lawsuit or seek any other legal or equitable remedy either Party may have.

8. Accounting and Deposit of Grant Funding

A. Separate Accounting of Funding Disbursements: Recipient shall account for the money disbursed pursuant to this Agreement separately from all other Recipient funds. Recipient shall maintain audit and accounting procedures that are in accordance with generally accepted accounting principles and practices, consistently applied. Recipient shall keep complete and accurate records of all receipts and disbursements. Recipient shall require its Subgrantees to maintain books, records, and other documents pertinent to their work in accordance with generally accepted accounting principles and practices. Records are subject to inspection by GRANTOR during regular business hours.

B. Fiscal Management Systems and Accounting Standards: The Recipient agrees that, at a minimum, its fiscal control and accounting procedures will be sufficient to permit tracing of grant funds to a level of expenditure adequate to establish that such funds have not been used in violation of State or Federal law or this Agreement.

C. Disposition of Money Disbursed: All money disbursed pursuant to this Agreement shall be deposited, administered, and accounted for pursuant to the provisions of applicable law.

9. Equipment

Any equipment purchases must be included in the Scope of Work and Budget, and must contain:

- A description of the equipment
- How the equipment will be used
- A justification in support of the equipment's necessity
- The disposition of the equipment at the end of the project

Equipment is defined as:

- Non-expendable (having a normal life expectancy of one year or more);
- Tangible (can be appraised for value);
- Free standing (complete in itself, does not lose its identity when affixed to or installed in other property); and
- Having an acquisition cost of $5000 or more.

10. Force Majeure

Neither party shall be liable to perform as required by this agreement to the extent such failure to perform is caused by any of the following: labor disturbances or disputes of any kind, accidents, failures of any required governmental approval, civil disorders, acts of aggression, acts of God, energy or other conservation measures, failure of utilities, mechanical breakdowns, material shortages, disease, or similar occurrences.

11. Subgrantees

A. Recipient agrees to comply with all applicable Federal, State, and local laws and regulations during the term of this Grant Agreement. All Subgrantees must have the proper licenses/certificates required in their respective disciplines.

B. Recipient must follow its documented procurement procedures when contracting with a Subgrantee. The procedures must reflect applicable Federal, State, and local laws and regulations.

C. Recipient shall comply with all applicable laws and regulations regarding securing competitive bids and undertaking competitive negotiations in Recipient's contracts with other entities for acquisition of goods and services, with grant funds under this Agreement.

D. A subgrant under this Agreement must be a written agreement between the Recipient and the Subgrantee, and must state the activities to be performed, the time schedule, the policies and requirements that apply to the Subgrantee, the amount of the subgrant, and a justification determining allowable costs.

E. The subgrant must not affect the Recipient's overall responsibility for the management of the project, and the Recipient must reserve sufficient rights and control to enable it to fulfill its responsibilities under this Agreement.

F. If the Recipient provides funds to any third party ("Subgrantee"), excluding any agency or department of the United States, to accomplish any of the work of this Agreement, the Recipient shall first enter into a written agreement with each Subgrantee by which the Subgrantee agrees to indemnify and hold harmless the GRANTOR and the State of Californiaa, a and its officers, agents, and employees from any and all liabilities, losses, claims, demands, damages, or costs, including without limitation litigation costs and attorney's fees, resulting from or arising out of the Subgrantee's performance under its agreement with the Recipient, but only in proportion to and to the extent such liability, loss, expense, attorneys' fees, or claims for injury or damages are caused by or result from the negligent or intentional acts or omissions of the Subgrantee, its respective officers, agents or employees. The foregoing does not limit any breach of contract action that the GRANTOR may have against the Recipient. Nothing contained in this Agreement or otherwise creates any contractual relation between the GRANTOR and any Subgrantee, and no contract relieves the Recipient of its responsibilities and obligations hereunder. The Recipient's obligation to pay its Subgrantee is an independent obligation from the GRANTOR's obligation to make payments to the Recipient. As a result, the GRANTOR has no obligation to pay or to enforce the payment of any moneys to any Subgrantee.

G. Recipient will perform their standard due diligence review of all Subgrantees before executing subgrants.

12. Audits

GRANTOR reserves the right to conduct an audit at any time between the execution of this Agreement and the completion of the project, with the costs of such audit borne by GRANTOR. After completion of the project, GRANTOR may require Recipient to conduct a final audit to GRANTOR's specifications, at GRANTOR's expense, such audit to be conducted by and a report prepared by an independent Certified Public Accountant. Failure or refusal by Recipient to comply with this provision shall be considered a breach of this Agreement, and GRANTOR may elect to pursue any remedies provided in the "Default Provisions" section of this Agreement,

Pursuant to Government Code Section 8546.7, the Recipient shall be subject to the examination and audit by the GRANTOR for a period of three years after final payment under this Agreement with respect to all matters connected with this Agreement, including but not limited to, the cost of administering this Agreement. All records of Recipient or its Subgrantee shall be preserved for this purpose for at least 3 years after project completion or final billing, whichever comes later.

13. Non-Discrimination Clause

With respect to this section, "contract" means this Agreement; "contractor" means University; and "subcontract" means Subaward.

During the performance of this contract, contractor and its subcontractors shall not unlawfully discriminate against any employee or applicant for employment because of race, religious creed, color, national origin, ancestry, physical disability, mental disability, medical condition, genetic information, marital status, sex, gender, gender identity, gender expression, age, sexual orientation, or military and veteran status. Contractors and subcontractors shall insure that the evaluation and treatment of their employees and applicants for employment are free of such discrimination. Contractors and subcontractors shall comply with the provisions of the Fair Employment and Housing Act (Gov. Code, § 12900 et seq.) and the applicable regulations promulgated thereunder (Cal. Code Regs., tit. 2, § 11000 et seq.). The applicable regulations of the Fair Employment and Housing Council implementing Government Code section 12990, set forth in Subchapter 5 of Division 4.1 of Title 2 of the California Code of Regulations are incorporated into this contract by reference and made a part hereof as if set forth in full. Contractor and its subcontractors shall give written notice of their obligations under this clause to labor organizations with which they have a collective bargaining or other agreement.

Contractor shall include the nondiscrimination and compliance provisions of this clause in all subcontracts to perform work under the contract.

14. Unenforceable Provision

In the event that any provision of this Agreement is unenforceable or held to be unenforceable, then the parties agree that all other provisions of this Agreement have force and effect and will not be affected thereby.

15. Excise Tax

The State of California is exempt from Federal excise taxes and no payment will be made for any taxes levied on employees' wages. The State will pay for any applicable State of California or local sales or use taxes on the services rendered or equipment or parts supplied pursuant to this Agreement. California may pay any applicable sales and use tax imposed by another State.

16. Right to Terminate

A. Both parties reserve the right to terminate this Agreement for Good Cause upon thirty (30) days written notice. Good Cause is defined as impossibility of performance or frustration of purpose. Good Cause does not include substantial breach (defined under the Disputes/Default clause above) or termination for convenience. Upon receipt of the GRANTOR's notice of termination or upon Recipient providing GRANTOR a notice of termination, the Recipient shall take reasonable efforts to limit or terminate all financial commitments and shall not incur new obligations under this Agreement. The GRANTOR shall reimburse the Recipient for costs incurred up to the effective date of termination and for costs incurred due to non-cancellable obligations, up to the undisbursed balance of funds authorized in this Agreement.

B. In the case of early termination, the Recipient will submit, within ninety (90) days of the termination date, an invoice and a report covering services up to the termination date. Any deliverable as described in Attachment 1 to this Agreement that is fully or partially completed up to the termination date (work product), shall be provided to the GRANTOR.

C. Upon receipt of the invoice, progress report, data, and work product, a final payment shall be made to the Recipient. This payment shall be for all costs incurred in accordance with this Agreement, and shall include labor and materials purchased or utilized (including all Non-cancellable Obligations) up to the termination date, and pro rata share of indirect costs as specified in the proposal budget.

D. Pursuant to a Governor's Executive Order or equivalent directive, such as a court order or an order from a federal or state regulatory agency, the GRANTOR may issue a Suspension Notice. The Notice shall identify the specific Executive Order or directive and the Agreement number(s) subject to suspension. Work charged to the GRANTOR shall stop immediately upon receipt of the Notice. The Recipient retains the right to reimbursement of costs incurred to date, including non-cancellable obligations, and reserves the right to seek reimbursement through administrative or legal action.

E. The Recipient shall include in any contract with any Subgrantee retained for work under this Agreement a provision that entitles the Recipient to suspend or terminate the agreement with the Subgrantee for any reason on written notice and on the same terms and conditions specified in this section.

17. Amendments

Changes to Scope of Work, Budget or the Project term, shall be requested in writing to the GRANTOR Grant Manager via letter, fax or email. GRANTOR Grant Manager shall respond in writing via letter, fax or email as to whether the proposed changes are accepted prior to implementing any change. Any accepted changes to the Scope of Work, Budget, or Project term must be subsequently approved by GRANTOR and an amendment signed by both Parties within 90 days of acceptance by the Grantor Grant Manager.

18. Confidentiality and Public Records

The Recipient and the GRANTOR understand that each party may come into possession of information and/or data which may be deemed confidential or proprietary and marked "Confidential" by the person or organization furnishing the information or data ("Confidential Information"). Such information or data may be subject to disclosure under the California Public Records Act, commencing with Government Code, Section 6250, or the Public Contract Code. The GRANTOR agrees not to disclose such information or data furnished by the Recipient and to maintain such information or data as confidential when so designated by the Recipient in writing at the time it is furnished to the GRANTOR, only to the extent that such information or data is exempt from disclosure under the California Public Records Act and the Public Contract Code.

19. Right to Publish

A. Subject to any restrictions on the publication, disclosure, dissemination and use of Confidential Information set forth in this Agreement or under any applicable law, the Recipient shall have the right to publish, disclose, disseminate and use, in whole and in part, any data and information received or developed under this Agreement.

B. The Recipient will provide publications, presentations and other public releases directly resulting from work performed under this Agreement to the GRANTOR for review at least thirty (30) calendar days prior to publication and will identify the proposed recipient(s). During the first fifteen (15) calendar days of such review period, the GRANTOR may provide notice to the Recipient that it intends to rebut some or all aspects of the presentation, publication or other public releases. Upon Recipient's public disclosure of the presentation, publication or other public release, the GRANTOR may prepare and submit such rebuttal to the recipient(s) identified by the Recipient. Within the review period, the GRANTOR may provide feedback to the Recipient; the Recipient shall give good faith consideration to such feedback, but has no obligation to make any changes in said material, other than the removal of any material whose disclosure is prohibited or restricted by this Agreement or by any applicable law. Any of the above referenced time periods may be modified upon agreement of both Parties. Neither Party may unreasonably deny such requests.

C. At the GRANTOR's sole discretion, the GRANTOR will require the Recipient to use one of the following disclaimers in any publication, presentation or other public release:
   1. "This project was funded by the <GRANTOR>. The contents may not necessarily reflect the official views or policies of the <Grantor> or the State of California."
   2. "This project was funded by the <GRANTOR>. The contents do not represent the official views or policies of the <GRANTOR>, or the State of California."

20. Copyrights

A. All rights in copyrightable works first created by the Recipient in the performance of the Scope of Work, Attachment 1, under this Agreement are the property of the Recipient. The Recipient shall grant the GRANTOR a royalty-free, nonexclusive, irrevocable license to use, reproduce, prepare derivative works, and distribute copies of the Deliverables identified in Attachment 1 to fulfill the GRANTOR's State purposes.

B. Notwithstanding the above, if the purpose of the Scope of Work is specifically to create a copyrightable work for use by the State and that fact is indicated as a deliverable in Attachment 1, which may be amended upon mutual agreement of the Parties, then all rights in such copyrightable work will be the property of the State, subject to a reserved right for the University to use the copyrightable work for educational and research purposes and to allow other educational and nonprofit institutions to do so for educational and research purposes.

C. Upon written request and subsequent amendment, the State may request delivery of computer software that is not identified as a deliverable on Attachment 1, but was first created in the performance of the Scope of Work. To the extent the University is legally able to do so, University shall grant a fully paid-up, royalty-free, nonexclusive, sublicensable, irrevocable license to use, reproduce, prepare derivative works, and distribute copies, to fulfill the State's government purposes, subject to restrictions, if any, identified on Attachment 1.

21. Patents

Patent Rights – Recipient

A. Subject to the requirements of law, all rights to any patentable inventions or discoveries conceived and first actually reduced to practice in the performance of the Scope of Work, Attachment 1, conducted under this Agreement ("Patentable Inventions") shall belong to the Recipient. The State shall have a nonexclusive, sublicensable, irrevocable, paid-up license to practice or have practiced such Patentable Invention for government purposes.

B. A State Confirmatory License (attached) will be executed by the Recipient to provide said license to any such Patentable Invention, within ninety (90) days after filing of patent application.

C. Recipient shall file, prosecute and maintain a patent application claiming a Patentable Invention described in paragraph A above within two years of disclosure of a Patentable Invention to Recipient by inventors and will diligently pursue broad application of such Patentable Invention. If State notifies University of a need that is not being met by Recipient, Recipient will take steps to meet such need or will offer sufficient field-of-use rights to State to address such unmet need.

D. If Recipient decides not to file a patent application within such two-year period or decides to abandon a patent or patent application claiming such Patentable Invention, and determines that it does not intend to pursue commercialization of such Patentable Invention, then Recipient will notify the State in sufficient time to preserve patent rights, and upon State agency request, Recipient agrees to assign title to State, subject to requirements of law, outstanding rights in third parties, and a reserved right to use the Patentable Invention for educational and research purposes and to allow other educational and nonprofit institutions to do so.

E. Copyrightable works that may be patentable are also subject to the Patent Rights clause, which will take precedence in case of a conflict.

22. Travel

Travel and reimbursement for Recipient employee travel costs shall be in accordance with the Recipient's travel policy in effect as of the date the cost is incurred. The Recipient's travel policy is found at: http://policy.ucop.edu/doc/3420365/BFB-G-28 [UC].

23. Closeout

The grant will be closed out after the completion of the project, receipt and payment of the final invoice and receipt and acceptance of the final report, resolution of any audit/desk review findings, and resolution of any performance or compliance issues.

Attachment 1
Scope of Work and Budget

Project Title: In Vitro Propagation of Citrus Rootstocks and Bioindexing Indicators

Project Summary:

Upgrade and optimize the shoot-tip grafting (STG) tissue culture protocol used in the CCPP for therapy and pathogen elimination from citrus materials. Standardize and optimal in vitro propagation protocol to produce citrus plant indicators and rootstocks species that are required for therapy and bioindexing procedures. Standardize optimal in vitro propagation protocols to produce different rootstocks genotypes, that can be potentially implemented by the citrus nursery industy in commercial scale.

Deliverables:

List all items that will be delivered by Recipient to GRANTOR under this Scope of Work:

Identify deliverable	Date due	Specify in this column if the deliverable is a copyrighted work subject to GRANTOR ownership as set forth in Exhibit C, Paragraph 20(B). If not checked, default is for Exhibit C, Paragraph 20(A) to control.
1. Annual Report	November 1, 2022	
2.		
3.		
4.		

Third-Party data, materials or IP:

Will any data, materials or intellectual property owned by a third party be knowingly utilized by Recipient in the performance of the project?

If Yes, list third party data, materials and/or intellectual property here.

Will GRANTOR be restricted from its use of any deliverables, as identified above, due to any third-party encumbrance? If yes, describe restriction.

Detailed Project Description – see attached additional pages.

Budget – see attached additional pages.

CALIFORNIA CITRUS NURSERY BOARD

PROJECT PLAN

Fiscal Year: 2023 **Duration of Project:** 3 years

This project is: Ongoing, Year 2 of 3

Project Leader: Paulina Quijia-Lamina

Location/Department: Department of Microbiology & Plant Pathology, University of California, Riverside, CA 92521.

Phone: +909 602 6848
E-mail: paulinaq@ucr.edu

Collaborators: Georgios Vidalakis, UC Riverside, (951) 237 9948, vidalg@ucr.edu

Project Title: In vitro propagation of citrus rootstocks and bioindexing indicators

Keywords: Micropropagation, rootstocks, Bio-indexing indicators, production rates, tissue culture,

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EXECUTIVE SUMMARY

The citrus nursery industry depends on efficient and high production rates of selected rootstocks varieties of citrus. The conventional method of rootstock propagation consists of the germination of open pollinated, highly apomictic and polyembryony seeds. To maintain clonal uniformity, 1 to 40 % of zygotic origin seedlings (depending on the rootstocks genotype) are culled from nursery seed beds. Reducing the production rates significantly, especially in genotypes that exhibit low levels of nucellar embryony and therefore do not produce adequate quantities of nucellar seedlings. Reduction in seed production of some valuable rootstock species have also limited the supply seeds to citrus nurseries. Introduction into California of new citrus varieties via the Citrus Clonal Protection Program (CCPP) also requires constant rootstock supply for therapy (shoot-tip grafting) as well as a high number of plant indicators for bio-indexing (VI index). Slow production of rootstocks and bioindicators plants have limited bio-indexing activities resulting in increased time for a variety to be released from quarantine.

Other important superior rootstocks that provide high yield efficacy and potential tolerance/resistance to citrus diseases including Huanglongbing (HLB) have been developed recently. However, they produce few or no seeds thus making difficult to comply with the high demand of quality plant material required by the citrus nursery industry. *In vitro* culture techniques such as micropropagation, can be used as viable strategy to alleviate these problems. These techniques allow the production of large number of propagules in relatively small space, reducing

the necessity of a constant seed supply. Uniform, disease-free, and high-quality plant production can be obtained using this approach. Therefore, this study will be undertaken with two main objectives. One, to develop an optimized protocol for *in vitro* propagation of different citrus varieties/species used as rootstock and bio indexing indicators at the CCPP. Two, generate the basic knowledge for future use of micropropagation protocols in commercial scale in California nurseries for “seedless” rootstock production.

BENEFITS TO CITRUS NURSERY INDUSTRY

The citrus nursery industry relies on the rapid propagation and constant supply of citrus varieties used as rootstocks. Programs like the CCPP that provide, pathogen-tested, disease-free budwood to citrus nurseries in California, also require a reliable protocol to produce rootstocks and plant indicator species used during therapy and bio-indexing procedures. Open-pollinated seed germination and rooting of cuttings are methods traditionally used to propagate rootstocks and plant indicators. Many citrus species/genotypes exhibit nucellar embryony. Somatic embryos can develop from the nucellar mother tissue altogether with the zygotic embryos. The advantage of nucellar gemination is the reproduction of true-to-type offspring and clonal uniformity of the produced population. However, propagation by seed gemination is not suitable when genotypes have low levels of nucellar embryony and therefore do not produce adequate quantities of nucellar seedlings. Global reduction in clean seed production of some rootstock species due to the presence of pathogens or diseases and in some cases single or limited number of commercial seed suppliers limits the availability of “clean” seeds to citrus nurseries. High demands of quality planting material of rootstocks and plant indicators is difficult and, in some cases, cannot be fulfilled under these circumstances.

Rootstocks with superior characteristics such as high yield efficiency and tolerance/resistance to devastating diseases have been developed. However, some potentially valuable rootstocks produce few or no seeds, thus limiting their access to the citrus nurseries. That is the case of rootstocks US-1279, US-1281, and US-Super Sour 1, which exhibited outstanding field performance but the nucellar embryony phenomena is not present (Albrecht et al. 2020). *In vitro* culture propagation techniques can be a viable alternative to obtain uniform disease-free citrus rootstocks and plant bioindicators in high numbers not limited by the seasonal citrus seed supply and storage. Several crop species have been successfully propagated using tissue culture techniques (Gološin and Radojevic 1987; Teixeira da Silva et al. 2019; Hiti-Bandaralage et al. 2017). *In vitro* culture protocols, including micropropagation, for the propagation of some citrus genotypes have been reported in the literature as well (Carimi and De Pasquale 2003; Sharma et al, 2009; Chiancone and Germanà 2012).

Micropropagation can be defined as the true-to-type propagation of selected genotypes under *in vitro* culture conditions. *In vitro* propagation can be accomplished using 1. Enhanced axillary shoot proliferation (Shoot culture), 2. Node culture, 3. Adventitious shoot organogenesis, or 4. Somatic embryogenesis (Kane 2011). Although shoot and node culture are not the more efficient procedures, they provide genetic stability and has been used as a reliable method to propagate

many plant species. A micropropagation protocol has different stages to be successfully completed to obtain reliable rates and consistency of propagule multiplication (Fig. 1).

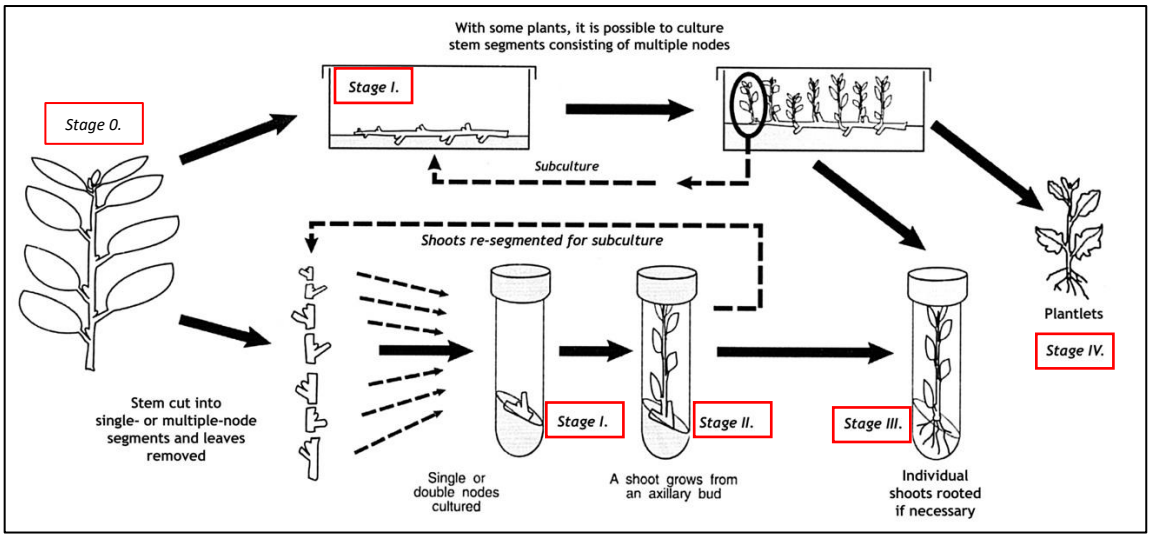

Figure 1. Micropropagation stages for plant production using node culture (Taken from https://users.ugent.be/~pdebergh/mic/mic2cd01.htm)

High cost of rootstocks propagated vegetatively under *in vitro* culture conditions and their presumable inferior root system (adventitious lateral roots) compared with seedlings (well-defined taproot) have been concerns driving the preference of seedlings rootstocks over tissue culture plants. However, studies on the influence of propagation methods (i.e., cuttings, tissue culture, and seed germination) in root architecture of different rootstocks cultivars demonstrated that differences in root architecture among plants derived from different propagation methods did not affect plant height and water and nutrients uptake capacity during nursery stages and after 1 year of growth (Albreacht et al. 2017; Albreacht et al. 2020). In addition, during 2 years of growth in the field root systems were not determined by the propagation method as it was observed at the nursery stages and a well-defined taproot was not observed in seed propagated rootstocks (Fig. 2) (Pokhrel et al 2021).

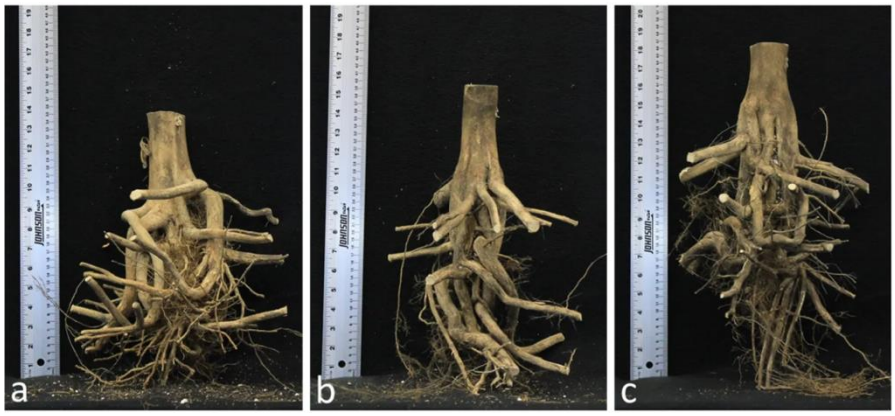

Figure 2. Root systems of field-grown Valencia trees grafted on a) seed-propagated, b) cuttings-propagated and c) tissue culture-propagated rootstocks after 2 years in the field (Pokhrel et al 2021)

Under current HLB conditions that drastically affect the citrus industry (70% reduction in Florida citrus production) it is imperative that the CCPP upgrade and optimize its shoot-tip grafting (STG) protocol to meet the increasing demand of clean plant propagation material Shoot tip grafting has been the foundation for pathogen elimination and therapy of citrus propagative materials produced in the CCPP since 1975 (Navarro et al. 1975). Protocols developed by Navarro et al. 1975 have been used in the CCPP without modification or improvement, resulting in high contamination rates and low production of shoots, leading to high frequency of budwood material loss (Fig. 3)

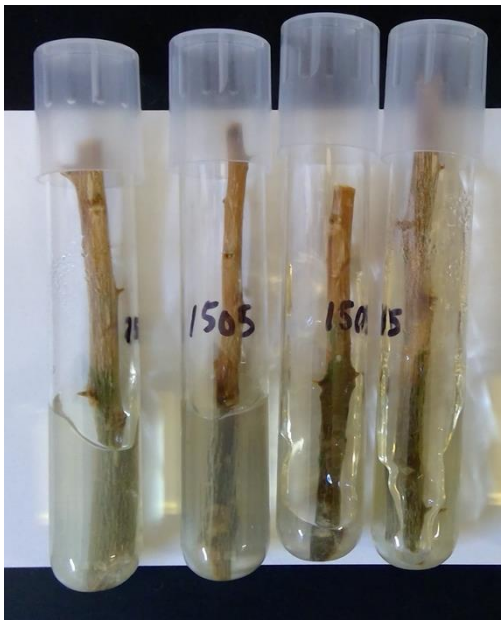

Figure 3. Death and contaminated Budwood culture of a citrus variety introduced in the CCPP pipeline

Shoot-tip grafting is a tissue culture technique that required the optimization of budwood culture and seed gemination under *in vitro* culture conditions. Shoots sprouting from a sterile budwood culture are used to obtain shoot tips to be grafted on *in vitro* geminated rootstocks seedlings. Successful surface sterilization, optimal seed germination, and seedling development and reliable budwood culture protocols need to become part of the routine protocols used at the CCPP. Scientific information and standardized protocols obtained from this project will be critical to improve the protocols currently used in the CCPP and can be potentially use and implemented by the citrus nursery industry within their production lines.

In summary, this project will establish standardized in vitro culture protocols upgrade CCPP's therapy procedures. Micropropagation pipeline will be stablished to produce plant bioindicators and rootstocks use for variety index (VI). Foundational knowledge will be obtained for *in vitro* culture production of citrus rootstocks (especially of the ones that do not produce seeds) for future use by California citrus nurseries in commercial scale. Results and scientific knowledge from this project will satisfy one of the CCNB research priorities which is the improvement of rootstocks management including growing, planting, and sprouting.

OBJECTIVES

1. Upgrade and optimize the shoot-tip grafting (STG) tissue culture protocol used in the CCPP for therapy and pathogen elimination from citrus propagative materials.
2. Standardize an optimal *in vitro* propagation protocol to produce citrus plant indicators and rootstocks species that are required for therapy and bioindexing procedures.
3. Standardize optimal *in vitro* propagation protocols to produce different rootstocks genotypes, that can be potentially implemented by the citrus nursery industry in commercial scale.

WORKPLANS & METHODS

Proposed protocol

Stage 0: Donor plant selection and preparation

Different rootstocks and plant indicators species will be used for this project. For example, the commonly used trifoliate and trifoliate rootstocks (e.g., Carrizo citrange, C35 etc.) as well as rootstocks recently released by USDA-ARS (e.g., US-802, US-942, US 1283 etc.) will be used. We will also request the input of this committee as well as that of individual citrus propagation nurseries for rootstock species to be used in this study. In addition, the collaborator Dr. G. Vidalakis, CCPP Director, will provide guidance on the seven citrus species used in bioindexing (e.g. Mexican lime, Sweet orange, Dweet tangor etc.). Donor plants will be maintained in CCPP greenhouses.

Stage 1: Establishment of Aseptic cultures

Stem nodes with 1-2 lateral buds ca. 3 cm long will be cut from the donor plant and used as explants. Leaf petioles will be removed. Explants will be washed under running tap water for 10 minutes and disinfected with ethanol 70% for 60 s followed by 1.65% sodium hypochlorite with 2 drops Tween 20 for 12 min and then washed 3 times with sterile water, one minute each.

After surface sterilization, an explant will be introduced in 25 x 150 mm glass culture tubes containing 15 ml stage 1 media (SM1) consisting of 1X Murashige and Skoog salts (Murashige and Skoog, 1962) supplemented with 0.4 mg/L thiamine, 100 mg/l myo-inositol, 0.5 mg/L Nicotinic acid, 0.5 mg/L Pyridoxine-HCl (Full vitamins), 0.5 mg/L glycine, 30 mg/L sucrose, and 2.5 uM of different types of cytokines. The media will be adjusted to pH 5.7 with 0.1 M KOH, solidified with 8g/L TC agar and sterilized by autoclaving at 1.2 Kg/cm2 pressure for 20 minutes at 121°C.

Cultures will be maintained in a plant growth incubator at 25 °C and 16-h photoperiod with a photon flux density of 40 µmol m^2 s^{-1} provided by cool-white, fluorescent lamps and sub-cultured periodically every 3-4 weeks during several cycles of culture until achieve consistent growth rates.

Stage 2: Multiplication of propagule

To increase shoot multiplication, nodes and apical shoots from stable Stage 1 cultures will be introduced in Magenta GA7 vessels containing 80 ml stage II media (SM2) consisting of 1X Murashige and Skoog salts (Murashige and Skoog, 1962) supplemented with 0.4 mg/L thiamine, 100 mg/l myo-inositol, 30 mg/L sucrose, 0.5 µM Indol-3 acetic acid and different molar concentration of different cytokinin types. The medium will be adjusted to pH 5.7 with 0.1 M KOH, solidified with 8g/L agar and sterilized by autoclaving at 1.2 Kg/cm2 pressure for 20 minutes at 121℃. Cultures will be maintained under the same culture environmental conditions described for Stage 1. Effect of different Cytokinins on shoot multiplication rate, shoot number and length of will be examined.

Stage 3: Pre-transplant (rooting)

Individual regenerated shoots from Stage II cultures will be transferred into GA7 vessels containing 80 ml stage III media (SM3) consisting of ½ X Murashige and Skoog salts (Murashige and Skoog, 1962) supplemented with 0.4 mg/L thiamine, 100 mg/l myo-inositol, 30 g/L sucrose

and different molar concentration of Auxins. Medium will be adjusted to pH 5.7 with 0.1 M KOH, solidified with 7g/L agar and sterilized by autoclaving at 1.2 Kg/cm2 pressure for 20 minutes at 121℃. Effect of rooting percent, number and length of roots will be examined.

Stage 4: Transfer to natural environment

Rooted shoots will be transferred to plastic trays containing soil substrate and placed under the CCPP greenhouses conditions. Trays will be kept under shade with low natural light levels (15-20 µmol m^2 s^1) at 25 ± 2 °C. The trays will be covered to keep high humidity 80 - 90 %. Trays covers will be gradually removed to allow humidity also decreased gradually. Trays will be also transitioned to high natural levels to complete the acclimatization process. Micropropagated plantlets will be ready to be used as rootstocks or in the case of plant indicators will be used in the bio-indexing procedures at the CCPP.

PROJECT MANAGEMENT AND EVALUATION

This project will be performed at the tissue culture facilities of the Citrus Clonal Protection Program (CCPP). Research activities including protocol development, experimental design, experiment set up data collection, and statistical analysis will be supervised by Paulina Quijia-Lamina, Ph.D. Dr. Quijia-Lamina has an extent experience on tissue culture techniques over the last 9 years of research working in a high technology tissue culture facility at the Environmental Horticulture Department at the University of Florida, under the advice of Dr. Michael E. Kane, a Distinguished Professor and world recognized researcher in the area of tissue culture of different crops and native plants.

Dr. Quijia-Lamina is currently the lead therapy expert of the CCPP, performing shoot-tip grafting (STG) in the varieties introduced at the CCPP. Therefore, funding from this project will be used to support again one tissue culture assistant so Dr. Quijia-Lamina will be able to carry out the experiments, maintenance of cultures, elaboration of media and data collection without affecting the core CCPP STG activities. The assistant has been trained by Dr. Quijia-Lamina before performing the mentioned activities and Dr. G. Vidalakis, CCPP director has been providing overall project direction and connections to citrus nurseries.

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PROGRESS REPORT

OBJECTIVE 1.

Optimization of the shoot tip grafting procedure includes standardization of protocols for citrus seed germination to obtain seedlings rootstocks, and budwood culture for shoot production. Shoot tips explants obtained from budwood cultures are micrografted in rootstocks seedlings. Successful shoot tip grafting is affected by the quality of the rootstock seedlings which are germinated in the dark under in vitro culture conditions. Different factors influence the optimal in vitro seed germination and seedlings development of citrus, including seed quality, culture medium, sterilization process, and pre-treatments of seeds (Niedz, 2018). To optimize the seed germination and seedling development protocol to obtain seedlings rootstock used for STG, four asymbiotic seed germination media were tested for their effectives to promote seed germination and seedling development 1) Control: 1X Strength Murashige & Skoog (MS; *Phyto*Technology Laboratories, Shawnee Mission, KS, Cat# M524, Murashige and Skoog 1962). 2) 1/10X MS. 3) 1/4X MS, and 4) 1/2X M. All media except the control was supplemented with 10 g/L sucrose. A

0.8% TC agar (*Phyto*Technology Laboratories, Shawnee Mission, KS, USA Cat# A175) was used for all medium treatments.

Carrizo (CRZ) was used for this study as it is the most common seedling rootstocks used in the CCPP. Seed were collected and stored under 4 °C after drying until their used. Peeling of the first seed coat was used as scarification method. After 24 h at room temperature for hydration, seeds were surface sterilized with a 50% ethanol solution for 1 minute followed by 20 minutes in a 1.65% sodium hypochlorite solution. The NaOCl solution was prepared adding 20 ml Clorox Bleach, 80 ml sterile deionized (d) water and two drops of Tween 20. Once sterilization was completed under constant agitation, seeds were then rinsed three times for 1 minute each with sterile d water. Seeds were placed on the surface of the germination medium and incubated in the dark at 28 °C. Germination data was collected daily for 21 days to generate reverse survival curves or germination curves used to determined germination rates (Fig. 4).

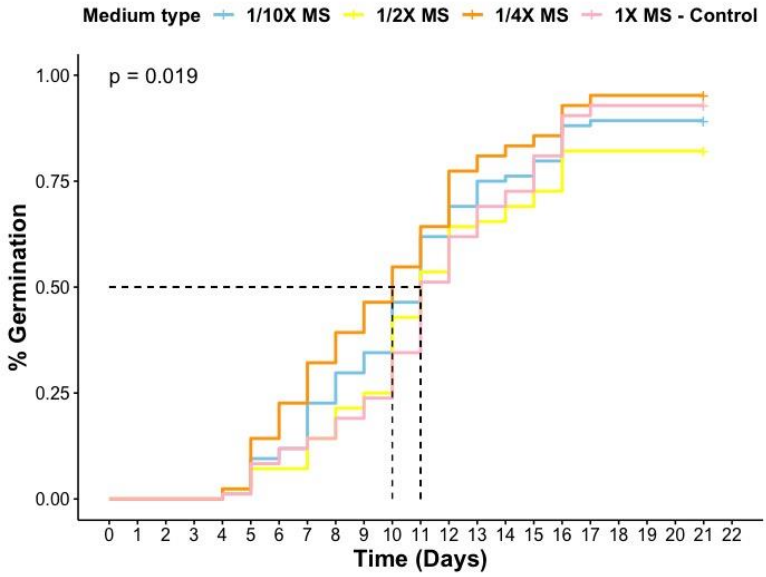

Figure 4. Germination survival curves for four different seed germination media

High germination percentages were observed in all treatments (>82%) at the end of the study, however significantly faster and higher germination (93%) rates were achieved when seed were sown in a medium containing ¼ strength of MS. Higher number and longer seedlings were also obtained in this treatment (Fig. 5). The control treatment included in our study has been used as the standard germination medium in the CCPP for decades, our results allowed the optimization of the medium composition to obtain more and longer seedlings rootstocks using less resources.

Budwood culture procedure has been also improved compared with the former standard protocol used in the CCPP, allowing us to maintain budwood cultures of the introduced varieties indefinitely or until a successful STG is obtained. Modifications included adding plant growth regulators to the culture medium to promote shoot production. Growth responses to different plant hormones are species/varieties dependent, some varieties required the presence of plant growth regulators to produce healthy shoots to be used for STG (Fig. 6).

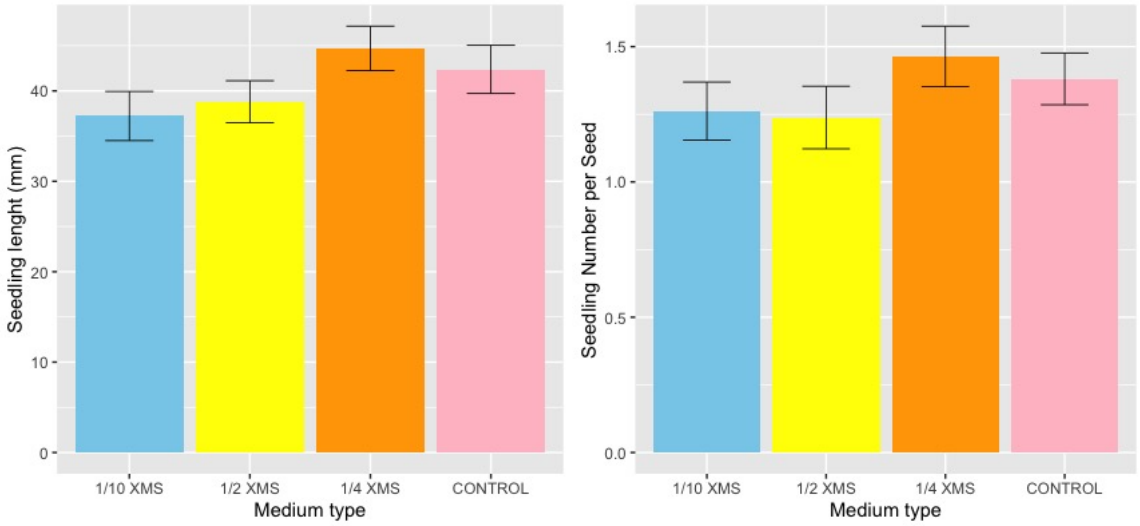

Figure 5. Carrizo seedlings number and length after 21 days from sowing in different media

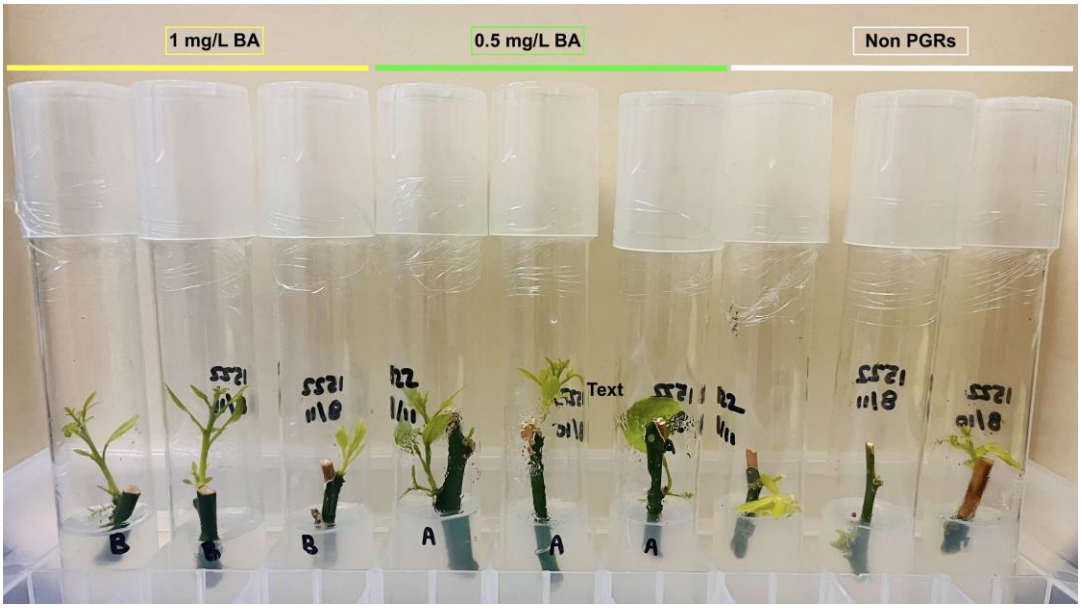

Figure 6. IPPN1522 Budwood cultured in media consisting of 1X MS supplemented with vitamins, 30 mg/L sucrose, and different concentrations of N6 benzyladenine (BA), a plant growth regulator (PGR). Notice the different patterns of shoot development especially in comparison to the Non-PGRs control, the standard 1970s method of culturing citrus budwood used by the CCPP.

OBJECTIVE 2 and 3.

Bioindexing indicators included in the first year of the project were: Dweet Tangor and Rusk Citrange. Reduction in seed production and germination have been observed in Dweet Tangor, which resulted in reduction of seedlings supply for bio-indexing procedures taking place at the CCPP. The popular US942 rootstock was also included in the project. According to our proposed protocol – Micropropagation stages (Figure 1), for all three varieties Stage 0 has been completed and STG 1 is currently undergoing.

Figure 7. US942 donor plant maintained in the CCPP greenhouses.

Stage 0 - Selection of donor plants has been stablished for all 3 citrus species. Donor trees plants are maintained in the CCPP greenhouses to ensure that the propagated material will be free of disease and pathogens (Fig. 7)

Stage 1: Establishment of Aseptic cultures: Successful surface sterilization (0% contamination) of explants has been achieved using the following protocol. Budwood plant material was collected from the donor plants. Nodal explants containing 1 or 2 buds were cut and surface sterilized using a 70% ethanol solution for 1 minute followed by 12 minutes in a 1.2% NaOCl sterilization solution. Sterilization solution was prepared adding 15 ml Clorox Bleach, 85 ml (d) water and two drops of Tween 20. Explants were then rinsed three times for 1 minute each with sterile d water.

Four stabilization medium types are being evaluated to achieve consistence growth rates of shoots after multiple subcultures. After sterilization was completed, explants were introduced in 25 x 150 mm glass culture tubes containing 15 ml stage 1 media (SM1) consisting of 1X MS supplemented with 0.4 mg/L thiamine, 100 mg/l myo-inositol, 0.5 mg/L Nicotinic acid, 0.5 mg/L Pyridoxine-HCl, 0.5 mg/L glycine, 30 mg/L sucrose, and 2.5 uM of different types of cytokines: N6 benzyladenine (BA), Kinetin, and 2-Isopentenyladenine (2-iP). A medium without plant growth regulators were included as control treatment. The media were adjusted to pH 5.7 with 0.1 M KOH, solidified with 8g/L TC agar and sterilized by autoclaving at 1.2 Kg/cm2 pressure for 20 minutes at 121˚C. Explants were transfer to a fresh SM1 medium every 4 weeks. Two cycles of 4-week subcultures have been completed with US942 (Fig. 8) and Rusk citrange (Fig. 9).

Differences in the number of shoots produced in the same media between cultures cycles have been observed with both US942 and Rusk. These responses were expected as the explants are stabilizing in the new *in vitro* environment. From our preliminary results, we observed that US942 not require the presence of plant growth regulators to produce shoots while Rusk explants produce shoot in both media supplemented with 2-iP or without PGRs. After more culture cycles, consistent production of shoots will be achieved in a specific media type. These shoots will be used to increase the production rates in the Stage 2 with a cytokine type defined in Stage 1.

Dweet Tangor explants exhibited oxidation therefore SM1 was supplemented with 50 mg/L Ascorbic and citric acid to overcome this issue and allow shoot development.

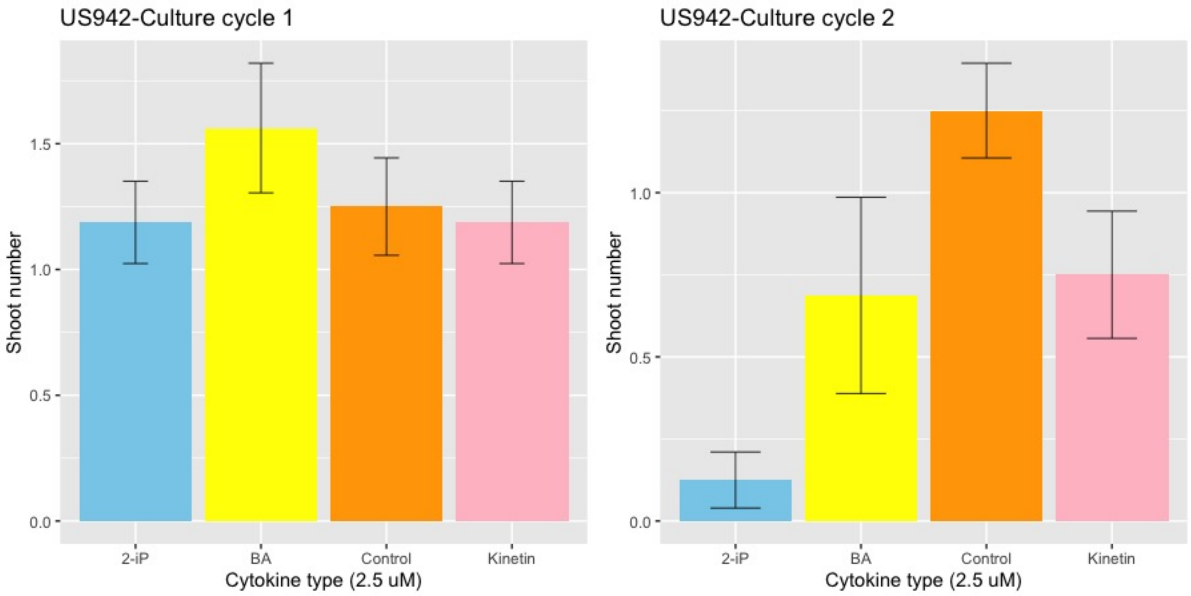

Figure 8. Number of shoorts produced from US942 budwood subcultured every 4 weekss in medium containind different cyrokines types.

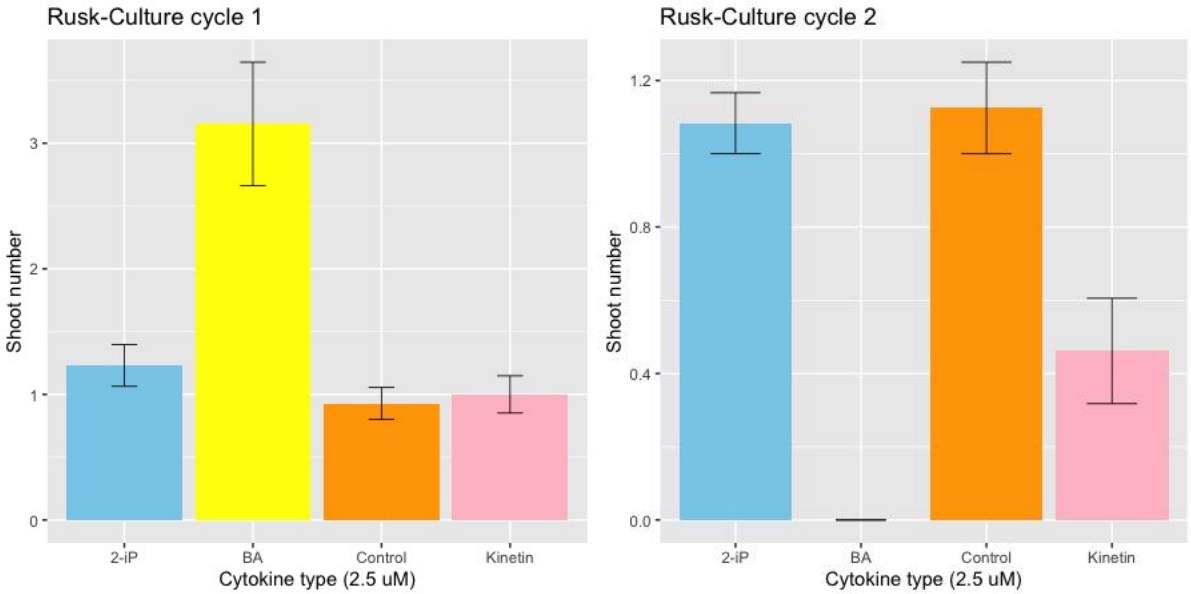

Figure 9. Number of shoots produced from Rusk budwood subcultured every 4 weekss in medium containind different cyrokines types.

BUDGET PROPOSAL

Project Title: In vitro propagation of citrus rootstocks and bio indexing indicators

Project Leaders: Paulina Quijia-Lamina, Georgios Vidalakis

Proposed Fiscal Year: 2023

A. PERSONNEL SERVICES:

Tissue culture Lab Technician (Tech 1)	$41,446
Benefits	$25,282
TOTAL PERSONNEL SERVICES	$66,728

B. OPERATING EXPENSES:

Equipment: Plant Growth Incubator (FY 2022 non-cost extension and reprograming is requested)	($22,000)
Equipment: Semimicro balance	$5,200
Tissue culture media components	$5,000
Petri dishes and tissue culture containers.	$3,500
Laboratory supplies	$4,000
Greenhouse supplies	$3,500
TOTAL OPERATING EXPENSES:	$21,200

C. TOTAL BUDGET REQUESTED: **$87,928**

Signatures of Requestors: _____ Date: 11-21-2022

_____ Date: _____

Signature of Cooperator: _____ Date: 11-21-2022

Department Chair: _____ Date: _____

LIAISON OFFICERLIAISON OFFICER_____ Date

QUI-23

Final Audit Report 2023-01-18

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