TISSUE CULTURE FOR AUSTRALIAN AVOCADOS

Project Information Pack

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

QAAFI
Queensland Alliance for Agriculture and Food Innovation

UNIVERSITY OF SOUTHERN QUEENSLAND

CQUniversity AUSTRALIA

RESEARCH WITH IMPACT

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4. Department of Agriculture and Fisheries

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We gratefully acknowledge and appreciate the following project partners:

Anderson Horticulture, Duravo, Delroy Orchards, Jasper Farms,

Donovan Farms, L&R Collins, and Mackays Bananas.

Cover photo: Tissue culture rootstock tree at Childers (24 months post-planting, prior to picking)

Project Summary

Nursery production of elite avocado clonal plants with desired high-quality rootstocks in expected quantities is challenging. This has imposed extended waiting periods for growers to obtain nursery trees. This project aimed to improve the efficiency of clonal avocado rootstock propagation through tissue culture (TC) technology to propagate true-to-type rootstocks of the highest quality year-round, to eliminate the constraints of conventional clonal propagation. Across a three-year timeframe, this project has delivered a holistic feasibility study of TC technology. This was achieved by developing the technology; undertaking in-field plant trials; evaluating economic commercial feasibility; and investigating industry readiness and perceptions about the use of the technology, through a partnership between The University of Queensland, Central Queensland University and The University of Southern Queensland.

Key learnings and outcomes:

- Developed the world's first commercial-scale pipeline for avocado TC, with complete proof-of-concept from lab to nursery to orchard.
- The commercial pipeline is licensed for rootstock Reed, with significant progress established for the rootstocks Velvick, Zutano, Kidd and Dusa.
- Our system can produce up to 500 clonal plants from a single shoot-tip in culture within 8 to 12 months, depending on the rootstock.
- TC Reed rootstock performance has been validated in field trials across five sites in QLD and WA.
- The economic assessment is complete at farm and industry level for production costs and market demand/saturation, with a potential 21% return on investment to growers.
- Year round in-lab production of rootstocks, which is independent of seedlings, is set to increase accessibility to trees for planting.
- Survey results highlight the readiness of the industry to learn about and use TC, and describe the interest in opportunities for future growth.

About Tissue Culture Technology

Tissue culture is an efficient means of plant propagation that has been widely applied in the horticulture and forestry industries. It provides high multiplication of genetically true-to-type and disease-free plants.

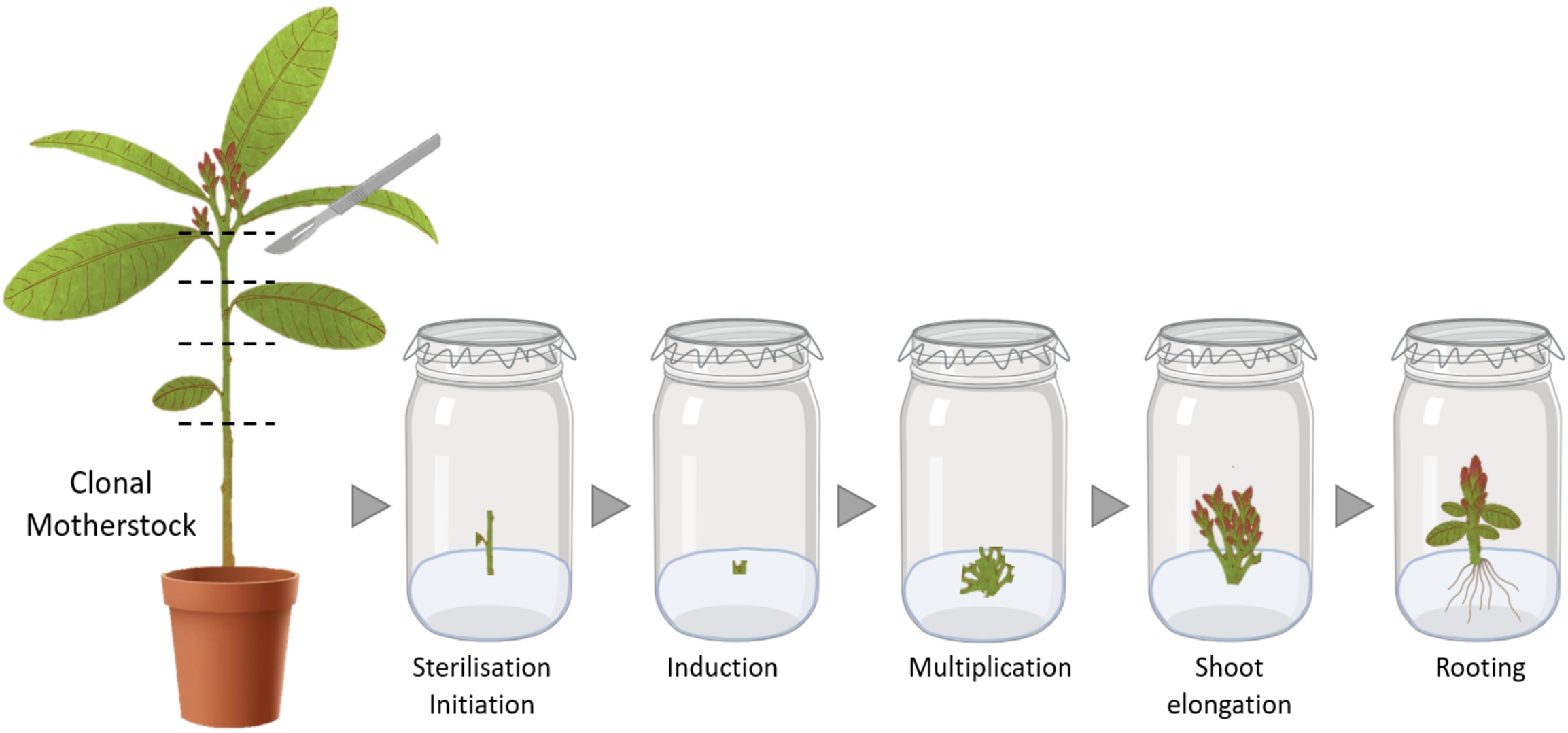
Our plant tissue culture (TC) for avocado is:

- A propriety technology to produce up to 500 plants starting from a single mature shoot-tip
- Pest- and disease-free
- High multiplication (>500x throughput)
- Independent of seed, enabling year-round climate-derisked rootstock production
- True-to-type to the mother plant
- Demonstrated to have fewer plant losses during propagation

Above: Avocado tissue cultured shoots of rootstock Velvick

Tissue Culture Lab Process

1. A small number of disease-free mature plants (mother plants) are maintained under glasshouse conditions to obtain material for tissue culture.
2. Cuttings are taken from the mother plant and sterilised using an optimised process to ensure viability of the material is not compromised.
3. Sterilised cuttings are placed on a sterile, nutrient-rich medium in a controlled environment growth room.
4. A wide range of different nutrient compositions (culture media) are then optimised to multiply and elongate a large number of shoots with healthy leaves, and then finally produce roots.
5. Rooted plantlets are carefully introduced to nursery conditions (acclimatised).



Above: Diagrammatic representation of the step-wise avocado tissue culture process

By providing the perfect conditions for plant growth and development, these explants will generate a large number of new plants that are genetically identical to the mother plant. Using our system for avocado, hundreds of clonal plants can be produced from a single shoot-tip, making this process extremely high-throughput, sustainable and resource-effective.

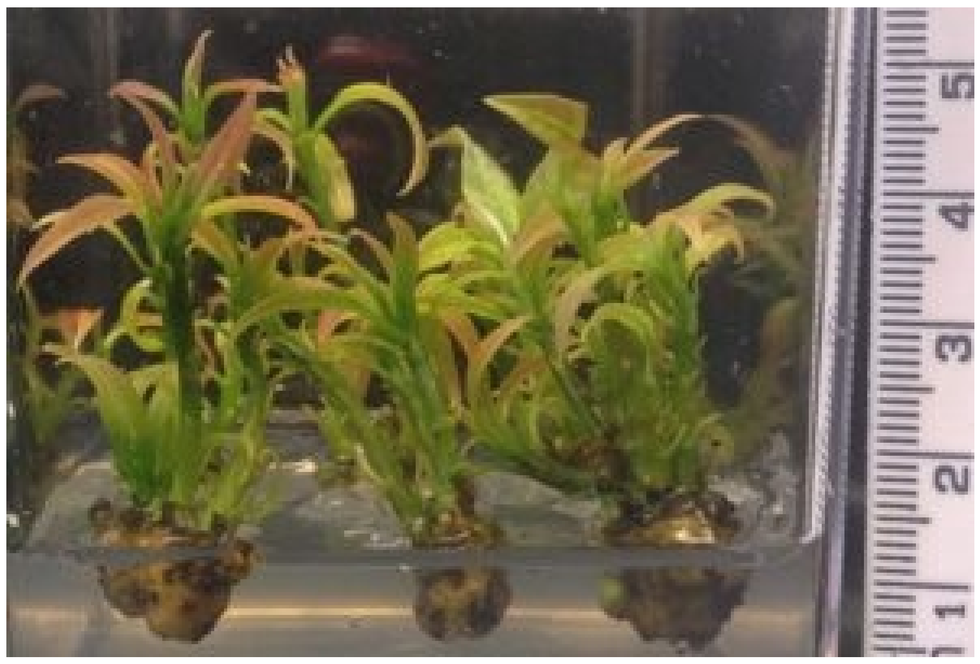
Key Avocado Tissue Culture Learnings:

- High throughput multiplication possible for avocado.
- Every stage of the tissue culture process occupies a different highly optimised media (nutrient composition).
- Each avocado cultivar trialed to date at UQ required a large number of individualised media formulations.

Avocado Tissue Culture Growth Stages

1. Meristem Induction

2. Multiplication



3. Shoot Elongation

4. Single Shoots

5. In-vitro Hardening

6. Root Induction

7. Acclimatised Plant

Images 1 - 7: Tissue cultured avocado at each stage of the rootstock propagation process (Reed pictured)

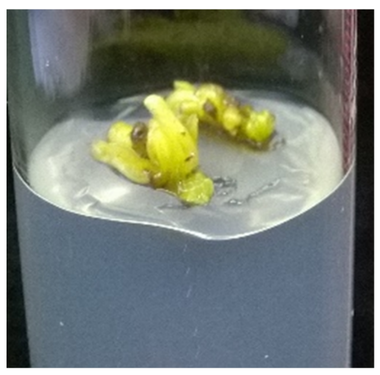
Summary of Rootstock Progress in Culture

The below table shows the progress of various rootstocks through TC optimisation. Every rootstock is variable in its response to each stage and requires individual optimisation.

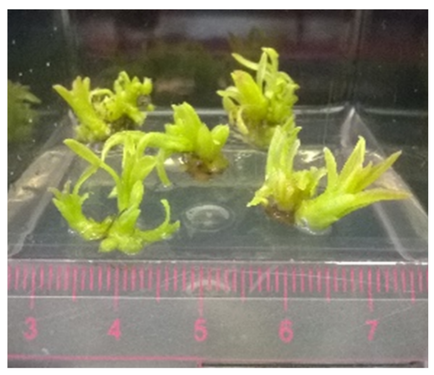
TC stage	Reed*	Velvick	Zutano	Kidd	Dusa
Multiplication	√	√	√	√	√
Elongation	√	√	√	√	√
Hardening	√	√	√	√	√
Rooting	90%	57%	40%	83%	80%
Acclimatisation	95%	83.3%	90%	97%	50%
Multiplication rate	*1x >500	*1 x >200	TBD	TBD	TBD

*Validated on commercial scale. TBD = To Be Determined.

Velvick

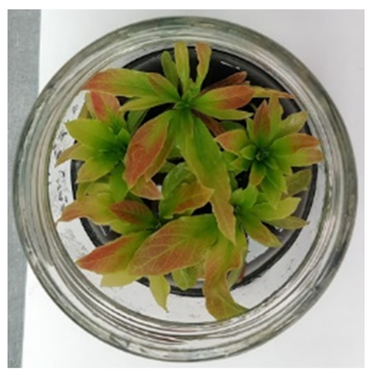


1. Induction



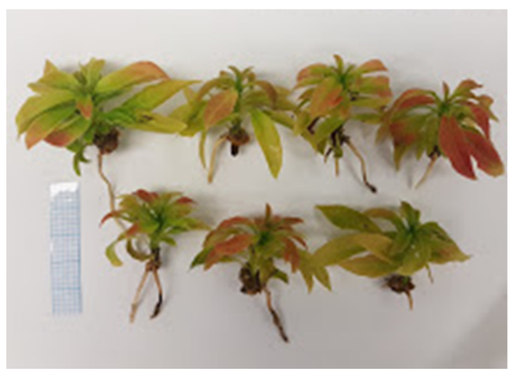
2. Multiplication

3. Shoot Elongation



4. In vitro Hardening

5. Rooting



6. Rooted Plants

7. Acclimatised Plant

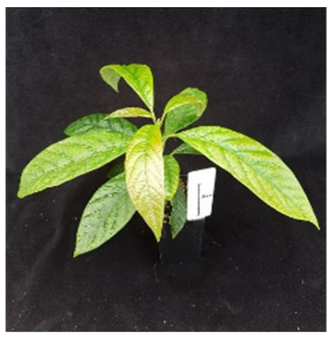
Kidd

1. Multiplication

2. Shoot Elongation

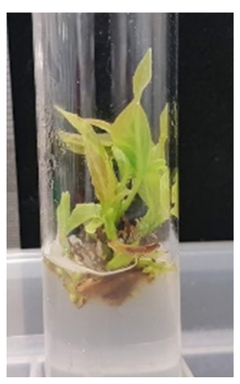
3. In-vitro Hardening

4. Rooting



5. Acclimatised Plant

Zutano



1. Induction

2. Multiplication

3. Shoot Elongation

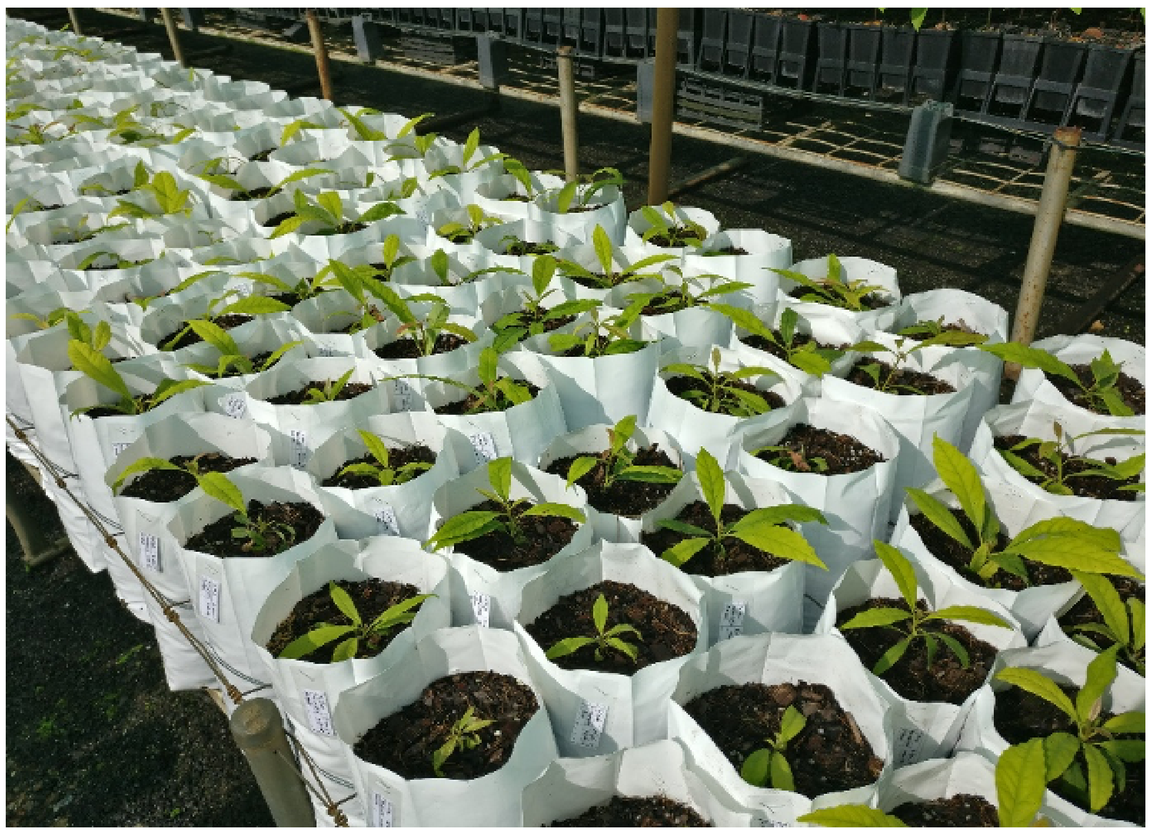
4. Rooting

5. Acclimatised Plant

From Lab to Nursery

Acclimatised Reed plants were transplanted, allowed to grow to a suitable height, grafted, hardened off in full sunlight and shipped. No difference in grafting success was noted between a tissue culture rootstock and seedling rootstock when grafting with either Hass or Maluma scions.

TC root system



Transplanted TC-produced Reed rootstock

Once an appropriate size, rootstocks grafted with desired scion (Hass/Reed)

TC Hass/Reed rootstock plants packed and ready for shipping

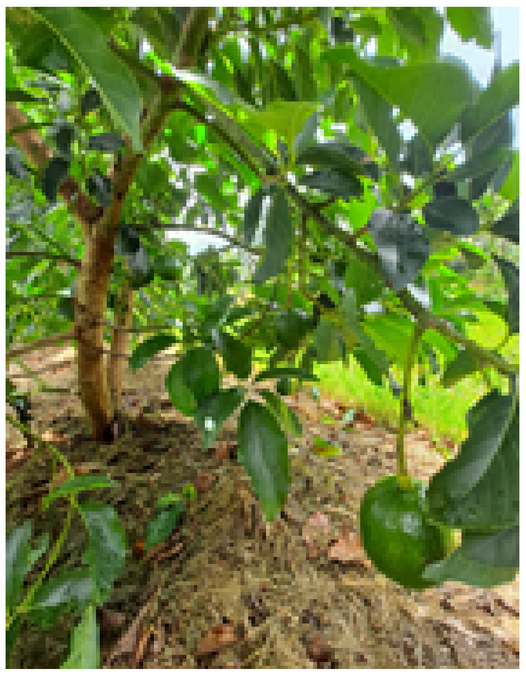
Validation of TC Plant Morphology in Field

For every species that goes through tissue culture, it is important to assess resulting plants for normal development, physiology, and phenology (such as seasonal flowering and fruit production).

For our avocado tissue culture rootstocks, once we have acclimatized plants, they are established as ungrafted trees in research field plots for this purpose. Examples of Reed and Velvick ungrafted trees showing normal development and fruit production in the nursery and after establishment in the field at Anderson Horticulture, Duranbah, NSW, are shown below.

Reed

Velvick



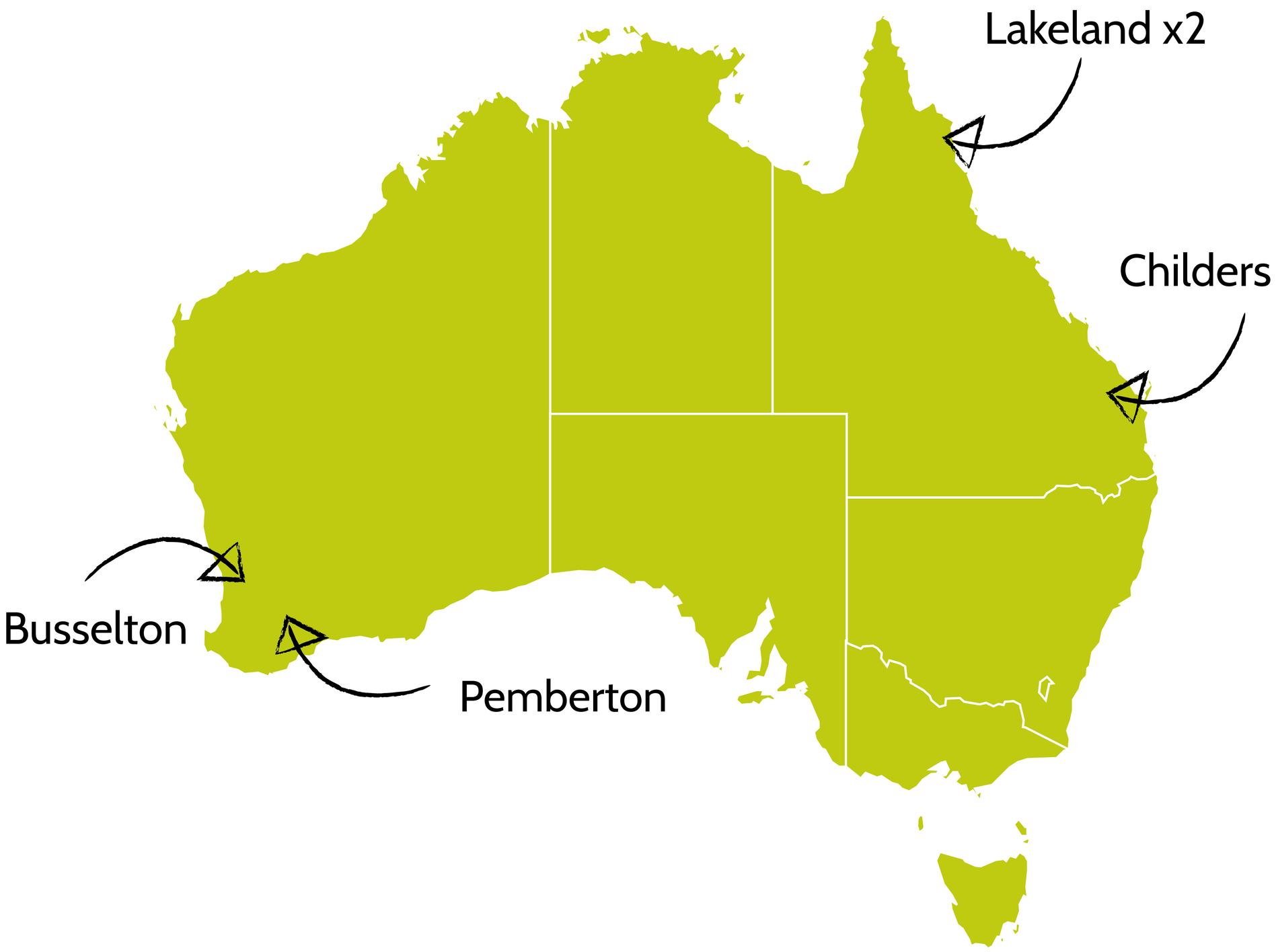
Grower-Led Field Trials

Five tissue culture avocado field trials of Reed cultivar rootstocks are operating across Australia. The trials were established in 2018 to demonstrate seamless integration of tissue culture-produced rootstocks into existing commercial management strategies in a variety of Australian production regions.

These trials are testing the performance of grafted tissue culture rootstocks in comparison to seedling rootstocks in a wide climatic range in predominant avocado regions across Australia, using both conventional and innovative management strategies (see page 9).

Field trials are proudly established in collaboration with Donovan Family Investments, Mackays Bananas, Jasper Farms, L & R Collins and Delroy Orchards with nursery support from Anderson Horticulture.

Locations:



Field Trials: 5 Locations; 4 Geographies

Location	Climate Range (°C)	Avg Annual Rainfall (mm)	Trial Planted date	Trial Design	Establishment Success
Childer's QLD (Donovan Family Investments)	Summer 20.0-30.8 Winter 9.9-21.7	1071	April 2018	43 replicates of Reed seedling and Reed TC Rootstocks. All grafted with Hass scion.	97.6% surviving Reed Seedling 100% surviving Reed TC
Lakeland QLD (Mac Farms)	Summer 24.3 - 32.0 Winter 18.1 - 26.3	1529	September 2018	50 replicates of Reed seedling and Reed TC Rootstocks. All grafted with Maluma scion. These trials are being trained onto a trellis with due central leaders under a shade cloth	96% surviving Reed Seedling 90% surviving Reed TC
Busselton WA (Jasper Farms)	Summer 24.3 - 32.0 Winter 7.5 - 16.3	807	October 2018	50 replicates of Reed seedling and Reed TC Rootstocks. All grafted with Hass scion.	96% surviving Reed Seedling 100% surviving Reed TC
Lakeland QLD (L&R Collins)	Summer 24.3 - 32.0 Winter 18.1 - 26.3	1529	November 2018	50 replicates of Reed seedling and Reed TC Rootstocks. All grafted with Maluma scion.	96% surviving Reed Seedling 90% surviving Reed TC
Pemberton WA	Summer 13.6 - 26.5 Winter 7.2 - 15.2	1185	November 2018	50 replicates of Reed seedling and Reed TC Rootstocks. All grafted with Hass scion.	98% surviving Reed Seedling 100% surviving Reed TC

Field Performance Images

Trees are being managed in accordance with the practices established by the host grower and as such, each site has different spacing, water and nutrition regimes, disease management and canopy management strategies. This document showcases data from the Childers site as it was the first to be established and is the site with preliminary yield data. Data collection from the other trial sites continues. Below are pictures from those sites.

Busselton planting day

Childers planting day

Pemberton planting day

Lakeland Mackay trellis planting day

Lakeland Collins planting day

Field Trial Spotlight: Childers

PLANTING DAY

6 MONTHS

12 MONTHS

24 MONTHS

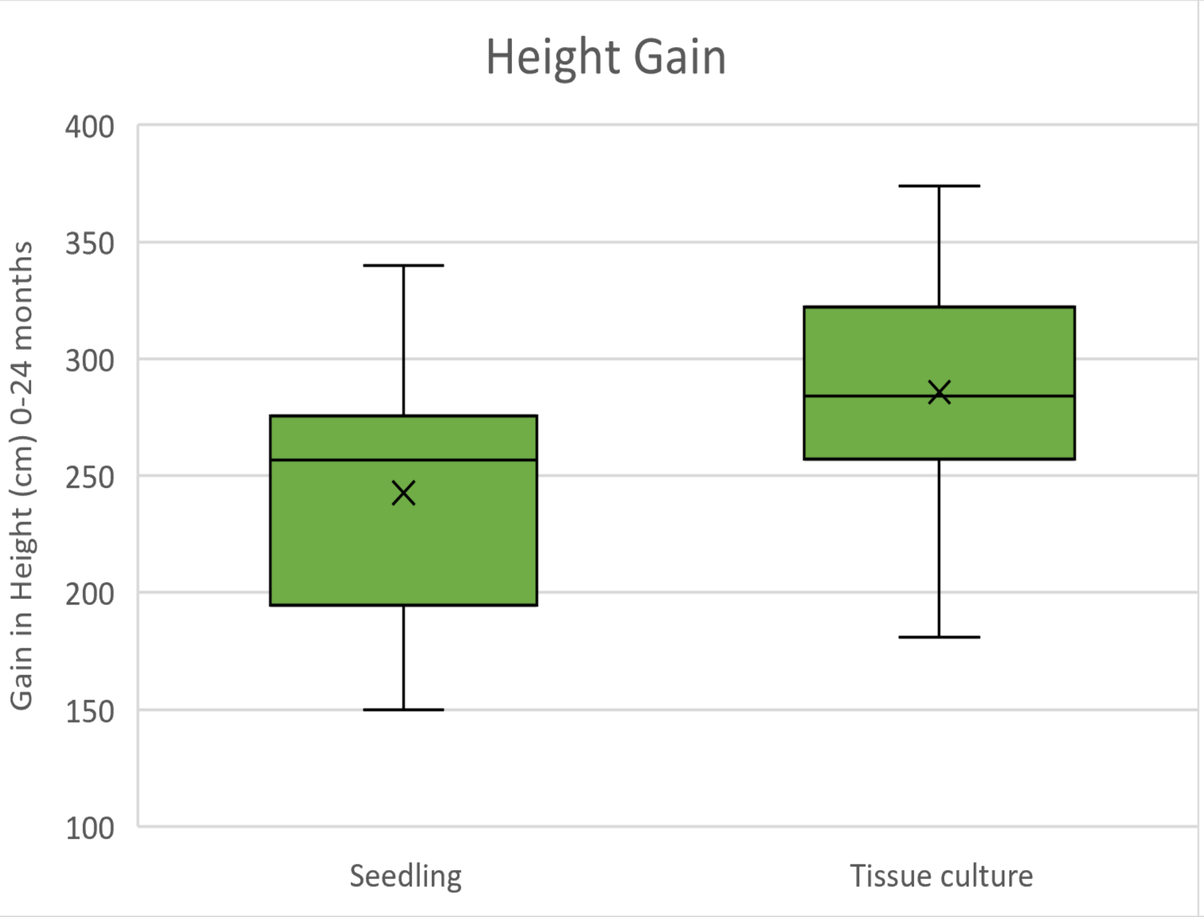
Above: Growth of trial plants (Reed seedling and Reed tissue culture rootstock with Hass scions) over first two years in Childers, QLD.

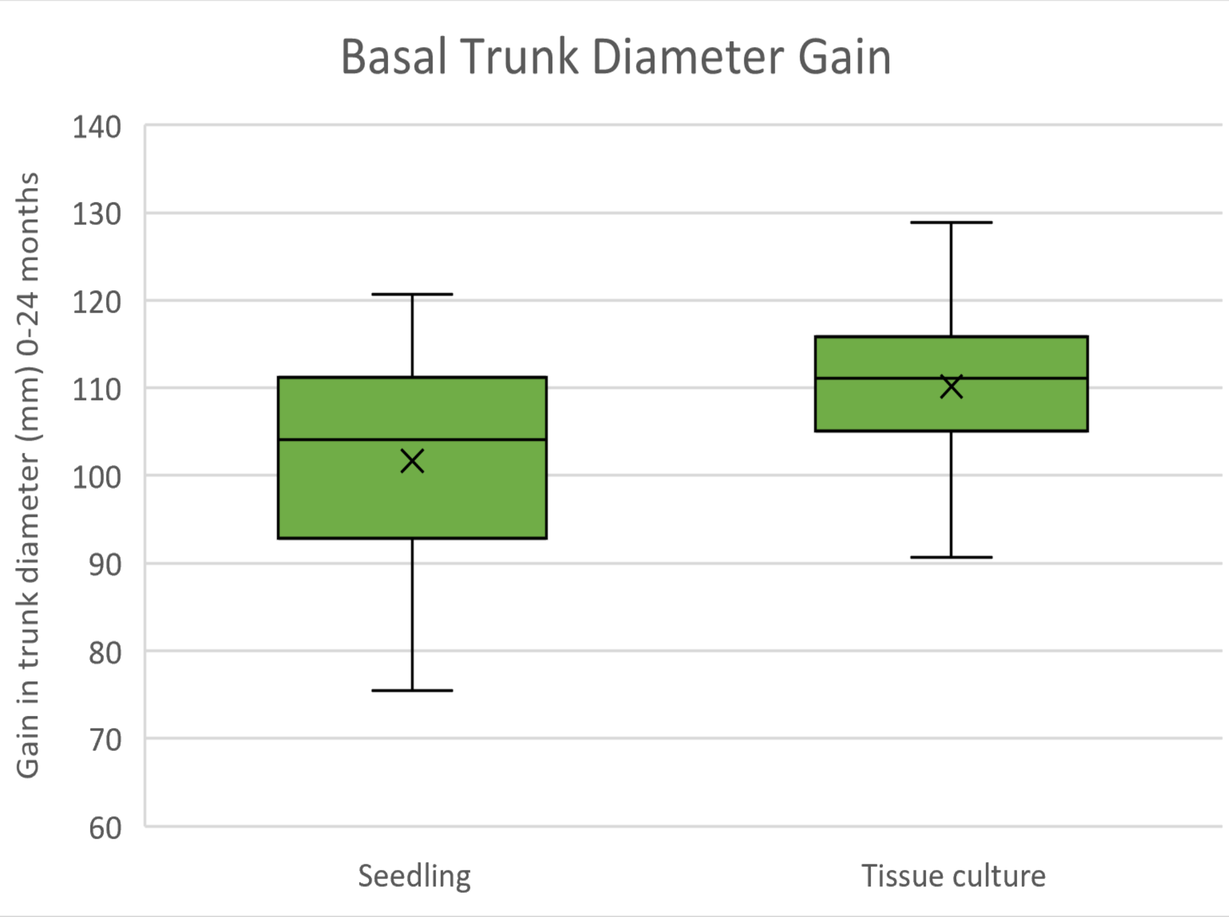
Hass grafted with TC rootstock tree at Childers (24 months post-planting); prior to picking.

Field Performance Measures

Spotlight: Childers, QLD

Data shown here is tree measurement at 24 months post-planting relative to its size at planting day to demonstrate growth over this time period.





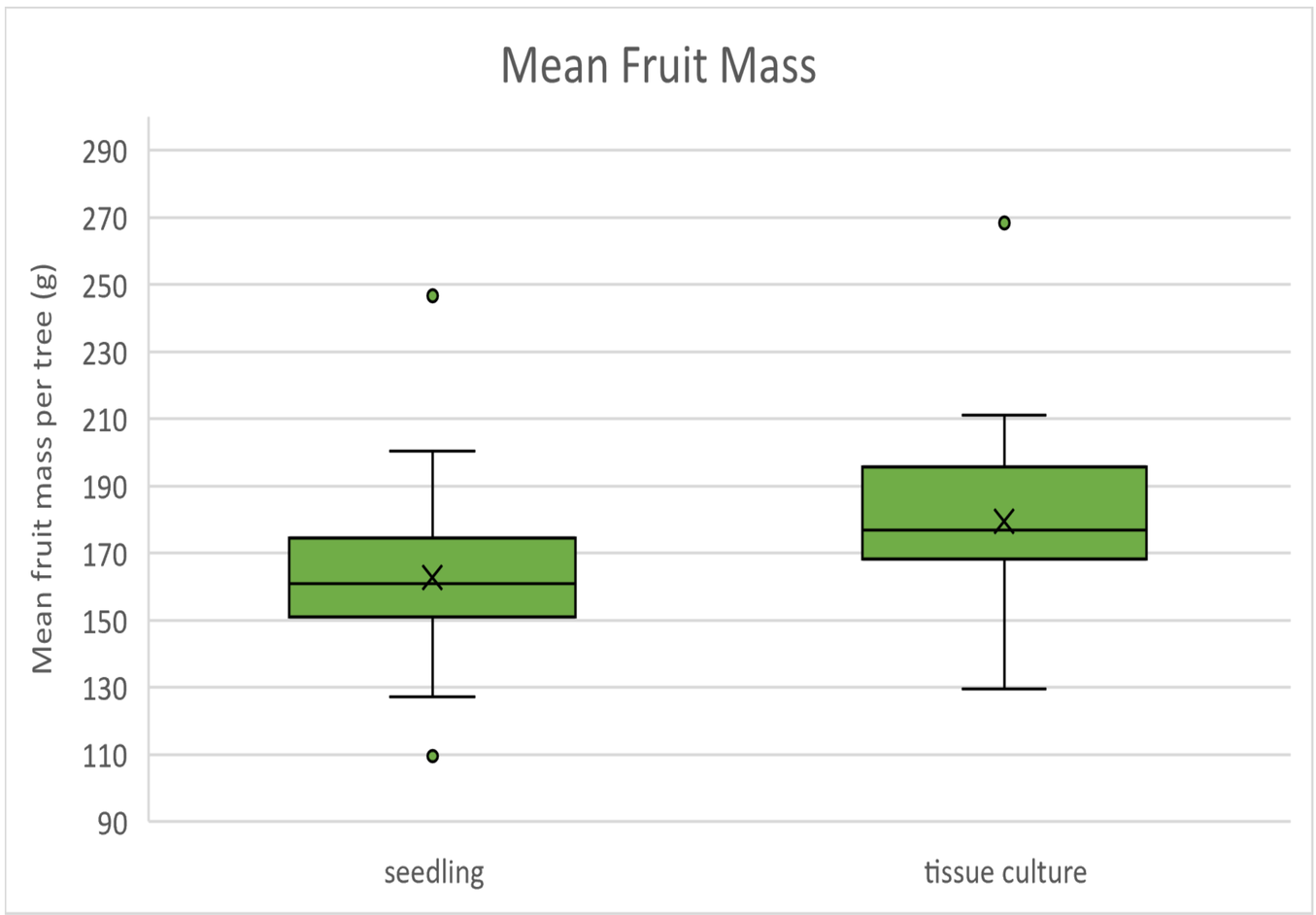
Above: Box and Whisker plot of gains in tree height and gains in basal trunk diameter between planting day and 24 months in the ground for trial trees at Childers, QLD. Reed rootstocks were either seedling controls or tissue culture propagated, and all trial trees were grafted with Hass.

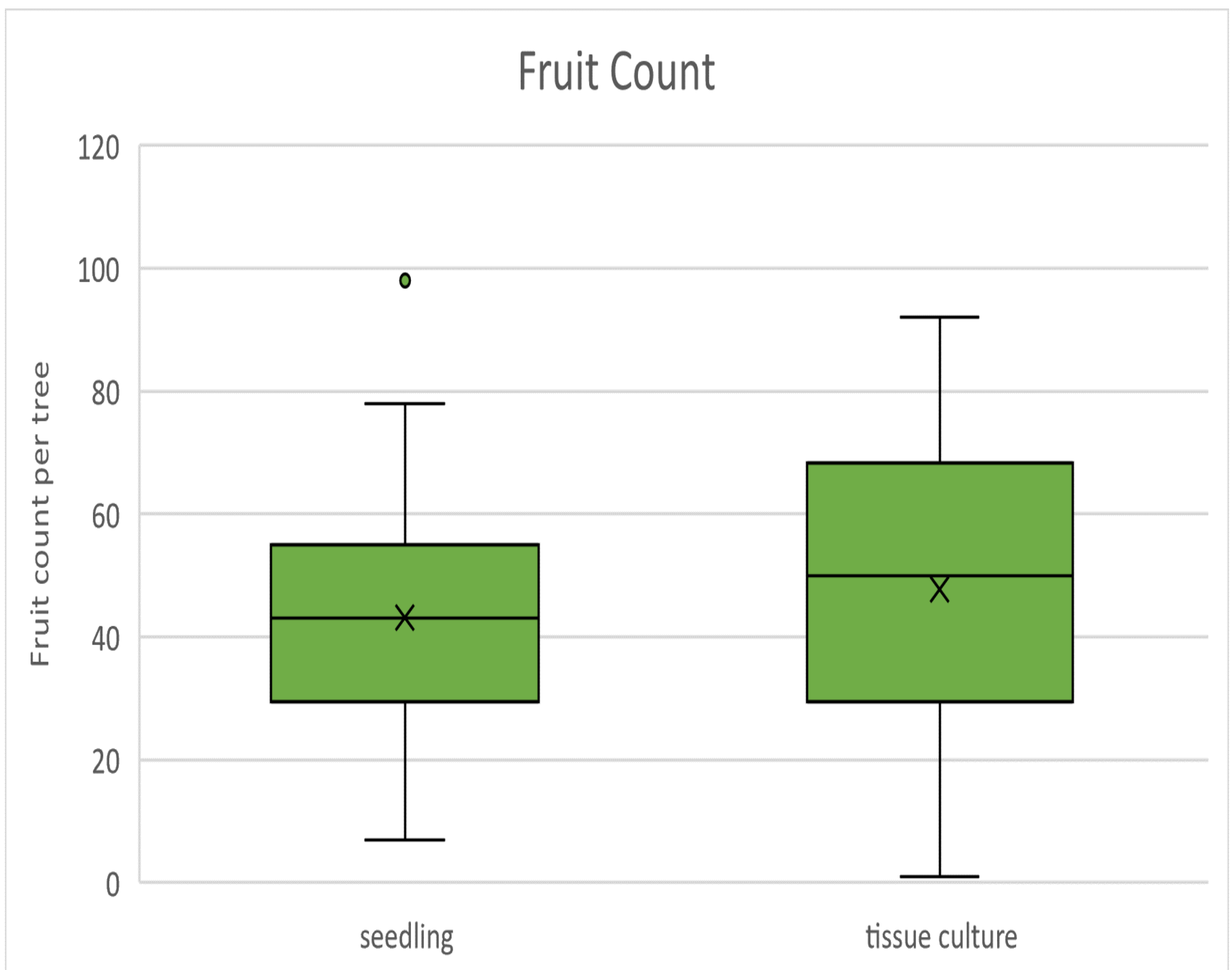
Box and Whisker plots contain 50% of the data in the 'box' part, have a middle line representing the median and an 'x' representing the mean. These plots are therefore fantastic at representing spread of data.

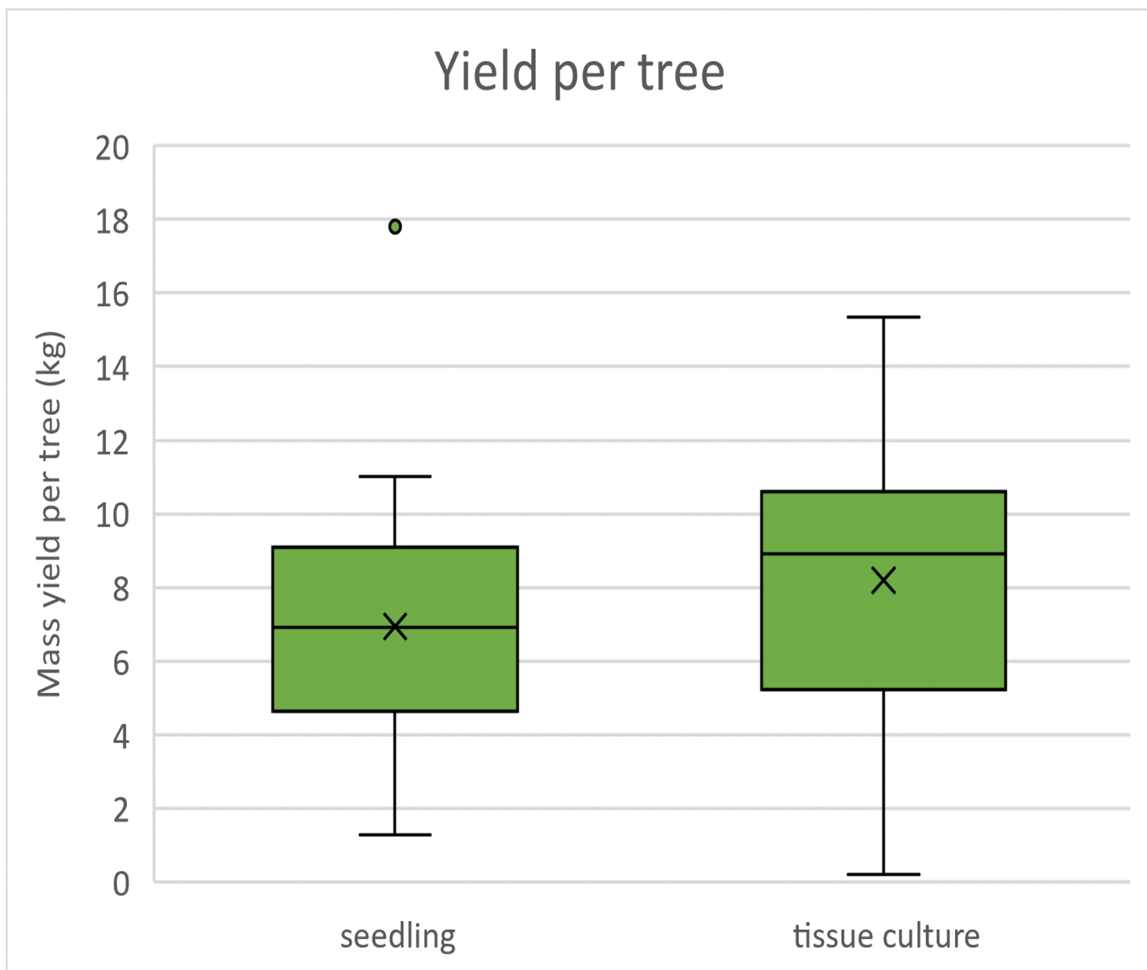
As the tissue culture rootstock 'box' is smaller for both gains in height and gain in trunk diameter, this suggests that growth of tissue culture trees is more uniform than seedling controls. Additionally, tissue culture rootstock trees have grown on average ~40 cm taller than seedlings (i.e. have a higher mean, 'x') during their first two years in ground.

Preliminary Yield Data

The first fruit was picked from the Childers trial in May 2020, when the trees were just over 2 years old. Below, the graphs show that Hass fruit picked from Reed tissue culture rootstock trees was on average larger than seedling rootstock trees (Graph a). However, there was no statistically significant difference observed in fruit count per tree (Graph b) or yield (kg) per tree (Graph c) between TC and seedling rootstock types.







Above: Fruit data from Childers trial two years post planting; a) mean fruit mass per tree (g), b) fruit count per tree and c) total yield (kg) per tree were all recorded.

Domestic Industry Perspectives

We surveyed Australian avocado industry members about technology adoption and their views of tissue culture (TC). Respondents were growers/farmers (42%), researchers/breeders (19%), agronomists (11%), consultants (11%) and nursery/suppliers (8%).

The sample included:

- Mostly participants who had worked on-farm with avocados (73%)
- An even split between those who had been in the industry for <10 years and for >10 years
- 69% of participants from QLD
- Participants ranging in age from 22 to 80, with an average age of 50 years

Overall, the domestic industry perceived tree supply as limited

- > 72% cannot access enough plants
- > 56% cannot access the quality of plants they want
- > 53% cannot access the cultivars they want

Perception around existing TC skills and knowledge, or ability to learn

- 40% indicated they already have the skills and knowledge needed to work with TC trees
- 83% agreed that they could develop the necessary knowledge and skills if the right information were available

Perceived resources required to use TC trees: money, not staff

- Money was rated highly as a perceived required resource, indicating that Australian industry members believe TC trees would be more costly than the plants they currently use
- Staffing resources were rated slightly lower, indicating that participants did not see TC trees as requiring additional staff to manage

Domestic vs. International Industry Perspectives

We explored international industry members' views and compared the findings against the domestic results. Respondents were growers/farmers (11%), researchers/breeders (36%), consultants (9%) and nursery/suppliers (25%), based in South Africa, the USA, New Zealand, Mexico, South America, India and Israel.

DOMESTIC

VS

INTERNATIONAL

Tree Supply

Access to tree quantity, quality and desired cultivars is problematic, but tree cost was of neutral concern.

Tree quantity and the cost of trees is problematic. Tree quality is of neutral concern. Access to desired cultivars is nonproblematic.

Confidence & Skills

40% were confident that they already had the required knowledge and skills to use TC trees

49% were confident that they already had the required knowledge and skills to use TC trees.

Resources

Money was the resource perceived to be most needed for using TC, while staffing resources were seen as least required.

Money, time and effort were the resources perceived to be most needed for using TC.

Uptake

66% plan to investigate using TC trees, with 70% agreeing that pending availability, they were likely use TC trees in the future.

93% plan to investigate using TC trees, with 88% agreeing that pending availability, they were likely use TC trees in the future.

Opportunities and Future Growth
Identified in Domestic Survey

1

TC BELIEVED TO BE BENEFICIAL

Two thirds of domestic industry respondents believed that TC would be beneficial for the industry.

2

WHAT IS WORKING WELL?

High productivity (57%);

Collaboration between researchers and industry (53%).

3

OPPORTUNITIES TO IMPROVE

Production costs (40%);

Policy development that supports production (30%).

4

MOST DESIRABLE CHARACTERISTICS OF TC FOR INDUSTRY GROWTH

Quality of TC root system;

Disease tolerance/disease free status;

Quality of fruit produced.

5

MOST LIKELY TO INFLUENCE TC ADOPTION

Field-trial observations (83%);

Professional recommendations (80%) .

6

ASPECTS OF SUPPLY CHAIN MOST LIKELY TO POSITIVELY IMPACT TC ADOPTION

Access to TC with desirable scion/rootstocks combinations (48%);

Competitive pricing of TC (27%);

Reliable and timely access to TC quantities (13%).

*Percentages represent the percentage of domestic survey participants who endorsed each item

Economies of Production with Clonal Tissue Culture

An economic assessment of TC for avocado orchards was done by comparing the traditional propagation scenario (S1) with six different TC propagation scenarios (S2-S7). The model assumptions, parameters, and key financial matric were based on industry interviews with growers, agronomists, and researchers.

Data and assumption sources:

- Growers visit
- Nursery visit
- Agronomist
- Academic & researchers
- Industry reports

Key financial matric

- Gross margin
- Net present value
- Annuity
- Maximum negative cash exposure
- Payback period
- Internal rate of return

Analysis parameters: Biological and economic

- Planting density: 312 trees/ha
- Plant mortality: 10% (in year 1)
- Plant price for traditional and TC: $35/tree
- Modelled orchard business horizon: 20 years
- Indicative mid-size orchard: 25 ha

Yield growth & other:

- Incubation stage: Years- 0-2 (0kg/tree)
- 1st bearing stage: Years 3 (12kg/tree)
- Growing bearing stage:
  - Year 4: 26kg/tree
  - Year 5: 38kg/tree
  - Year 6: 45kg/tree
  - Year 7-20 years: 50kg/tree
- Required rate of return: 7%
- Orchard capital structure: 100% equity

Above: Bioeconomic modeling parameters, data, and output used for all scenarios.

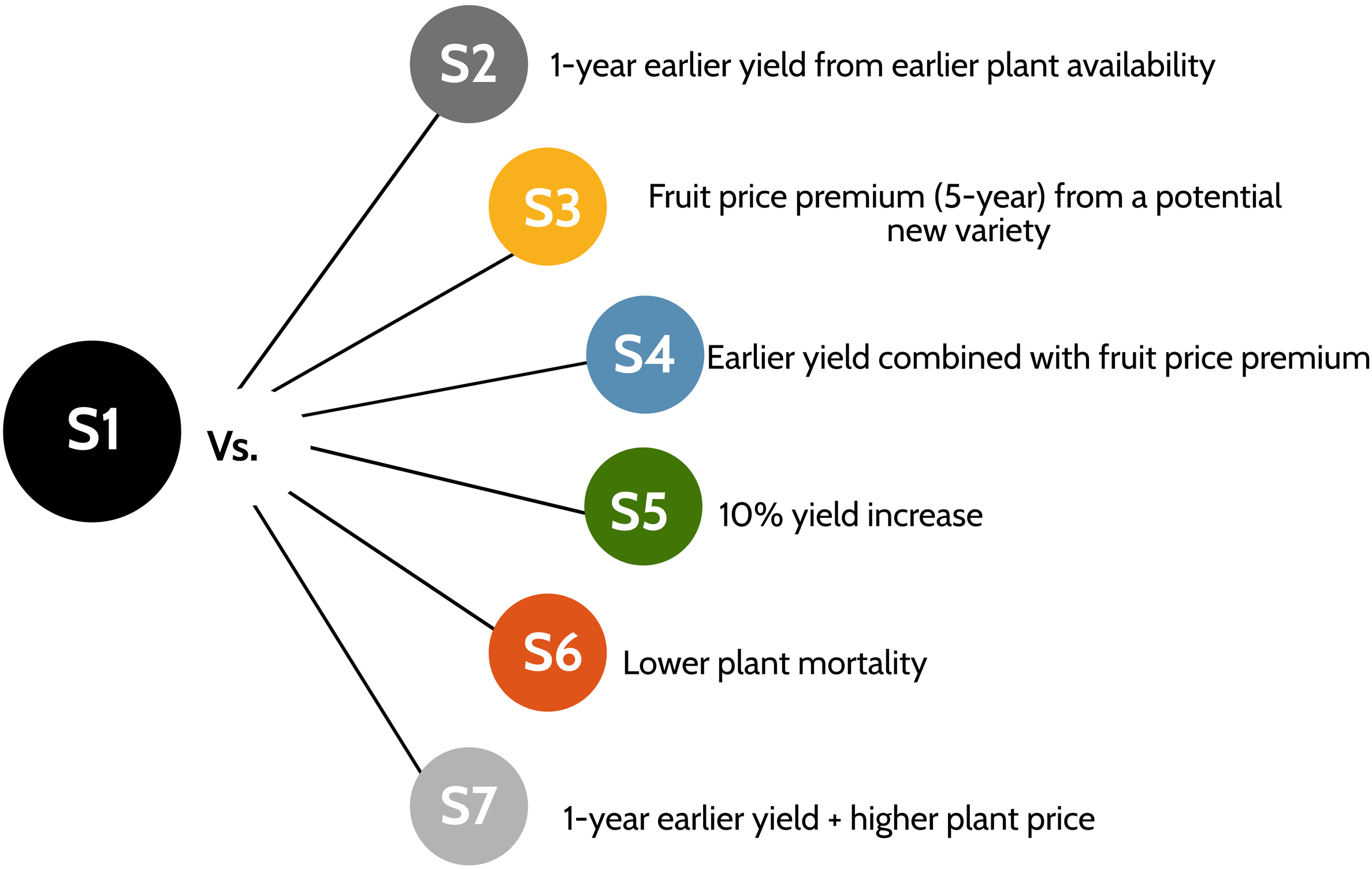
Comparing Traditional Propagation (S1) to Clonal Tissue Culture Propagation Scenarios (S2-S7)

All farm financial matric improved with the adoption of clonal tissue culture.

The additional benefit ranges from TC are:

- **Annuity**: increased A$77-A$7000/ha/year.
- **Cash exposure (investment):** decreased up to A$250K.
- **Payback period**: decreased 0.6 years-3.4 years.
- **Internal rate of return:** increased 0.38%- 29.61%/year.

Scenario Descriptions:



See the website for detailed financial analyses: qaafi.uq.edu.au/tissue-culture

Based on planting a 25 ha TC orchard, the below tables show the financial advantages of each scenario over S1 over a 20-year investment horizon.

	Cash flow annuity/ha/year	Maximum negative cash exposure	Payback period.	Internal rate of return (IRR)
S1	$19,000	$1.02 M	6.54 years	32%/year
	Additional $/ha/year	**Decrease in negative cash exposure**	**Decrease in payback period**	**Additional IRR**
S2 over S1	$3,159	$250K	2.36 years	12.91%/year
S3 over S1	$2,154	$0	1.50 years	5.51%/year
S4 over S1	$6,949	$250K	3.34 years	29.61%/year
S5 over S1	$3,346	$0	0.60 years	3.11%/year
S6 over S1	$77	$0	0.06 years	0.38%/year
S7 over S1	$2,099	$20K	1.70 years	5.42%/year

Grower Positioning Matrix: Tissue Culture Economic Benefits Summary

Tissue culture Scenarios	Additional cash flow annuity	Decreased negative cash exposure (investment)	Decrease in payback period	Additional internal rate of return
S2	🥑🥑	🥑🥑🥑	🥑🥑	🥑🥑
S3	🥑	N	🥑	🥑
S4	🥑🥑🥑	🥑🥑🥑	🥑🥑🥑	🥑🥑🥑
S5	🥑🥑	N	S	🥑
S6	S	N	S	S
S7	🥑	🥑🥑	🥑	🥑

Key: 🥑🥑🥑 TC scenario generating 1st highest economic additional benefits to growers;

🥑🥑 TC scenario generating 2nd highest additional economic benefits to growers;

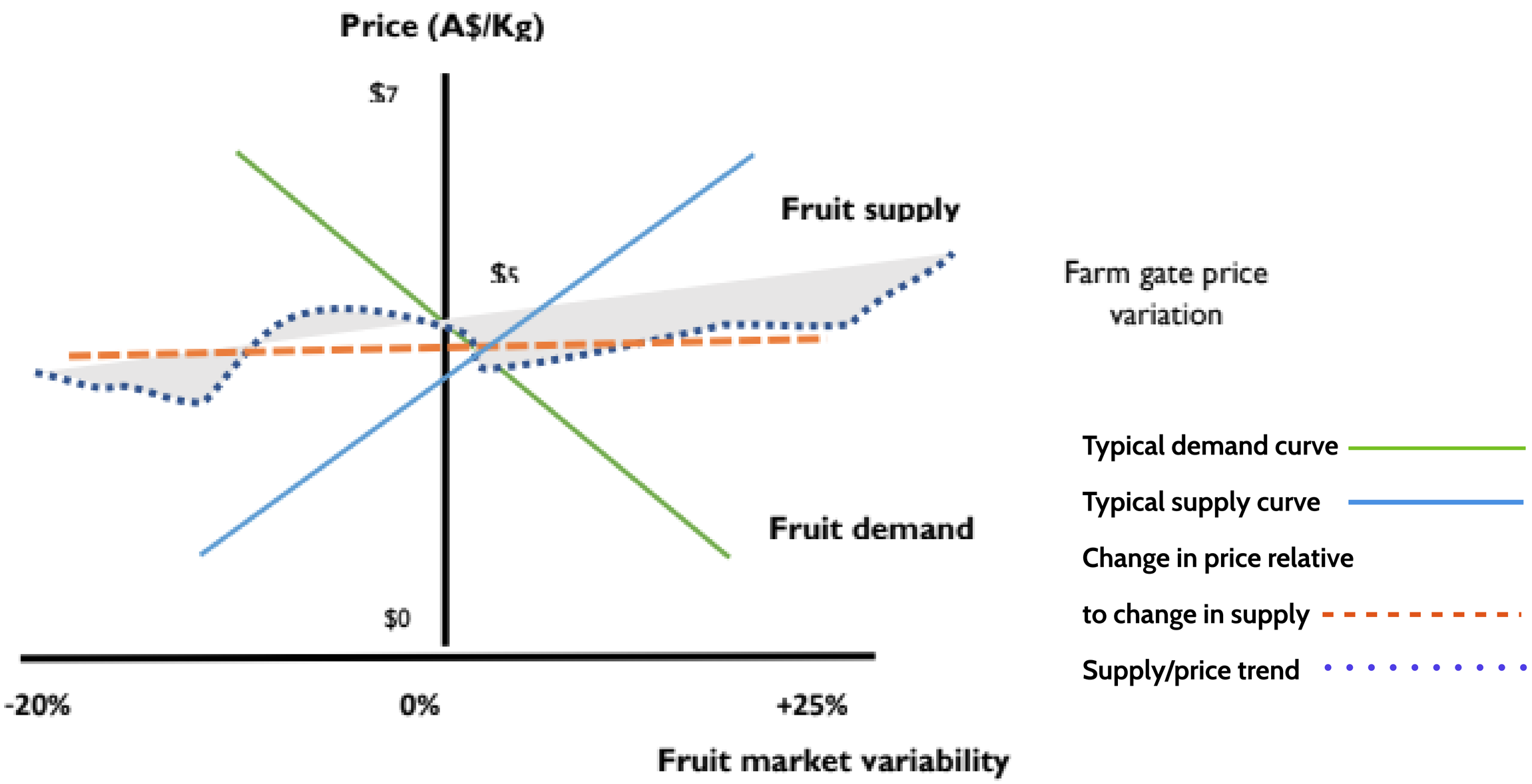
🥑 TC scenario generating 3rd highest economic additional benefits to growers;

S: slim additional benefits; ***N***: No change.

Fruit Market Assessment: Traditional Propagation (S1)

The analysis of historical avocado market performance (demand, price, and supply) over the last 20 years (as shown by the graph below) concluded:

- Real fruit prices varied from A$3.5/Kg to A$6.5/Kg
- Average observed fruit price: A$5/Kg
- Average fruit demand and fruit supply varied from -20% +25% from the long-term trend



Above: price variation in response to fruit market supply, demand, and real fruit prices over 20 years in Australia

Key learnings from the Market assessment: Traditional Propagation (S1)

- With the variation of fruit price and supply in the Australian market, no relationship between avocado supply and the market price was observed. Within the ranges observed over 20-years, supply is price inelastic in the Australian avocado market.

- This means the Australian avocado market is not yet saturated and has the potential to expand supply.

Fruit Market Assessment: Clonal Tissue Culture Propagation (S2-S7)

Future effects of clonal tissue culture on the baseline fruit market equilibrium have been analysed through two hypothetical cases:

Case 1: Hypothetical 100% adoption of TC plantings by the industry for established and new growers, with an *equal* increase in fruit supply and demand (status quo plants/fruit in the market)

Indicative market impacts: Short term

- An equal increase in fruit supply and demand.
- No significant change in fruit market price
- No detrimental change in consumer and producers' welfare

Case 2: Hypothetical 100% adoption of TC plantings by the industry for established and new growers, with fruit supply *exceeding* demand.

Indicative market impacts: Long term

- Both supply and demand increase.
- But the change in supply exceeds the change in demand
- Decrease in fruit market price due to supply (production) surplus.
- Increase in consumer welfare due to reduced price.
- Decrease in producer welfare due to reduced price.

Potential Australian Demand for Clonal Tissue Culture Plants

There are an estimated 1.87 million mature (equivalent) avocado trees in Australia in 2020. Approximate annual demand for avocado plants in 2020 was approximately 103,800 plants.

- 37,317 replacement trees per year
- 66,501 new plants per year

Fruit prices, demand, and supply

Tissue culture plants allow for fruit availability one year earlier by reducing the time-lag for the availability of trees, therefore decreasing total cash outlays (investment) and re-investment of saved funds can be used to purchase more trees within the avocado enterprise. This may speed up the development of new and existing orchards in Australia.

Key Economic Benefits of Tissue Culture

Bio-economic analysis learnings:

- Growers are likely to benefit financially from adopting tissue culture through:
  - shorter lead times for plant supply
  - increased capacity for capturing fruit price premiums
  - increased productivity potential
- Financial benefits for avocado nurseries from:
  - shortening plant production time
  - bringing new rootstocks to market sooner, and
  - increased supply capacity
- New rootstocks may be better **matched** to specific environments
- Plant price has no significant influence on orchard financial returns

Market analysis learning:

- Australian avocado industry is likely to get benefits from tissue culture through:
  - increased production
  - import substitution
  - export market development
  - potential greater varietal choice, fruit quality, and menu utility for consumers

Key Contacts

Project Teams

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Contact for:

- Queries about TC in the lab
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- Queries about domestic and international survey results
- Incorporating psychology into agricultural production studies

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Contact for:

- Queries about financial modelling of TC
- Commercialisation of agribusiness innovations

Where/how to purchase Reed tissue culture trees:

Anderson Horticulture

andersonhorticulture.com.au
management@andersonhorticulture.com.au
0438 390 441

Photos by: Jayeni Hiti Bandaralage, UQ

Further Information and Resources

For all project information, please head to qaafi.uq.edu.au/tissue-culture

Further project-related information:

Scientific publications on UQ tissue culture technology: qaafi.uq.edu.au/horticultural-science

Current reed tissue culture plant supplier: Anderson Horticulture
andersonhorticulture.com.au/

Avocados Australia: "Survey: grower knowledge of tissue culture."
avocado.org.au/newsletter/guac250119

Media:

ABC Catalyst: "Growing Avocados."
education.abc.net.au/home#!/media/3023914/growing-avocados

ABC News: "Avocado shortages to be a thing of the past as researchers trial faster, high-density growing techniques."
abc.net.au/news/rural/2017-08-23/technique-improves-avocado-tree-availability/8833476

ABC News: "Stem cell science being used to smash avocado shortage by speeding up tree growth."
abc.net.au/news/2019-03-17/meet-the-scientist-ensuring-the-future-of-smashed-avocado/10896312

Fresh Fruit Portal: "New Aussie tissue culture system supplies "500 times more avocado plants"."
freshfruitportal.com/news/2017/08/24/new-aussie-tissue-culture-system-supplies-500-times-avocado-plants/

Good Fruit & Vegetables: "Research bears higher yielding avocados."
goodfruitandvegetables.com.au/story/6700877/research-bears-higher-yielding-avos/

WIN News video: "World-first technique to double Queensland's avocado production and ease global shortage."
youtube.com/watch?v=itAcSPKEYhE

Queensland Government

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