

# Mitter Lab: Innovating Avocado

## We love Challenges....

## We love Avocado...

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# Innovating Avocado – The list keeps on growing...

## Avocado propagation



## BioClay – Sustainable crop protection



## Adventitious Rooting

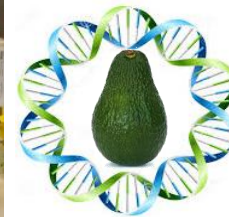
- MicroRNAs-master regulators
- Auxin Response Factors



## cryopreservation



## New frontiers - avocado genomics

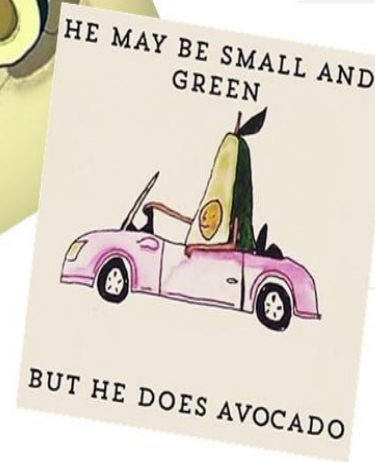
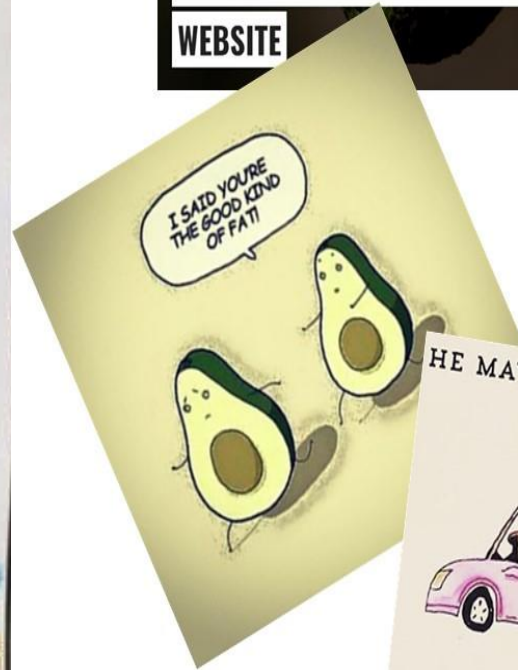


## Phase Change → Juvenile to Maturity





# Avocado: *The world's trendiest #food....*



avocadooil  
55,929 posts

avocadotree  
30,456 posts



# Avocado: *NOT* the world's trendiest #genome

NCBI 	<i>Avocado</i>	<i>Banana</i>	<i>Brussels Sprout</i>	<i>Tomato</i>	<i>Rice</i>
Nucleotides	<b>2,185</b>	267,558	237,763	92,228	769,512
Proteins	<b>1,589</b>	46,665	171,444	164,349	1,284,969
Genes	<b>131</b>	35,752	66,580	35,553	97,447
Genomes	<b>0</b>	3	1	4	22
Pubmed	<b>3,257</b>	<b>14,606</b>	<b>19,500</b>	<b>49,641</b>	<b>120,316</b>

Beaten by Brussels Sprouts !







# The avocado genome informs deep angiosperm phylogeny, highlights introgressive hybridization, and reveals pathogen-influenced gene space adaptation

Martha Rendón-Anaya<sup>a,b,1</sup>, Enrique Ibarra-Laclette<sup>a,c,1</sup>, Alfonso Méndez-Bravo<sup>a,d</sup>, Tianying Lan<sup>e</sup>, Chunfang Zheng<sup>f</sup>, Lorenzo Carretero-Paulet<sup>g</sup>, Claudia Anahí Perez-Torres<sup>a,c</sup>, Alejandra Chacón-López<sup>a</sup>, Gustavo Hernandez-Guzmán<sup>a,h,i</sup>, Tien-Hao Chang<sup>e</sup>, Kimberly M. Farr<sup>e</sup>, W. Brad Barbazuk<sup>j</sup>, Srikar Chamala<sup>k</sup>, Marek Mutwil<sup>l</sup>, Devendra Shivhare<sup>l</sup>, David Alvarez-Ponce<sup>m</sup>, Neena Mitter<sup>n</sup>, Alice Hayward<sup>n</sup>, Stephen Fletcher<sup>n</sup>, Julio Rozas<sup>o,p</sup>, Alejandro Sánchez Gracia<sup>o,p</sup>, David Kuhn<sup>q</sup>, Alejandro F. Barrientos-Priego<sup>r</sup>, Jarkko Salojärvi<sup>l</sup>, Pablo Librado<sup>s,t</sup>, David Sankoff<sup>f</sup>, Alfredo Herrera-Estrella<sup>a</sup>, Victor A. Albert<sup>e,l,2</sup>, and Luis Herrera-Estrella<sup>a,u,2</sup>

# HASS Genome Sequencing and Annotation

- Leaf Sample collected from Mother Hass tree located at Maroochy research station.
- Genomic DNA was extracted, Quantified and Quality Assured.
- Sent for Sequencing long read PacBio
- Long read sequencing (Nanopore) may be added.
- Genome scaffolding into chromosomes using Hi-C.
- Genome annotation (using transcriptome information).
- **Development of an improved publically available Browser for avocado community**

# Sequencing germplasm (>40)

Cultivars	Propagated as
A8	Rootstock
A10	Rootstock
<b>Ashdot</b>	Rootstock
Barr Duke	Rootstock
Belle Prima	Scion
Bounty	Rootstock
<b>Carmen Hass</b>	Scion
Duke7	Rootstock
<b>Dusa</b>	Rootstock
Edranol	Scion
Esther	Scion
Fuerte	Scion
<b>Gem</b>	Scion
Gwen	Scion
Hass	Scion
Hazzard	Scion
Kidd	Rootstock
Lamb Hass	Scion
Latas	Rootstock

- Samples collected from available cultivars in Australia.

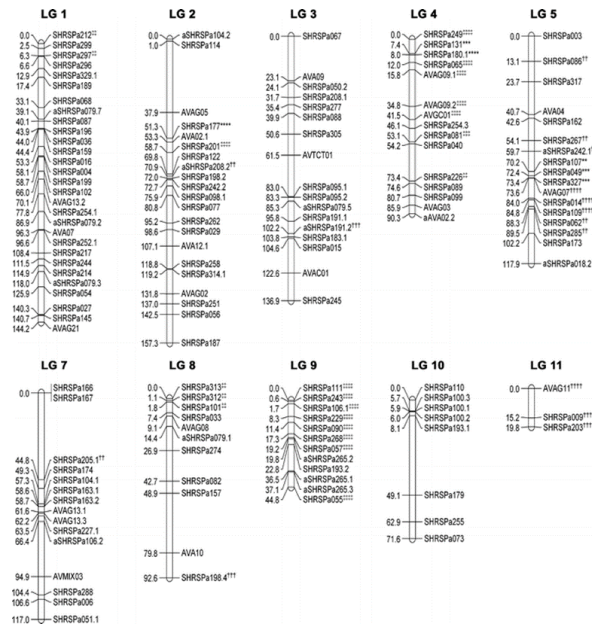
## Maluma:

- Total Reads Sequenced: 84 Million (84,389,829)
- For All samples At-least 99.9% of reads passed QC (99.95 for Maluma)
- 98.6% of reads Mapped to Available Hass Genome

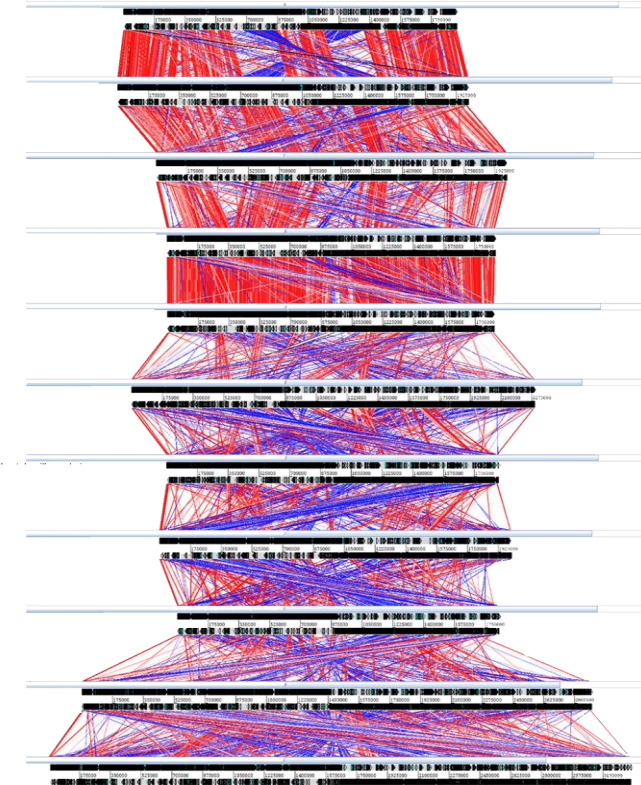
Cultivars	Propagated as
<b>Maluma</b>	<b>Scion</b>
Nabal	Rootstock
Parida	Rootstock & Scion
Pinkerton	Scion
Plowman	Rootstock
Reed	Rootstock & Scion
Sharwil	Scion
Shepard	Scion
SHSR-04	Rootstock
Simmonds	Scion
Skhirate	Scion
Thomas	Rootstock
Tora Canyon	Rootstock
Velvick	Rootstock
Whitsell	Scion
Wurtz	Scion
Zutano	Rootstock & Scion
Topa Topa	Rootstock

# What's Next – CULTIVAR SPECIFIC MARKERS

- Find the minimal set of markers required to uniquely identify specific cultivars.
- Develop an easy, lab-based assay for cultivar assignment
- **Unique genetic tag for each cultivar**



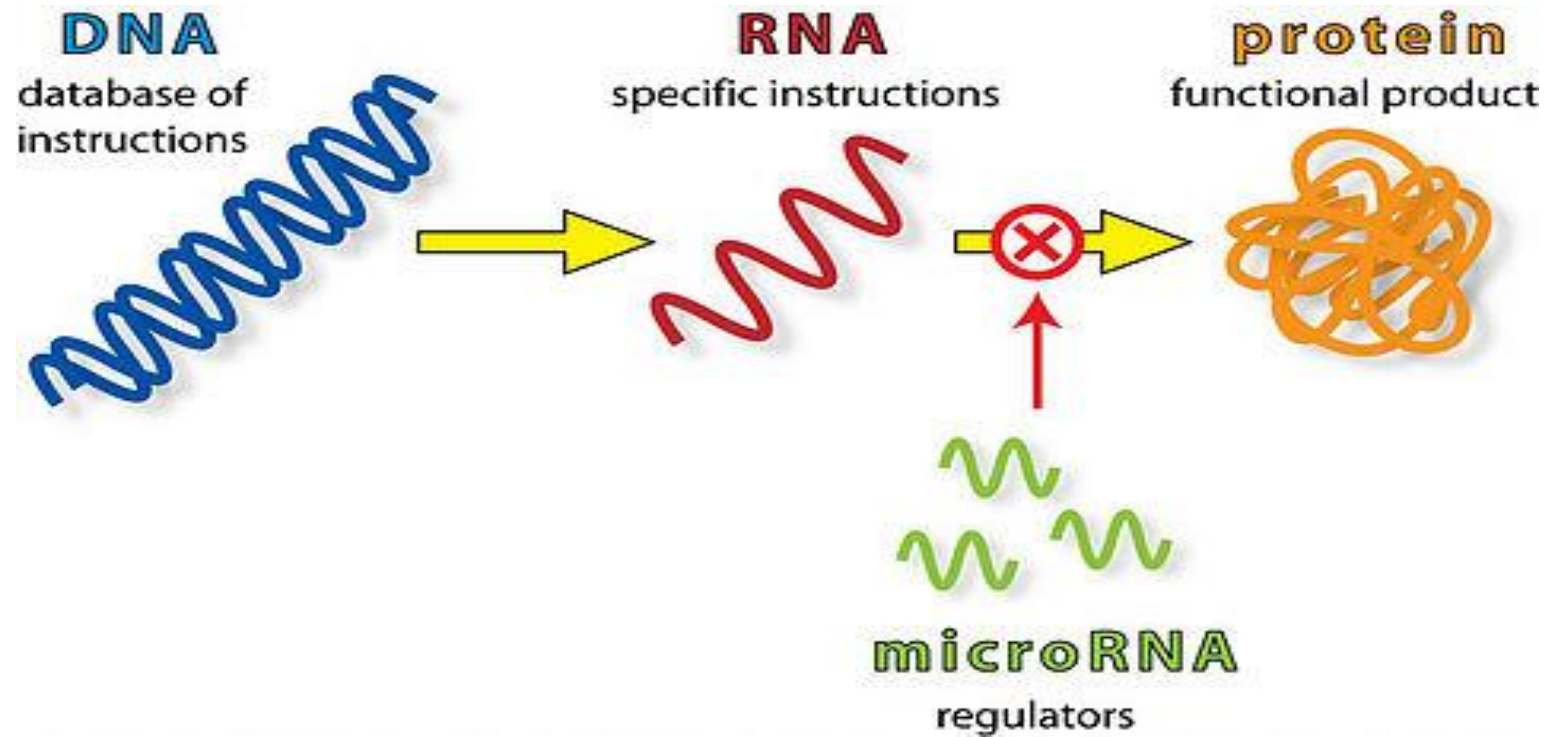
Hass 1 ATGCAACCTTCGAGAGCCTCATCGATATGTACGTCGACTTGC  
Hass 2 AAGCAACCTTCGAGAGCCTCATCGATATGTACGTCGAATTGC  
Hass 3 ATGCAACCTTCGAGAGCCTCATCGATATCTACGTCGAATTGC  
Velvick 1 ATGCAAAATTCGAGAGCCGCATCGATATCTACGTCGAATTGC  
Velvick 2 ATCCAAATTCGAGAGCCGCATCGATATGTACGTCGAATTGC  
Velvick 3 ATGCAAAATTCGAGAGCCGCATCGATATGTACGTGGAATTGC

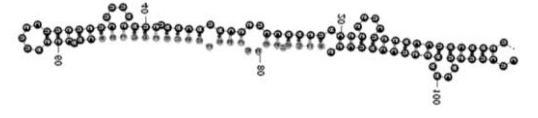
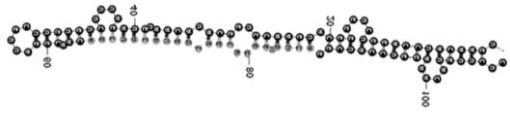




# MicroRNAs in Avocado

## What are miRNAs?





# microRNAs and Avocado-

## Adventitious Rooting

- miR160 → Root induction



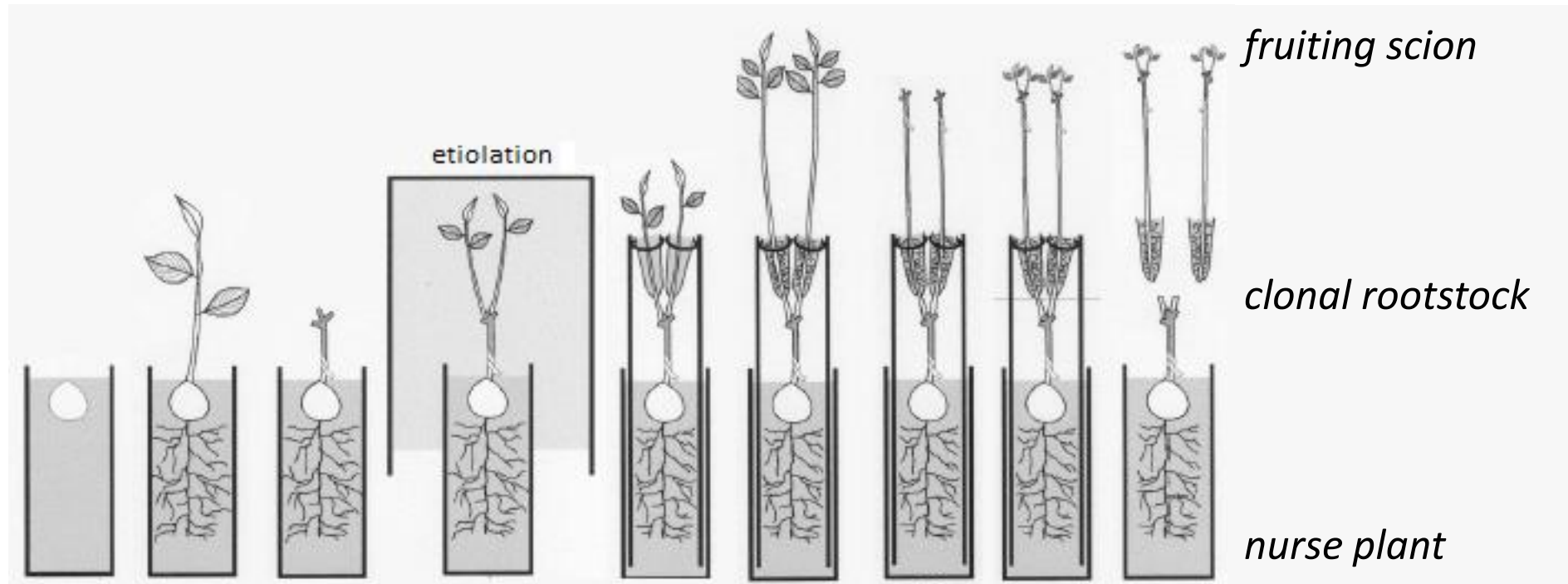
## Phase Change and Flowering

- miR156 → inhibits maturation
- miR172 → Promotes maturation and flowering



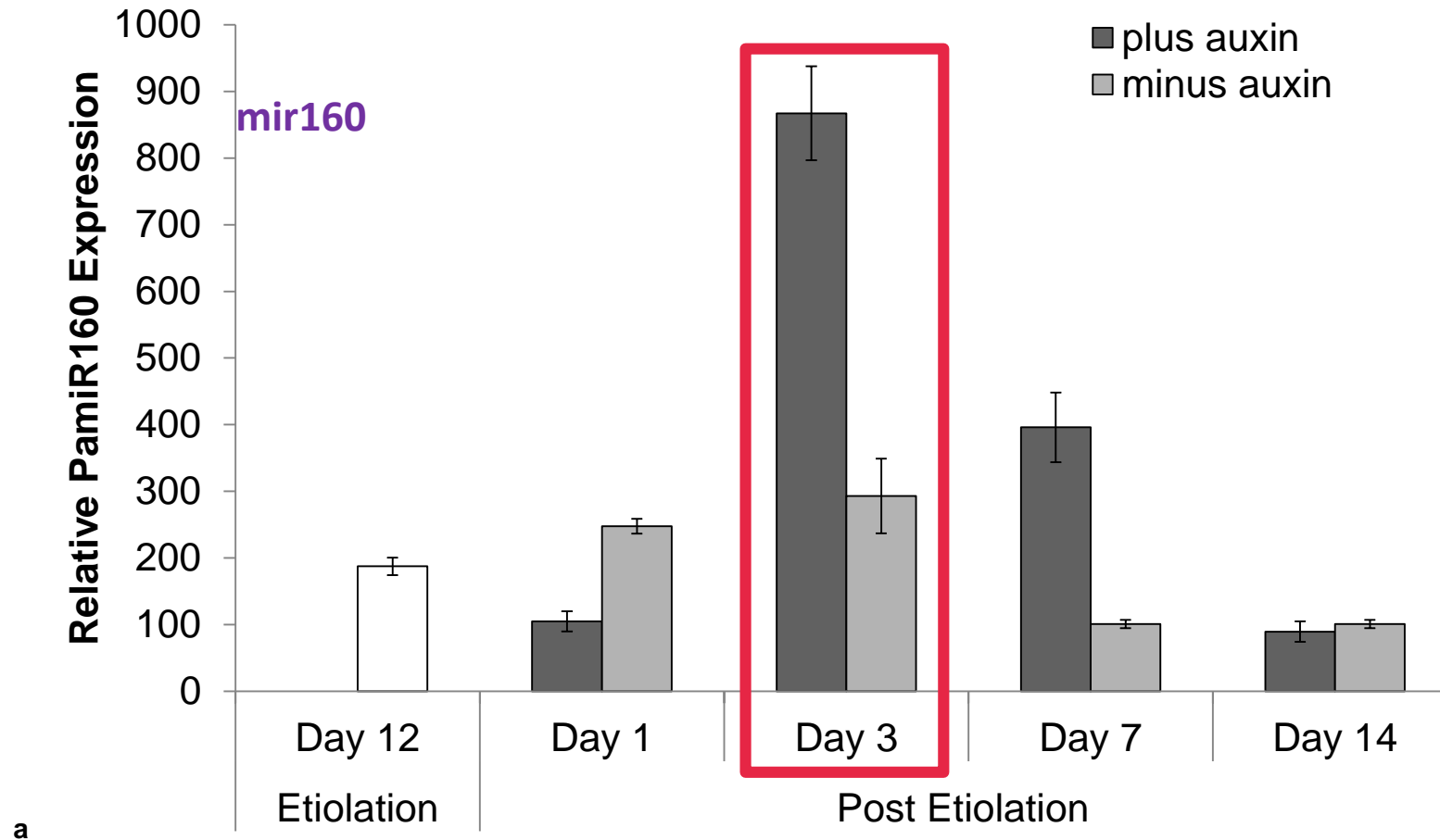
# Clonal Propagation and AR

Is miR160 involved in AR and if yes, when?





# It All Happens At Day 3



a

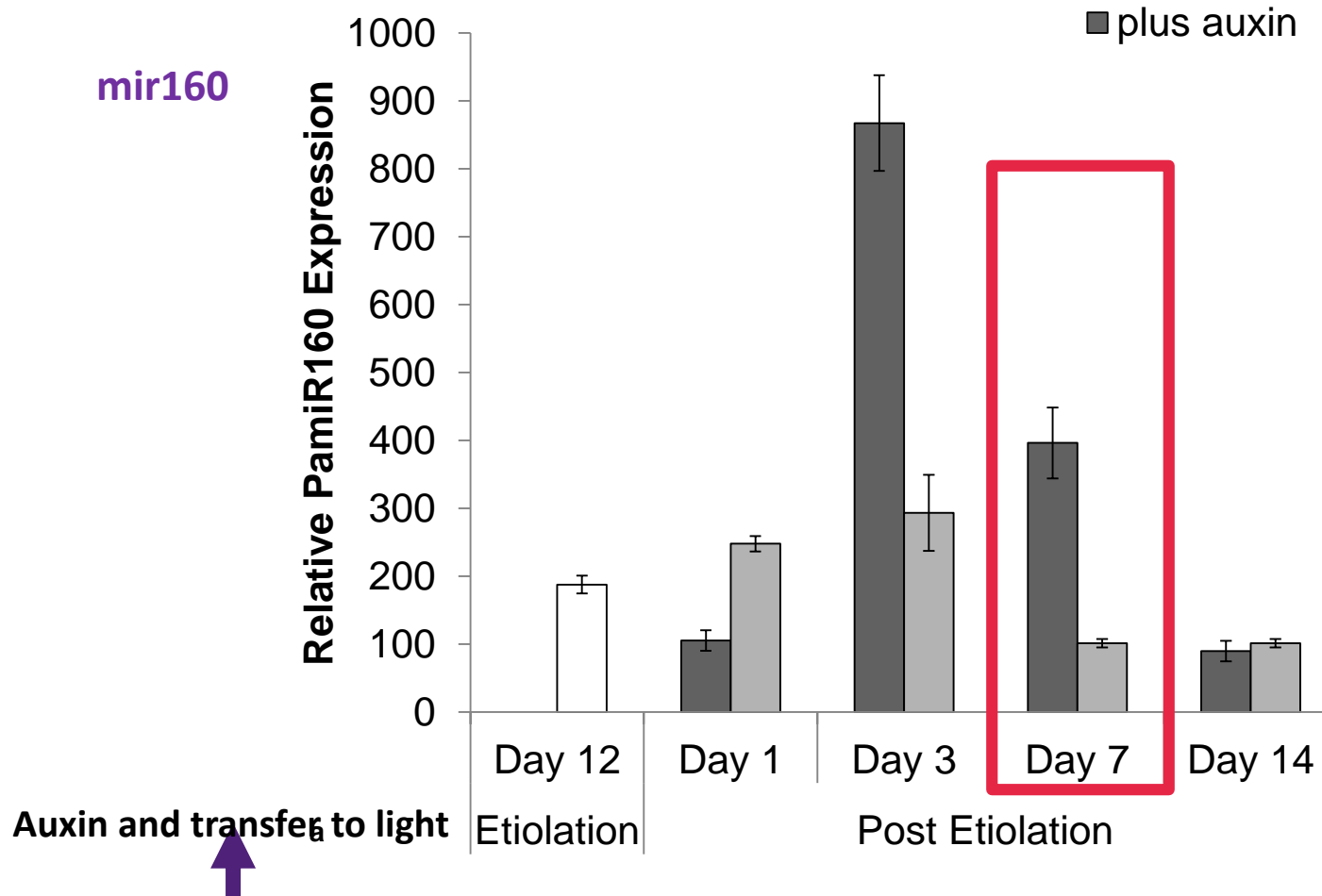


**Auxin and transfer to light**



	Treatment	% Rooted	Replicates
a	Etiolated, plus auxin	88.88%	9
b	Etiolated, minus auxin	0%	9

# ... And Gone By Day 7



## How can we turn this understanding to deliver outcomes:

- Topical Application of RNAs.
- Can we supply mir160 to the plants?
- Will it increase adventitious rooting?
- Will it improve our understanding of AR pathways ?



# MicroRNA control of Juvenility and Maturity in tropical/subtropical tree crops





# Juvenility and Vegetative Phase Transition in Tropical/Subtropical Tree Crops

*Muhammad Umair Ahsan<sup>1</sup>, Alice Hayward<sup>1</sup>, Vered Irihimovitch<sup>2</sup>, Stephen Fletcher<sup>1</sup>, Milos Tanurdzic<sup>3</sup>, Alexander Pocock<sup>3</sup>, Christine Anne Beveridge<sup>3</sup> and Neena Mitter<sup>1\*</sup>*

<sup>1</sup>Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD, Australia,

<sup>2</sup>The Volcani Center, Agricultural Research Organization, Institute of Plant Sciences, Rishon LeZion, Israel,

<sup>3</sup>School of Biological Sciences, The University of Queensland, Brisbane, QLD, Australia

# Tree Phase transition

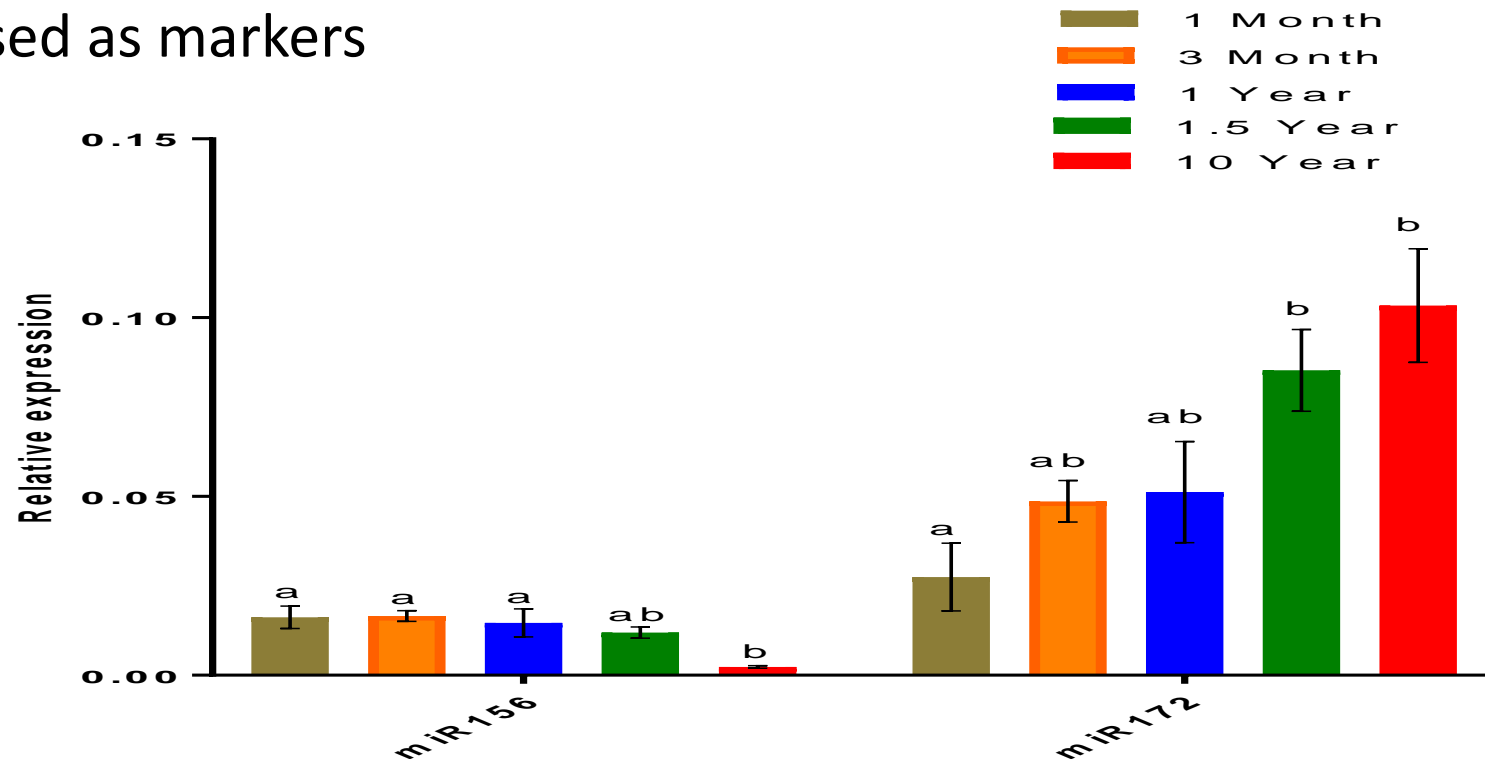
- Juvenile to Reproductive.
- miR156 – prolongs juvenility and delays flowering
- miR172 – Promotes flowering/reproductive phase transition





# Vegetative phase transition in Avocado

- High miR156 expression observed in young plants
- miR172 expression increases with age
- Can be used as markers



(Ahsan, Mitter et al., 2019)

Ahsan *et al.* *BMC Plant Biology* (2019) 19:382  
<https://doi.org/10.1186/s12870-019-1994-5>

BMC Plant Biology

RESEARCH ARTICLE

Open Access

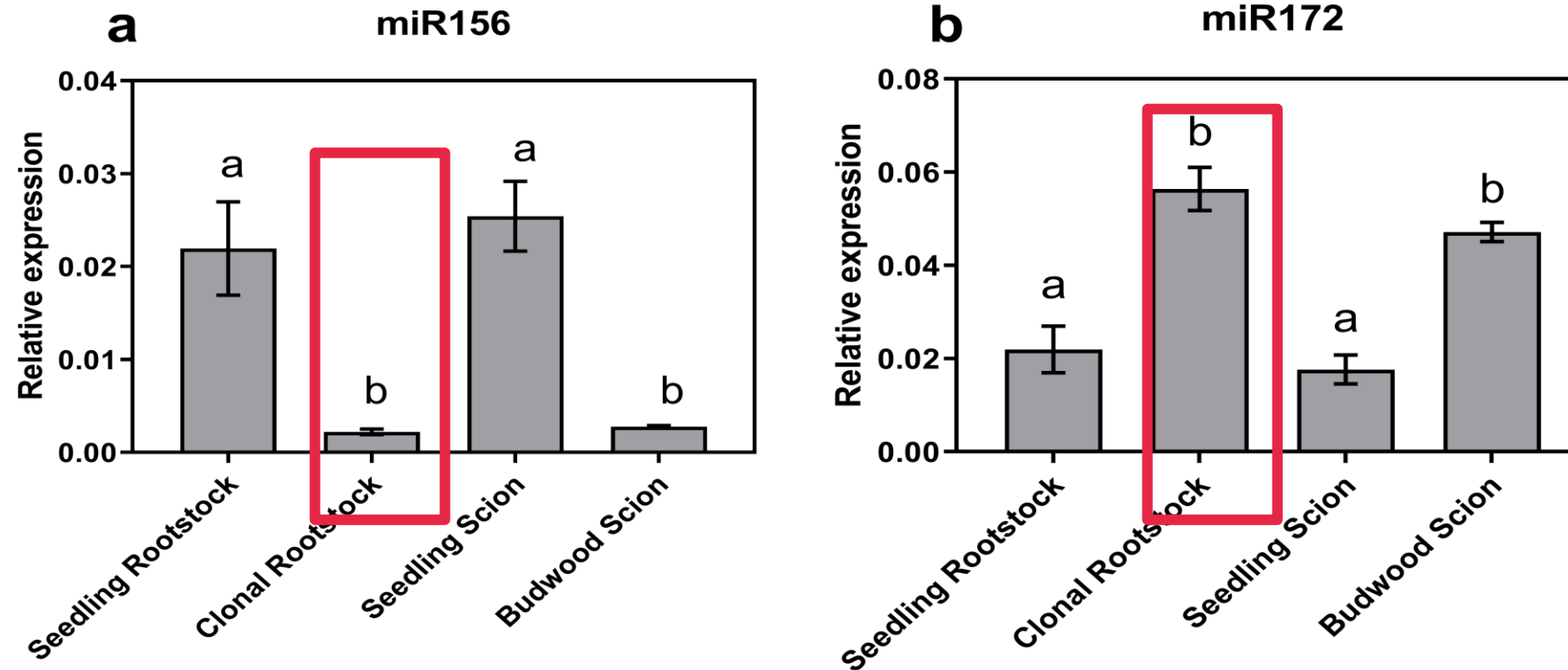
# Scion control of miRNA abundance and tree maturity in grafted avocado



Muhammad Umair Ahsan<sup>1</sup>, Alice Hayward<sup>1</sup>, Mobashwer Alam<sup>1</sup>, Jayeni Hiti Bandaralage<sup>1</sup>, Bruce Topp<sup>1</sup>, Christine Anne Beveridge<sup>2</sup> and Neena Mitter<sup>1\*</sup>

# Maturity of the propagation material

Clonal rootstock – low miR156 and high miR172 → molecularly ‘mature’



(Ahsan Mitter et al., 2019)



# Effect of clonal Rootstock defoliation on gene expression in scion

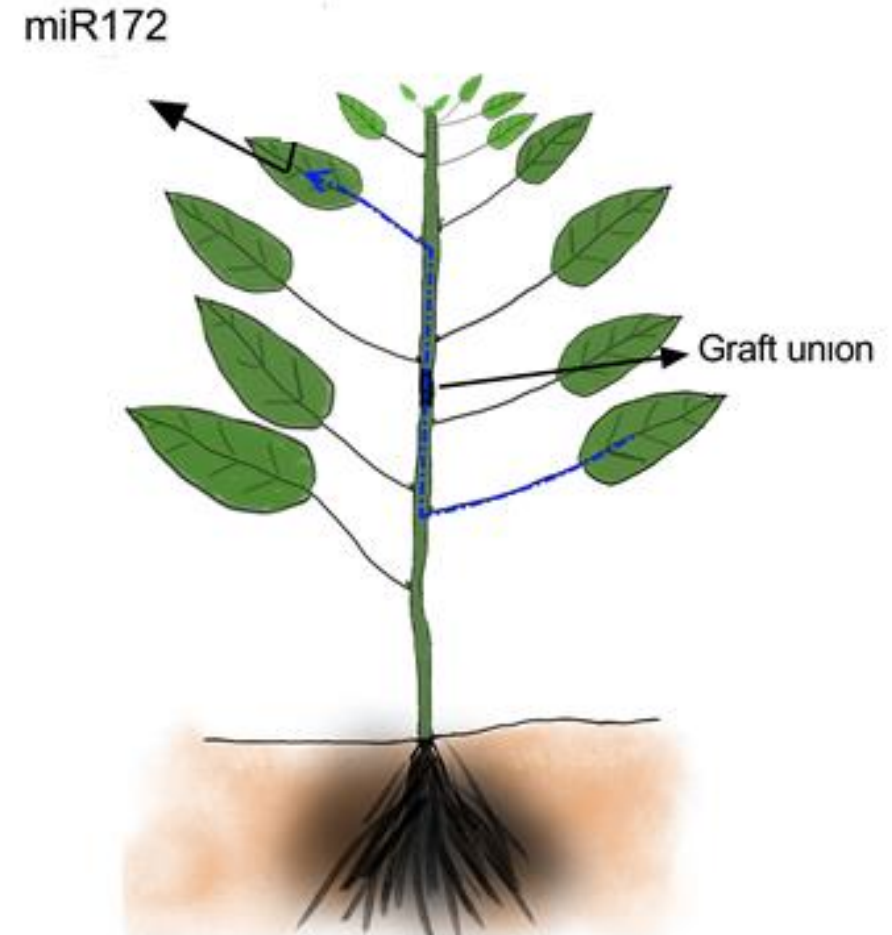
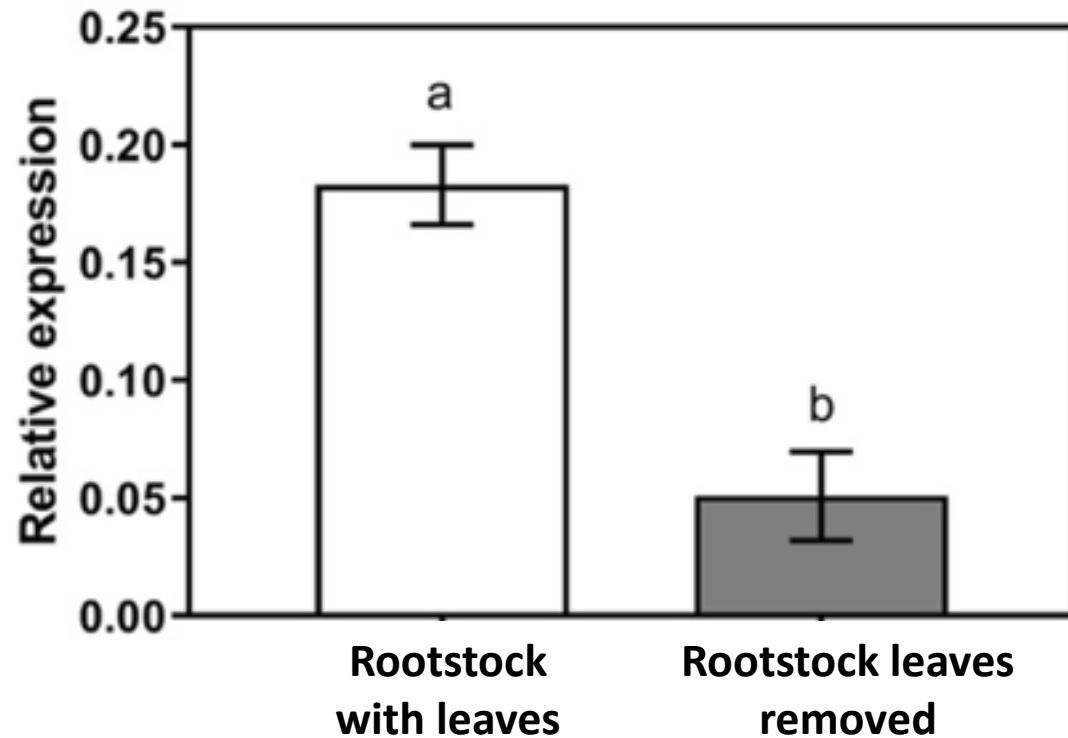
Defoliated rootstock

With leaves



# Leaves promote miR172 in Scion

miR172 expression in scion



# Take Home Messages

- **MicroRNAs are key players in plant development**
- **miR160 is involved in AR regulation**
- **miR156 and miR172 may control maturity and phase change in avocado**
- **In grafted plants leaf-derived signals from clonal rootstocks affect miR172 status**

# Cryopreservation of Avocado Shoot Tips for Germplasm Conservation



Avocado seeds are  
recalcitrant and heterozygous.



Recalcitrant seeds do not survive  
freezing and drying, therefore not  
amenable to seed storage.

Avocados are open-pollinated  
crops and seeds are heterozygous  
and have limited conservation  
value.

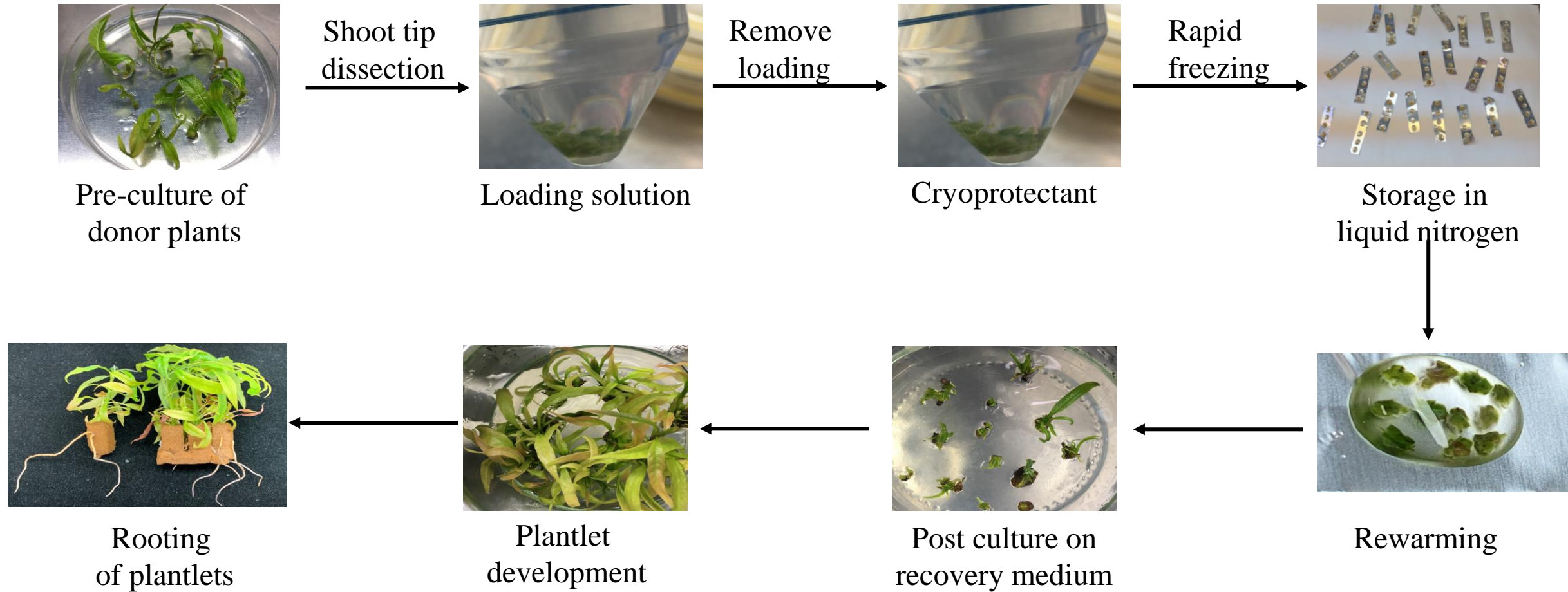


Cryopreservation is the storage of living tissues at ultra-low temperature in liquid nitrogen ( $-196^{\circ}\text{C}$ ).

Protocols have been developed for plant species using

- Apices
- Calli
- Cell suspensions
- Seeds
- Somatic embryos
- Zygotic embryos

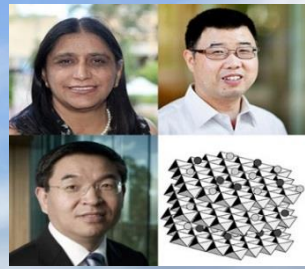




## CRYOBANK FOR AVOCADO GERmplasm



# RNA based Pesticides - BIOCLAY



- RNA as the biological active ingredient
- Clay particles as delivery agents

- **NON - GM**
- **NO RESIDUE**
- **SPECIFIC**
- **SUSTAINABLE**
- **SAFE**

**Inventors:**  
**Prof Neena Mitter,**  
**Prof Gordon Xu**  
**Prof Max Lu**

**BILL & MELINDA**  
*GATES foundation*



A person wearing a full-body white protective suit, a blue face mask, and yellow gloves is spraying a fine mist of pesticides onto a field of large green leafy plants. The person is holding a long-handled spray wand. The background is a dense field of similar green plants.

## PESTICIDES:

- RESISTANCE
- RESIDUE
- LACK OF SPECIFICITY
- NEW CHEMICALS



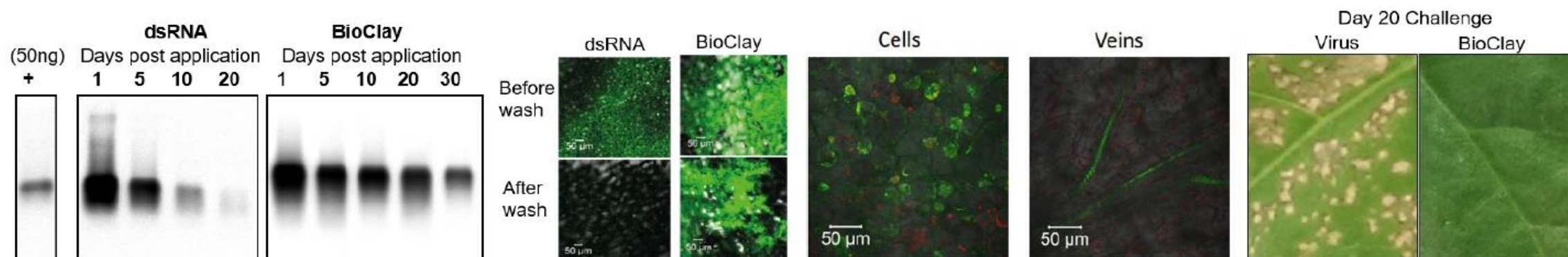
nature  
plants

ARTICLES

PUBLISHED: 9 JANUARY 2017 | VOLUME: 3 | ARTICLE NUMBER: 16207

# Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses

Neena Mitter<sup>1\*</sup>, Elizabeth A. Worrall<sup>1</sup>, Karl E. Robinson<sup>1</sup>, Peng Li<sup>2</sup>, Ritesh G. Jain<sup>1</sup>, Christelle Taochy<sup>1,3</sup>, Stephen J. Fletcher<sup>1,3</sup>, Bernard J. Carroll<sup>3</sup>, G. Q. (Max) Lu<sup>2,4</sup> and Zhi Ping Xu<sup>2\*</sup>





# BioClay for Tomato Spotted Wilt Virus on Capsicum



# Targeting *Phytophthora* root rot – Lupins as test host



No zoospores



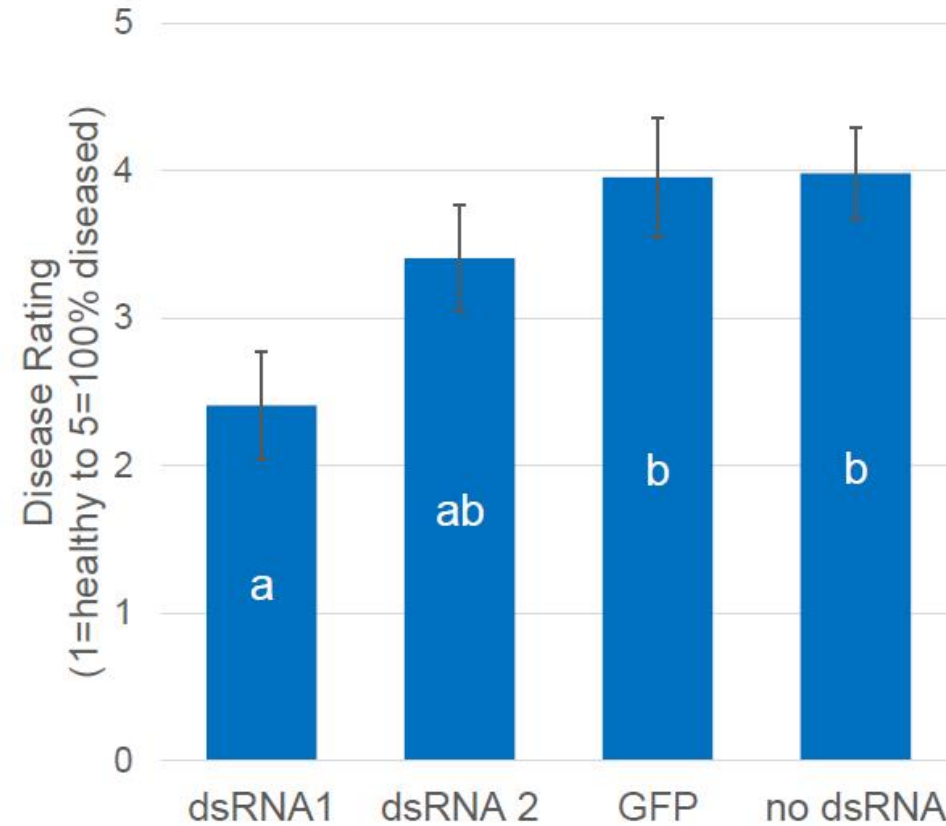
With zoospores



**Zoospores + dsRNA**  
shoots = 0.9 cm, roots = 7.6 cm  
10/10 seedlings healthy



# Targeting *Phytophthora cinnamomi* in pineapple

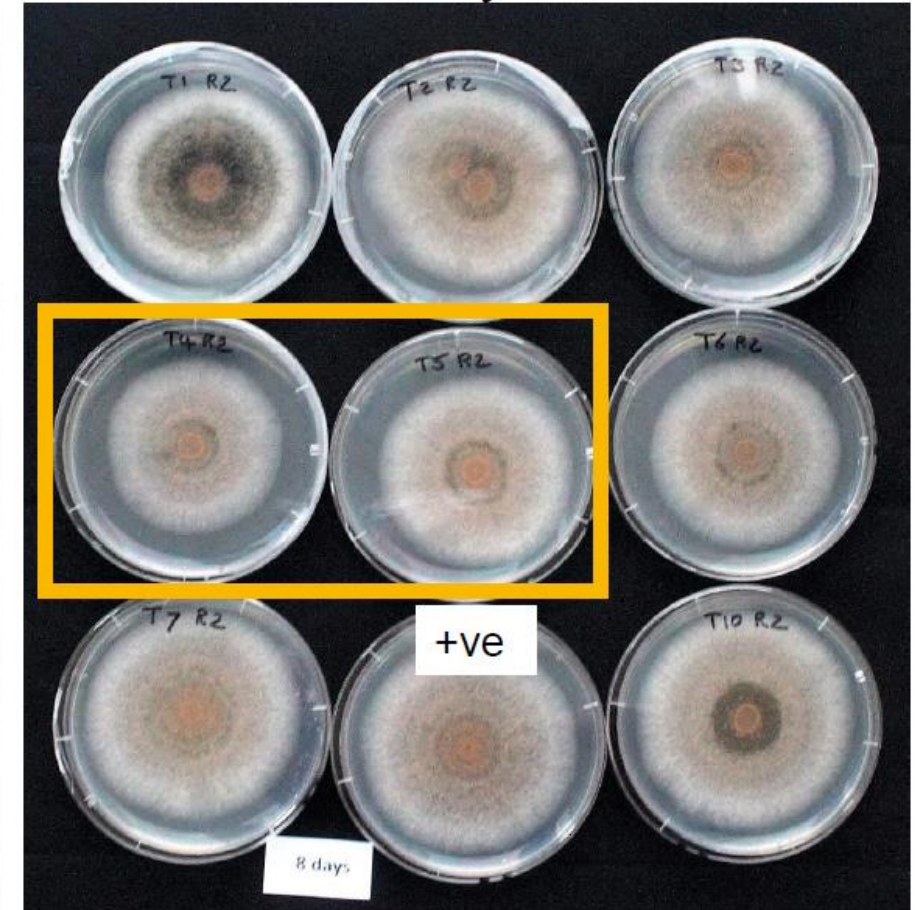
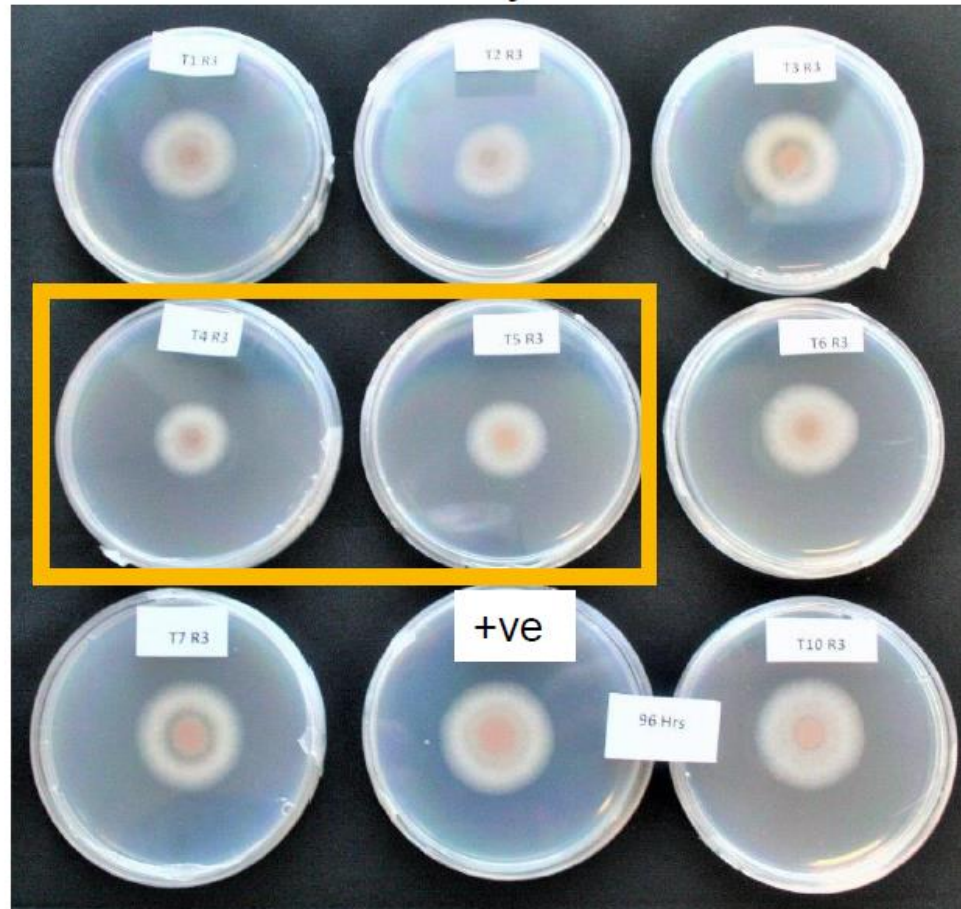


# Targeting *Colletotrichum* sp.

4 days

8 days

Two  
promising  
targets  
identified





# Avocado fruit bioassay





# Topical RNA application – NON-GM

In Australia, the Office of the Gene Technology Regulator has legislated  
topically-applied RNA is exempt from GMO regulations (Schedule 1A –  
Techniques that are not gene technology)



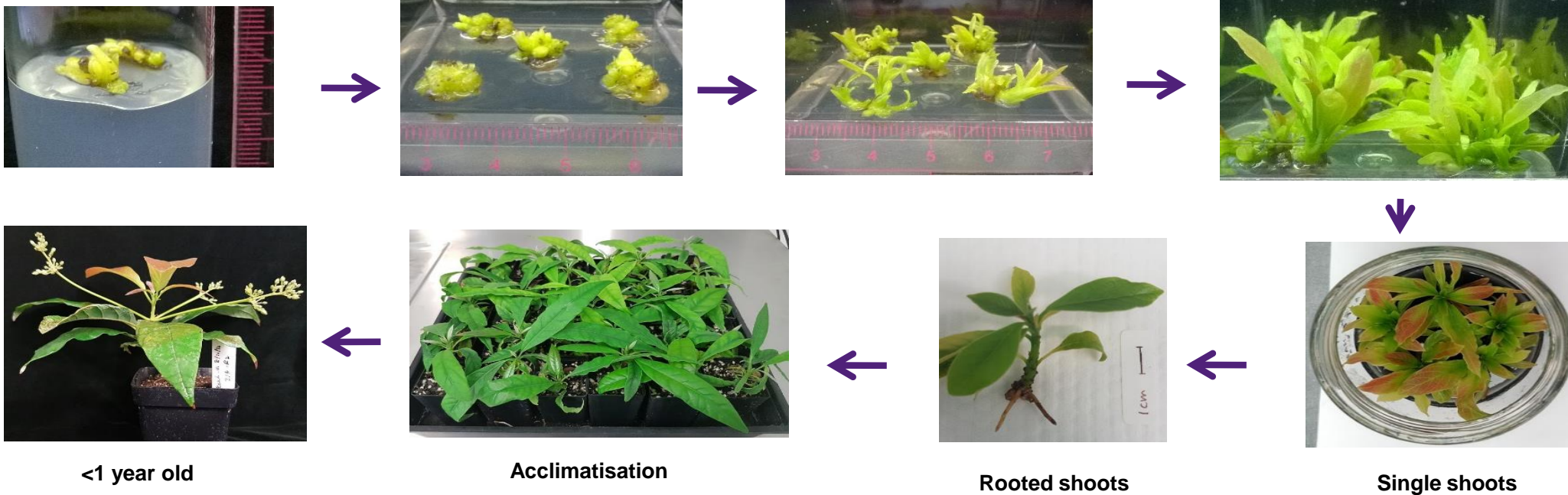
Gene Technology Amendment (2019 Measures  
No. 1) Regulations 2019

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This item provides that techniques involving applying RNA to an organism to temporarily induce RNA interference are not gene technology, provided that:

- the RNA cannot be translated into a polypeptide
- the organism's genome sequence cannot be altered as a result, and
- an infectious agent cannot be produced.

# AVOCADO PROPAGATION



37

***500 plants from one shoot tip***

Mitter Lab: Innovating Avocado



# FROM LAB TO NURSERY





# NURSERY TO FIELD









# TEAM NEENA

