

# Advances in Research Methods for Commercial Micropropagation Systems

Presented By

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# Overview

- Development of a Production Scheme

1. Multiplication Media Formulation and Optimization
2. Production Structure Optimization
3. Rooting Rate Improvement

- From the Multiplication Base to the Customer

1. Culture Vessels
2. Photoautotrophic Expression Systems
3. Plantlet Transitioning

Presentation Available on Website at

<http://www.tissuegrown.com/Browse/ResearchMethods>

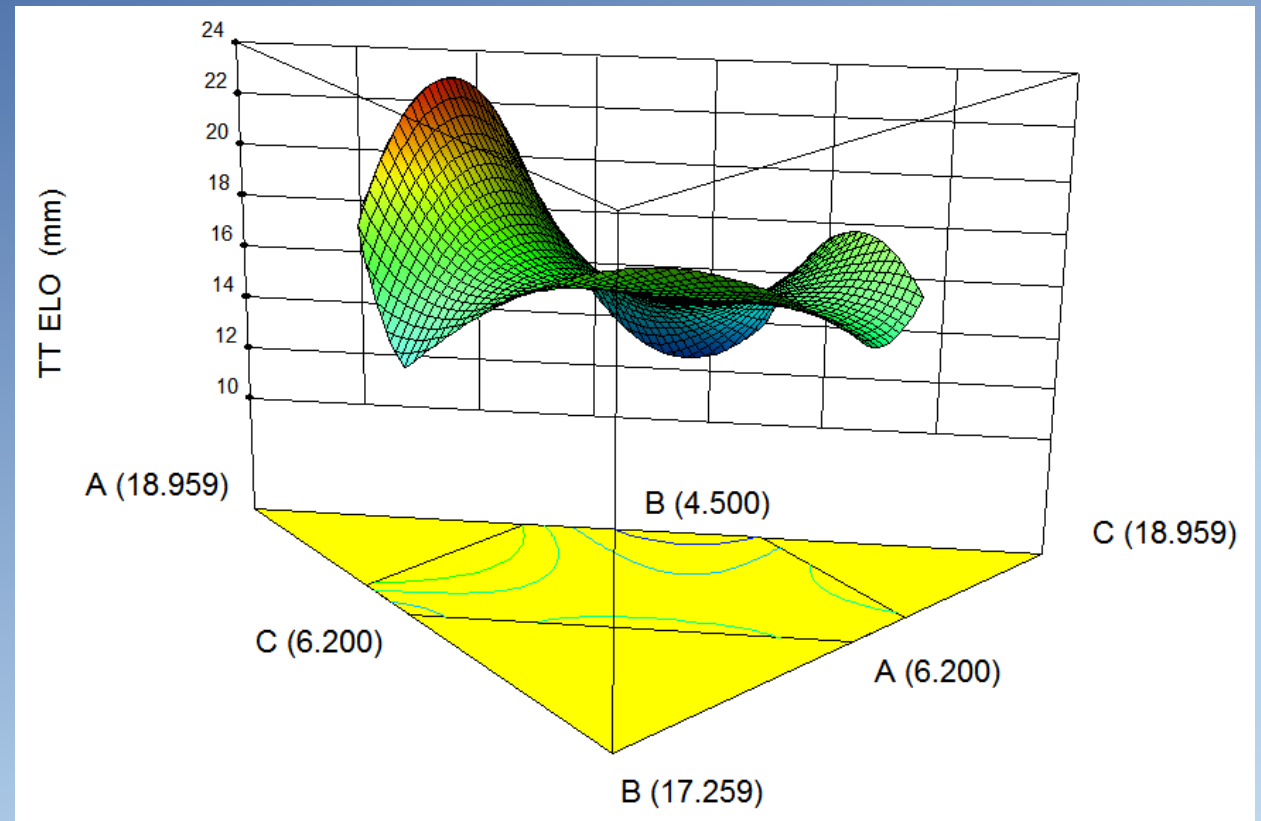
# Keys to Developing an Efficient Production Scheme

1. Multiplication Media Formulation and Optimization
2. Production Structure Optimization
3. Rooting Rate Improvement

# Media Development: Plant Mineral Nutrition

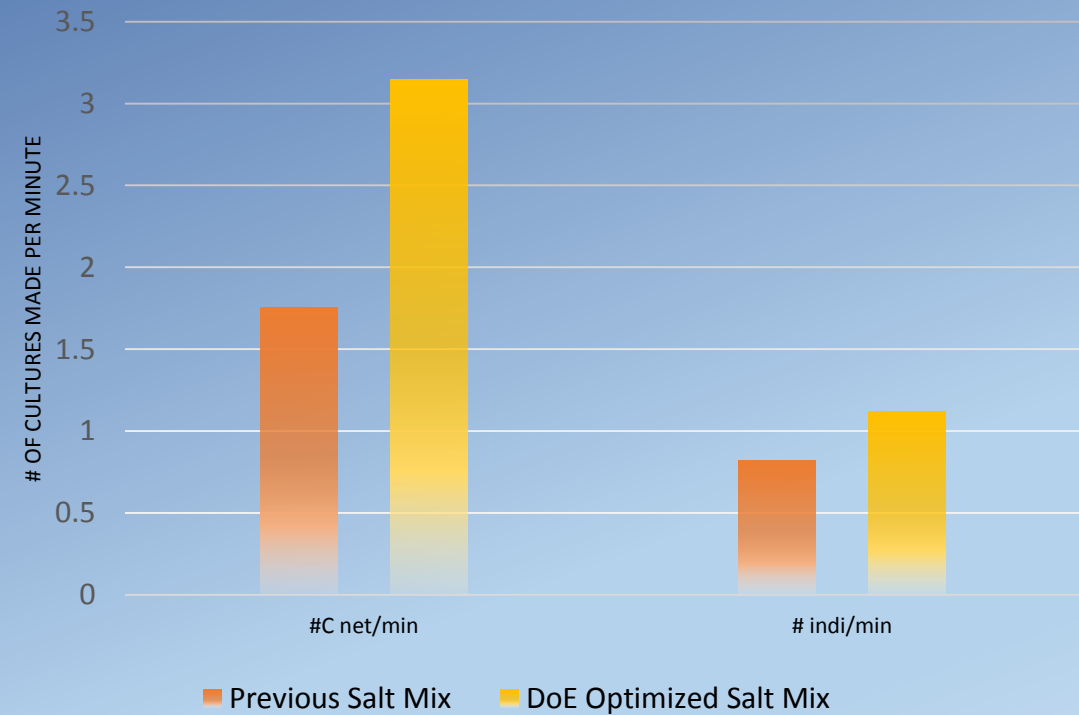
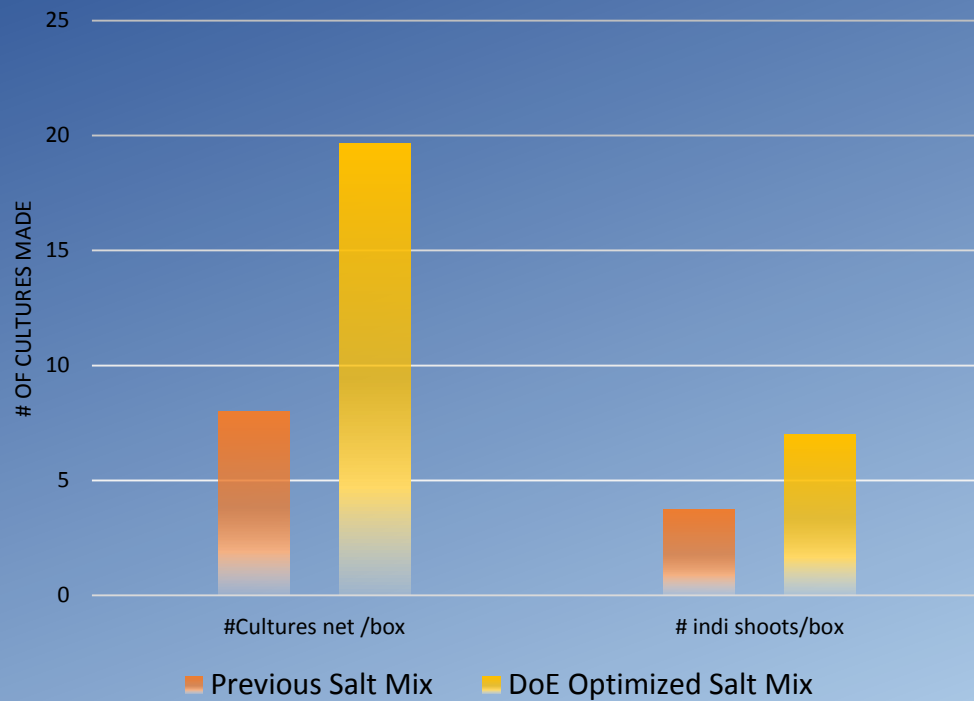
## Design of Experiment Advantages

- Allows “a direct estimation of ion-specific proportional and amount effects on plant tissue”(Dr. Niedz).
- Concurrent assessment of mineral requirements allows rapid media optimization.
- Software is available for those who don't want to spend extensive time calculating statistics



# Media Development: Plant Mineral Nutrition

## COMPARISON OF TISSUE CULTURE MEDIAS BEFORE AND AFTER DOE OPTIMIZATION



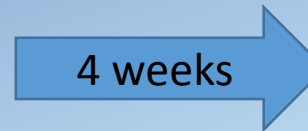
# Media Development: Plant Mineral Nutrition

## Design of Experiment: Challenges

- Large scale of experiments require very uniform environmental conditions, large amounts of plant material, and meticulous planning.



Starting Material





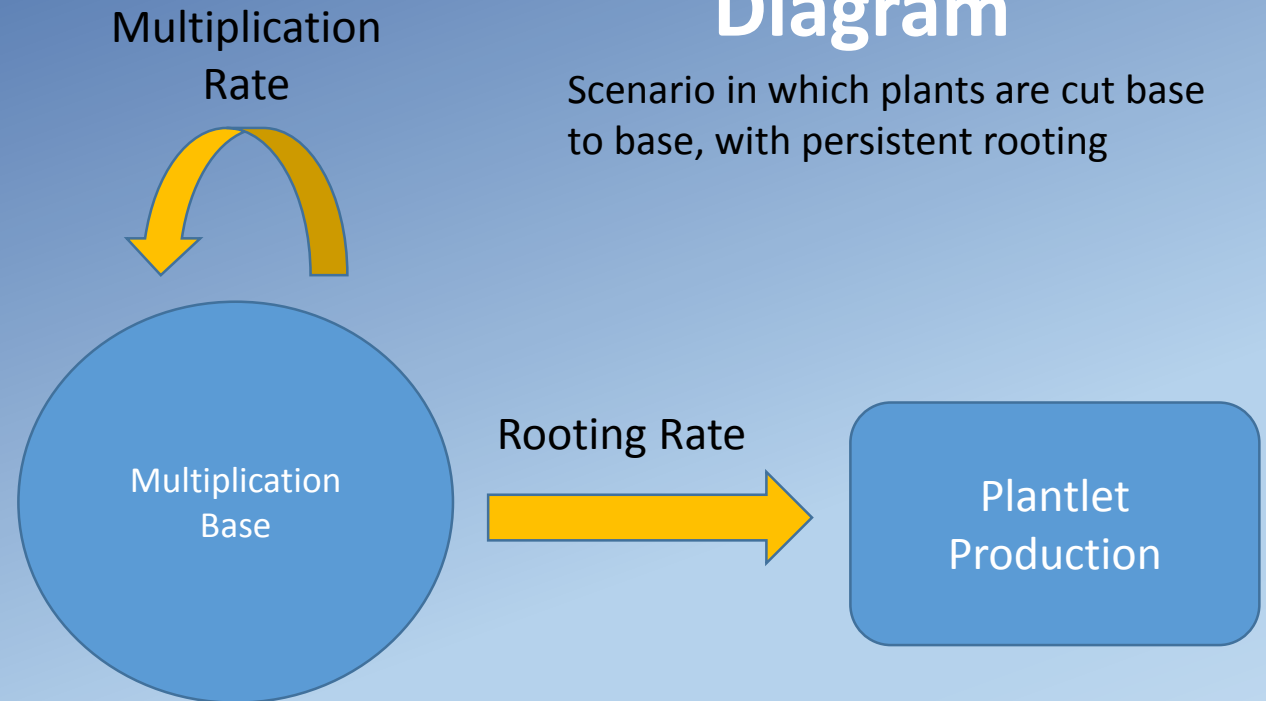
# Production Structure Optimization: Developing an Efficient Production System

## Keys to Success

- Collect average operator rates for every production step:
  1. # worked/hr
  2. # made multiplication/hr
  3. # made rooting stage/hr
- Use linear algebra to determine optimal balance between production steps which emphasize growth of multiplication base vs. rooted plantlet production
- Distribute labor pool accordingly to optimize production goals

## Basic Production Diagram

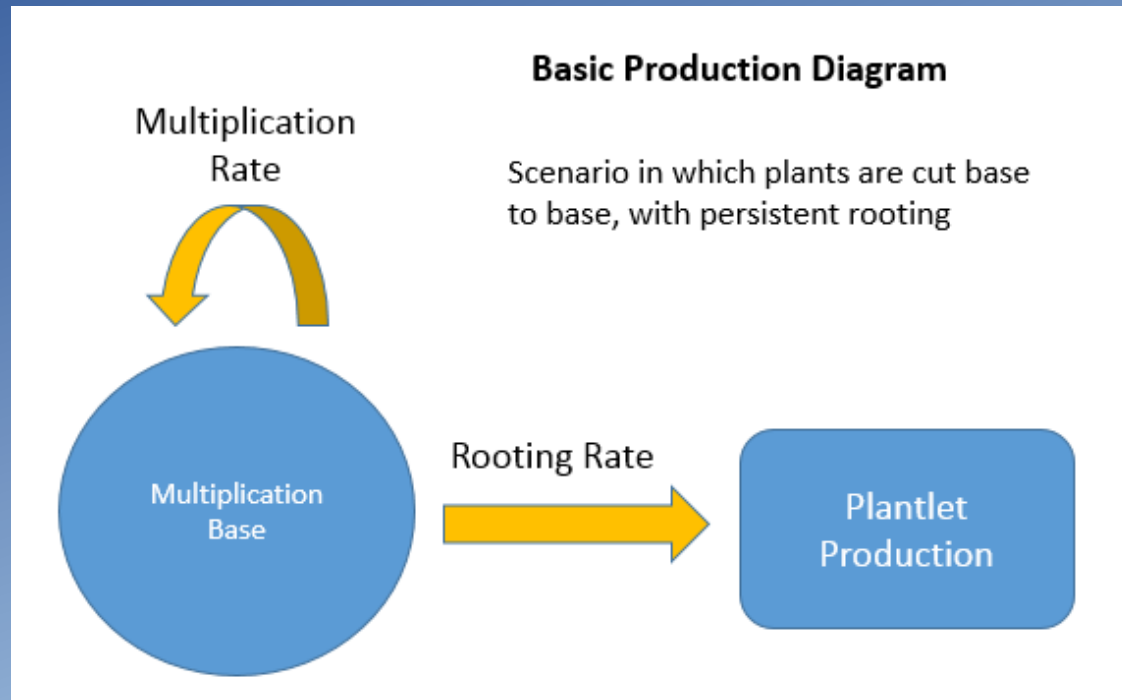
Scenario in which plants are cut base to base, with persistent rooting



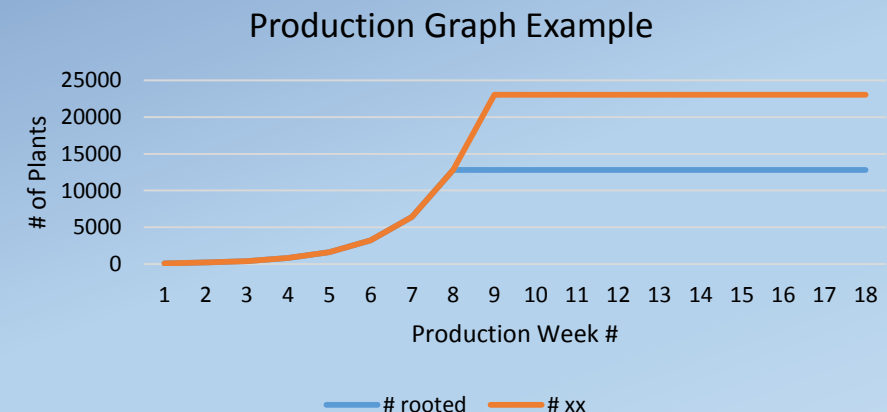
# Production Structure Optimization:

In this simple production scheme:

- Rooted Plantlet Production (#R) = (Rooting Rate: %R) x (Avg. Operator Speed ) x (# of Operators at Step)
- Total Number of Multiplication Plants Made = (Avg. Operator Speed) x (# Operators at Step) x (Multiplication Rate)

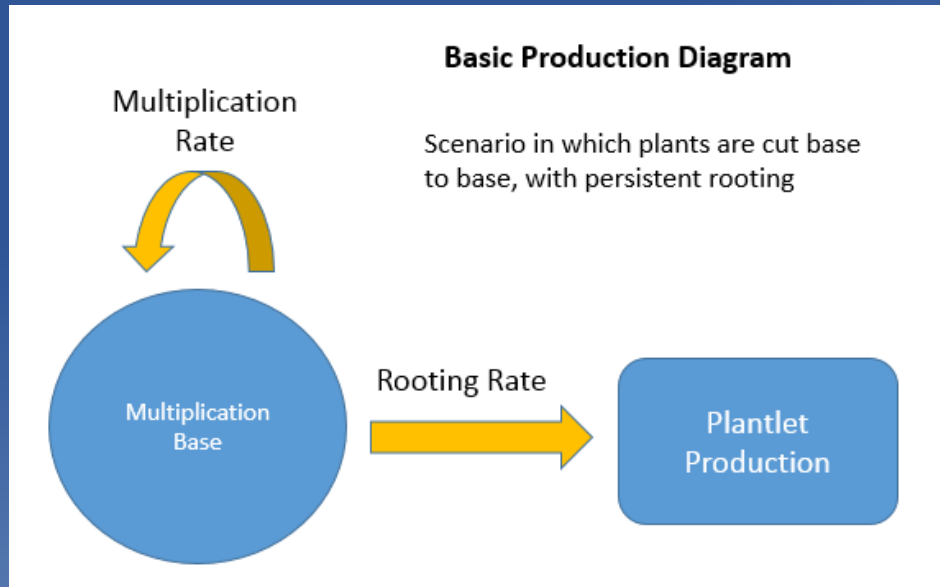


A production model of this type shows a typical exponential growth phase, until production numbers are met, growth of the base stops, and product is harvested in the form of rooted plants.



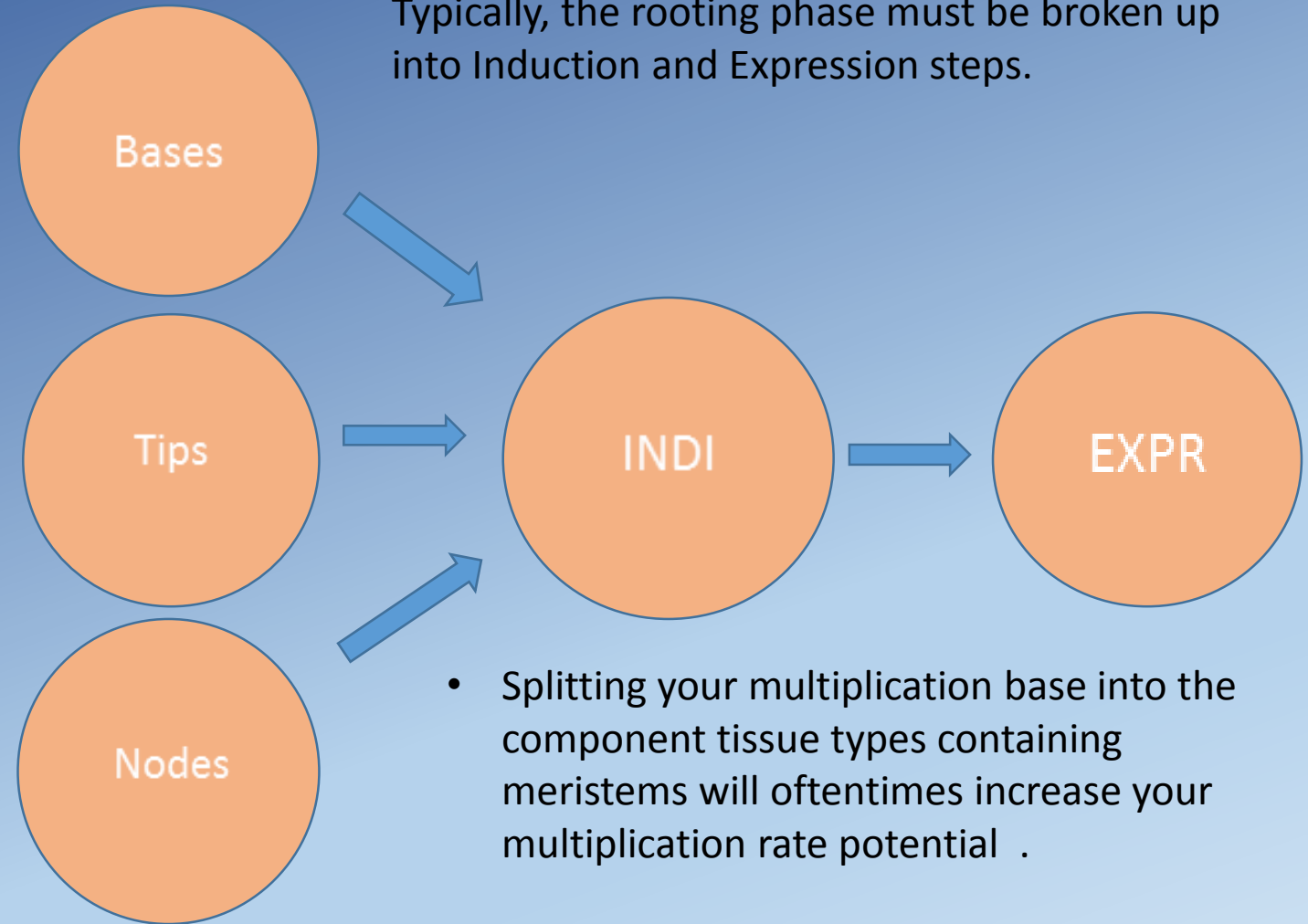


Sadly, a simple scenario such as this is hardly ever viable.



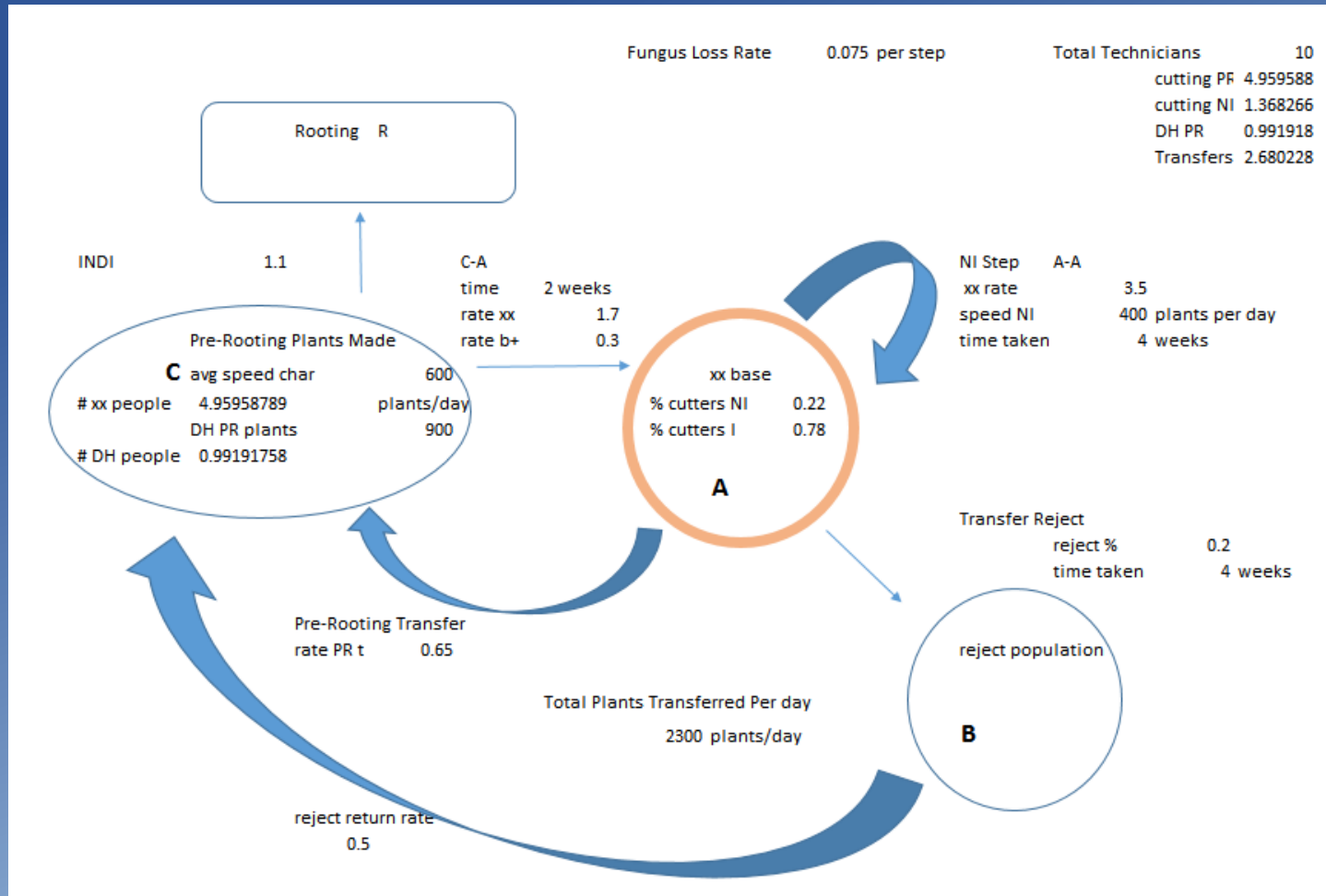
- In addition, by separating the multiplication and rooting step, it is normally possible to use media with higher cytokinin content to multiply plantlets, thereby achieving a higher multiplication and induction shoot rate.

- Rooting will not occur at production levels in recalcitrant species on a multiplication media. Typically, the rooting phase must be broken up into Induction and Expression steps.



- Splitting your multiplication base into the component tissue types containing meristems will oftentimes increase your multiplication rate potential .

# Production Structure Optimization: Inclusion of a Pre-Rooting Step



- In recalcitrant species, including a pre-rooting stage to allow plantlets to develop, and harden prior to root induction will likely increase rooting rates.
- It is necessary to perform a mathematical evaluation of technician's operation rates in order to determine whether the % rooting increase justifies the additional labor that must be spent to incorporate said pre-rooting stage.
- Use Excel's solver function to determine the optimal distribution of labor in order to achieve a desired multiplication rate, and rooted plantlet output. This process is not necessarily that math intensive.

Tracking the equilibrium multiplication rate requires you to calculate the product of the multiplication rates through each pathway (A-B-C-A, A-C-A & A-A), and multiply it by the proportion of technicians needed for said stage.

## Production Structure Optimization: Inclusion of a Pre-Rooting Step

Pistachio Plantlet Prior to Pre-Rooting Stage



- Plantlet prior to Pre-Rooting Stage: leaves are pale green, slim, and vitrified. Plantlets root at a lower rate, and transition less effectively.

Pistachio Plantlet After Pre-Rooting Stage



- Post Pre-Rooting Stage: leaves are dark green, thoroughly expanded, and less vitrified. Plantlet rooting rate is increased by 20%



# Culture Vessels



- Custom lids for magenta boxes improve aeration by providing ventilation lower than the uppermost leaves, and at the top, through a highly-permeable paper filter, thereby reducing commonly seen abnormalities, such as vitrification, resulting in higher overall plant quality.



- Specialized expression boxes allow in-vitro production of rooted plugs. An interchangeable filter allows you to adjust vessel humidity to meet the specific demands of root expression and subsequent transitioning.



# Why Most Commercial Tissue Culture Labs Still Use Vessels with Gelled Media



Walnut: Chandler



Peach-Almond: Cornerstone



Pistachio

- It is extremely common for desirable varieties-- especially selections from mature trees-- to contain a wide variety of non-pathogenic endophytes which become evident after several subcultures. It appears possible to continually propagate some varieties with a high bacterial titer in gelled media without significant losses to production potential. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3826729/> is a great example of the endophytic diversity within tissue culture plantlets.
- Gelled media generally produces a higher quality shoot, which, will likely root at a higher rate, and transition more effectively. While the multiplication potential is far lower in gelled media, the overall production potential of transitioned plantlets remains similar when one takes into account the expected losses throughout the entire production process for many varieties.



# Photoautotrophic Expression Systems

- Customizable Arduino microcontrollers allow affordable control of CO<sub>2</sub>-enriched microenvironments. A microcontroller equipped with a CO<sub>2</sub> sensor can be linked to an SD card to record historical information about the CO<sub>2</sub>-enriched environment.
- Open-source nature of the Arduino community means that an abundance of information is available on how to construct and code these devices. Check out [http://www.veetech.org.uk/Prototype\\_CO2\\_Monitor.htm](http://www.veetech.org.uk/Prototype_CO2_Monitor.htm) for an example, or ideas on how to construct your own.
- Our particular models were designed, constructed and installed by [drees.oellerich@chopperlight.com](mailto:drees.oellerich@chopperlight.com).



MG-811 CO<sub>2</sub> Gas Sensor Module and Arduino Microcontroller (2)





# Photoautotrophic Expression Systems

Kiwi: Ambient CO<sub>2</sub>



Kiwi: CO<sub>2</sub> Enriched to ~2500ppm



- Plantlets which tend to be highly heterotrophic in culture— as evidenced by the rapid sugar consumption rate in multiplication media, as measured by BRIX refractometer— will more readily continue growing under enriched CO<sub>2</sub> when placed in media without sugar.

- CO<sub>2</sub> enrichment in expression can significantly increase transitioning rates. Rooted plantlets tend to flush more extensively in a CO<sub>2</sub> enriched environment, thereby providing larger and hardier plants which are more easily transitioned (4).
- As there is no exogenous sugar in the media for a photoautotrophic expression systems, it is possible to get productivity from contaminated plantlets, i.e. fungus, or endogenous bacteria.

# Greenhouse Transitioning

- Ultrasonic fog benches ease transitioning, especially with difficult to transition plants. A semi-closed environment (still allowing some fog to escape for temperature regulation) with an enclosed humidistat allows you to steadily reduce the humidity around the plants thereby minimizing the transitioning shock to the plantlets. Regular watering is still necessary in order to control temperature, and because soil drying still occurs under ultrasonic fog.



Transitioned Peach-Almond Hybrids



- Transducers and fogger heads are available from <http://www.mainlandmart.com/foggers.html>



# Citations

1. Niedz, RP and Evens, TJ. "The effects of nitrogen and potassium nutrition on the growth of nonembryogenic and embryogenic tissue of sweet orange (*Citrus sinensis* (L.) Osbeck)." *BMC Plant Biology* 2008, 8:126. Accessed May 29, 2015. <http://www.biomedcentral.com/1471-2229/8/126>.
2. Liddament, M. "A Prototype Carbon Dioxide (CO2) Indoor Air Quality Monitor with Digital Display and SD Card Data Logger for under £55." July 18, 2013. Accessed May 29, 2015. [http://www.veetech.org.uk/Prototype\\_CO2\\_Monitor.htm](http://www.veetech.org.uk/Prototype_CO2_Monitor.htm)
3. Zhang, Powell, Benyon, Zhou, and Duan. "Deciphering the Bacterial Microbiome of Citrus Plants in Response to 'Candidatus Liberibacter asiaticus'-Infection and Antibiotic Treatments." *PLoS One*. 2013; 8(11): e76331. Accessed May 29, 2015. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3826729/>
4. Kozai, T. and Y. Xiao. 2006. A Commercialized Photoautotrophic Micropropagation system. IN: Gupta, S.D. and Y. Ibaraki (eds), Plant Tissue Culture Engineering, 335-371, Springer: Dordrecht.
5. Lawrence Berkeley Laboratory - Earth Sciences Division, 2015, Phylochip, <http://esd.lbl.gov/research/facilities/andersenlab/phylochip.html>, accessed May 29, 2015.