Plant Tissue Culture

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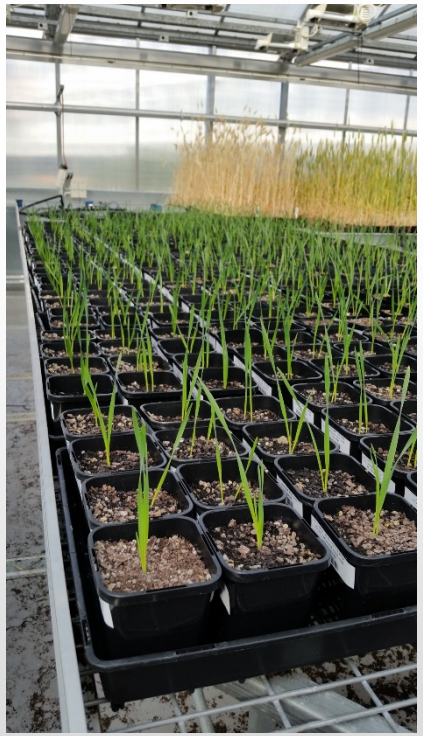
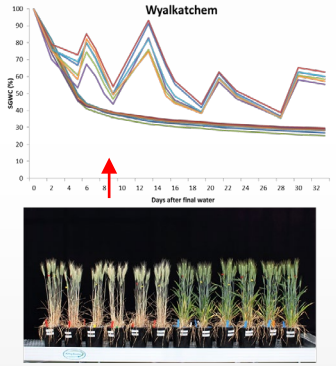
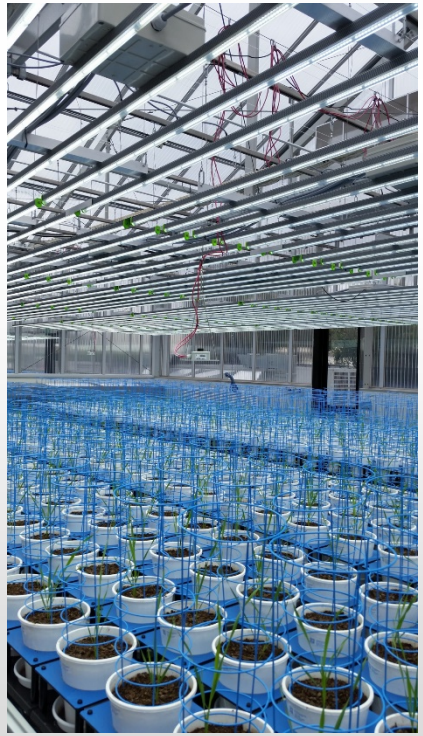
My back ground

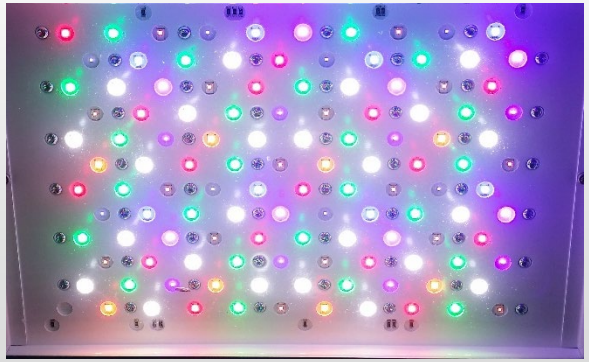
Plant phenomics and physiology to quantify plant performance to abiotic stress.

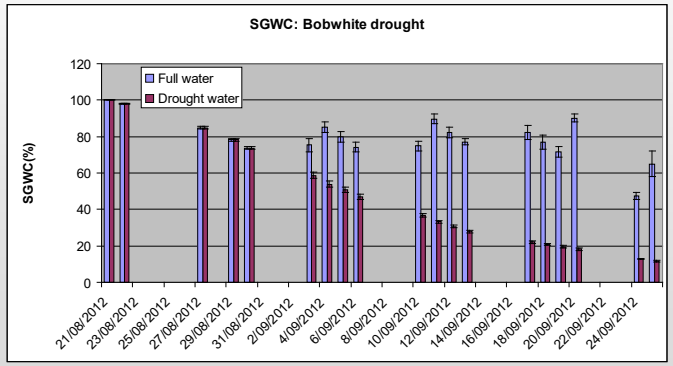
- Controlled environment research and breeding; new technology to automate.
- Design & management of plant performance assays; plant production to tight specifications; high throughput imaging & physiology to quantify performance.











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Summary

- Introduction
- History
- Applications
- Benefits and constraints
- Tissue culture facility requirements
- Micropropagation workflows and media
- Summary

Introduction to plant tissue culture

- *In vitro* propagation of plant parts, such as single cells, tissues and organs under sterile conditions.
- Used routinely in ornamental and horticultural crop nurseries:
  - Begonia
  - Rosa
  - Chrysanthemum
  - banana
  - potato
  - grapevine
- Industry-wide application of tissue culture:
  - potato seed certification schemes mandate the use of tissue culture to produce healthy primary stock.
  - tissue culture of banana provides Panama disease-free stock.

History of plant tissue culture

When	By whom	What
1838	Schwann & Schleiden	Cellular theory
1902	Haberlandt	Totipotency First attempt to in vitro culture
1904	Hanning	Nearly mature zygotic embryo developed into a plant in vitro
1925	Laibach	Development of inter-specific embryos in vitro
1948	Skoog	Kinetin could induce organogenesis in tobacco callus
1957	Skoog & Miller	Effects of hormone interaction in vitro
1962	Murashige & Skoog	MS medium formulation
1966	Guha & Maheshwary	Haploid plants from anthers
1971	Takabe	First Plant from protoplast

Applications of plant tissue culture

Disease elimination from unhealthy plants

- Meristem culture to re-establish healthy stock after viral, fungal or bacterial infection.

Transport and quarantine inspection

- Low volume & ease of observation, preferred form of cultivation by DAWR.

Germplasm Storage

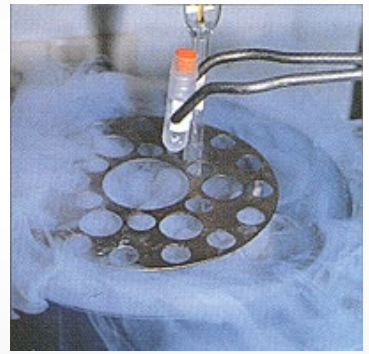
- Ease of manipulation & miniaturised plant organs convenient for preservation of live genetic resources.

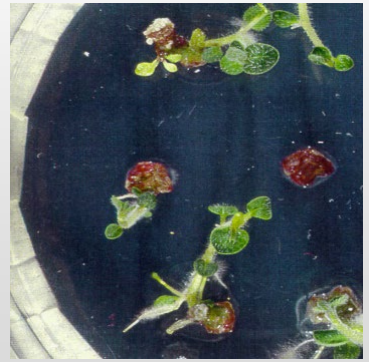
Genetic improvement

- Biotech approaches to genetic modification and the creation of novel variation.

Micropropagation

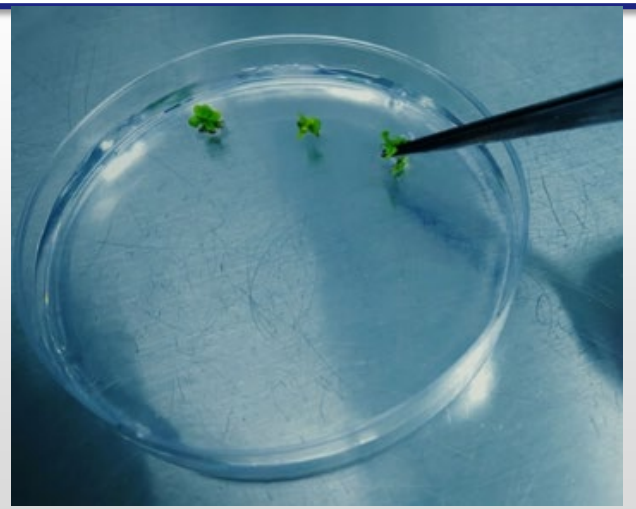
- Rapid multiplication of clean, clonal stock.





Advantages of micropropagation?

- Rapid and efficient plant multiplication at scale.
- Genetic and phenotypic uniformity (clonal multiplication).
- Production of high health plants, TC process is sterile & disease free.
- Pathogen testing for certification is readily applied *in vitro*.
    - Note: Recent phytoplasma infection in Cannabis report in USA.
- Reduced space requirements to store hundreds of varieties.
- Reduced-risk maintenance of high value stock genotypes.

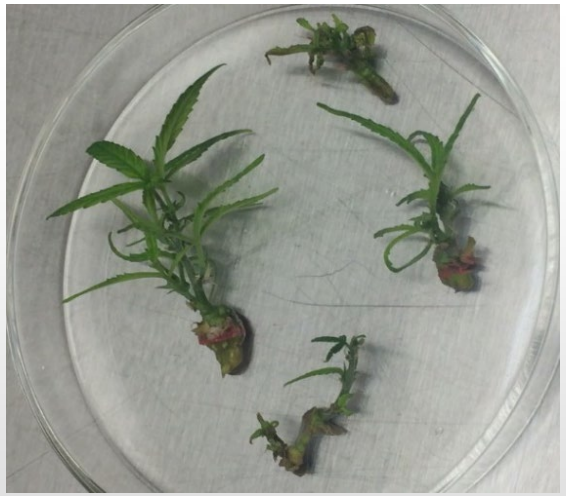


Disadvantages of micropropagation?

- Set-up costs for specialised laboratory and equipment.
- Skilled labour (aseptic technique).
- Cannabis may be difficult to establish *in vitro* and may require considerable time and effort to optimise growth conditions.
- Losses due to infection; potential for off-types/spontaneous mutations.

Micropropagation: critical factors

1. Facilities: laboratory, tissue culture growth room, nursery.
2. Mother plants: a source of healthy tissue to bring *in vitro.*
3. Workflow and sterile media formulation: optimisation, per variety.
4. Skilled staff: majority operational costs.



1. Micropropagation: facility requirements

- Media prep room: autoclave, accurate balance, pH meter, dispenser, 4 °C storage.
- Transfer room (clean room): laminar flow hoods, instruments and sterilisers.
- Growth room: temperature & humidity control, horticultural lighting.
- Nursery: acclimatisation and transplantation of rooted plantlets.

2. Micropropagation: mother stock

- A source of optimal health, elite phenotype explants (cuttings) is required.
- Not all genotypes of a species are amenable to *in vitro* propagation.
- Explants are nodal cuttings with axillary buds (meristem culture).
- After initial establishment, mother plants can be maintained *in vitro.*
- Establish & maintain a genotype library of high-health stock plants efficiently.

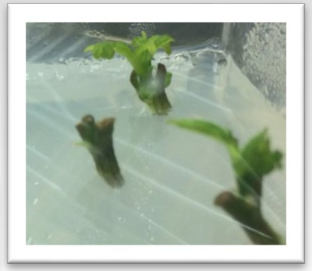
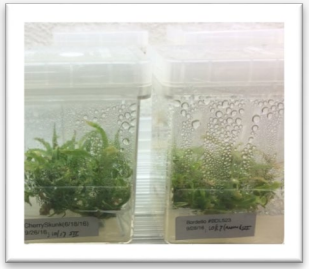
3. Micropropagation: workflows & media

1. Initiation (day 0)
   - Nodal cutting surface sterilisation
   - node isolated & embedded

2. Shoot multiplication (day 25)
   - aseptic sub-culture of shoots
   - shooting is plant hormone enhanced: **cytokinin**

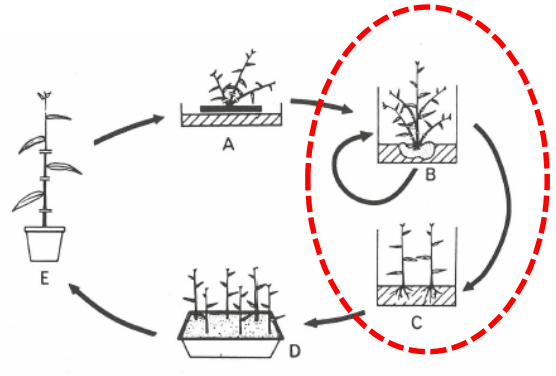
3. Rooting of shoots (day 50)
   - rooting is plant hormone enhanced: **auxin**

4. Acclimatisation/de-flask (day 75)
   - plant nursery, humidity gradually reduced
   - transplant into soil, rockwool, coco coir…

3. Micropropagation: workflows & media

1. Initiation
2. Shoot multiplication
3. Rooting of shoots
4. Acclimatisation



In Vitro Cell.Dev.Biol.-Plant (2009) 45:12–19
DOI 10.1007/s11627-008-9167-5

DEVELOPMENTAL BIOLOGY

Thidiazuron-induced high-frequency direct shoot organogenesis of *Cannabis sativa* L.

Hemant Lata · Suman Chandra · Ikhlas Khan · Mahmoud A. ElSohly

ak. J. Bot., 41(2): 603-608, 2009.

A MICROPROPAGATION SYSTEM FOR CLONING OF HEMP (*CANNABIS SATIVA* L.) BY SHOOT TIP CULTURE

REN WANG[1*], LI-SI HE[1], BING XIA[1], JIN-FENG TONG[1], NING LI[2] AND FENG PENG[1]

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Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

In vitro mass propagation of *Cannabis sativa* L.: A protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological, biochemical and genetic fidelity of micropropagated plants

Hemant Lata[a,*], Suman Chandra[a], Natascha Techen[a], Ikhlas A. Khan[a,b], Mahmoud A. ElSohly[a,c]

3. Micropropagation: workflows & media

Media formulation, key ingredients

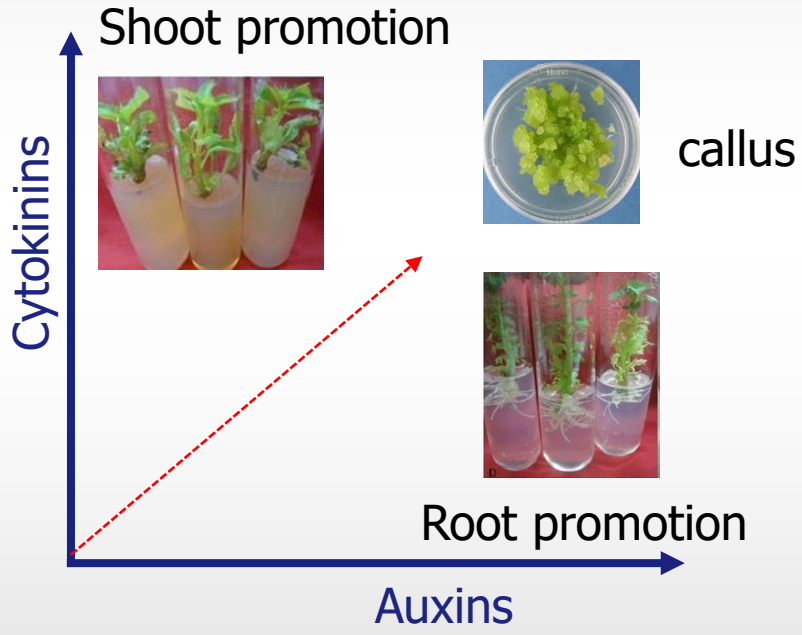
Gelling agent	Macro & micro nutrients	Carbon source	Hormones: cytokinins auxins
Provides physical support	Synthetic nutrients N, P, K…	Sucrose replaces light	Steer organogenesis

Additional considerations & ingredients: pH, osmotic potential, charcoal, vitamins, other organics…

3. Micropropagation: workflows & media

- Shoot & root proliferation is induced by specific media formulations.
- Hormones (plant growth regulators; PGRs) at the appropriate concentration promote organogenesis:
  - **Cytokinins:** shoot induction/elongation
  - **Auxins:** root induction

- Transfer between shooting & rooting media is required.
- Too much PGR can have unexpected effects.



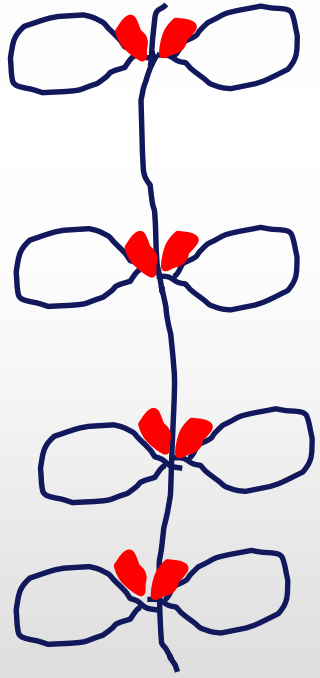
3. Micropropagation: workflows & media

(A) Mother (stock) plant. (B) Shoot formation. (C-E) Rooting.
(F-G) Established plants in nursery. (H) Field production.

Reference: Lata et al. (2016) J Applied Research on Medicinal and Aromatic Plants 3: 18-26

3. Micropropagation: workflows & media

- Micropropagation output is dependant on the:
  - sub-culture interval, approximately 25 days.
  - multiplication rate (MR).
- MR dependant on nodes per shoot and vegetative buds per node.



Vegetative bud

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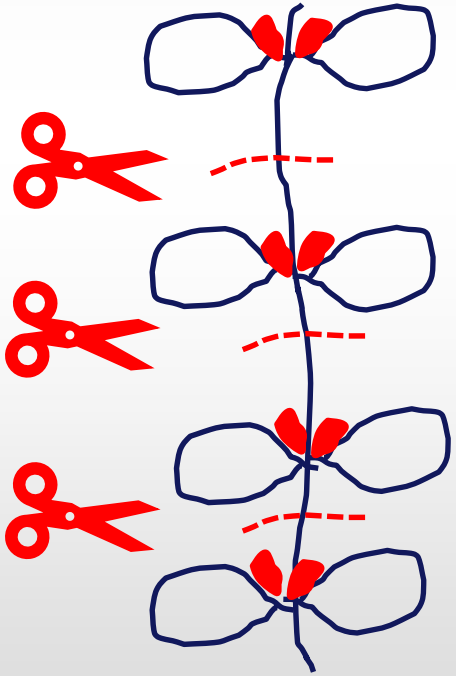
3. Micropropagation: workflows & media

- MR dependant on nodes per shoot and vegetative buds per node.

1 shoot



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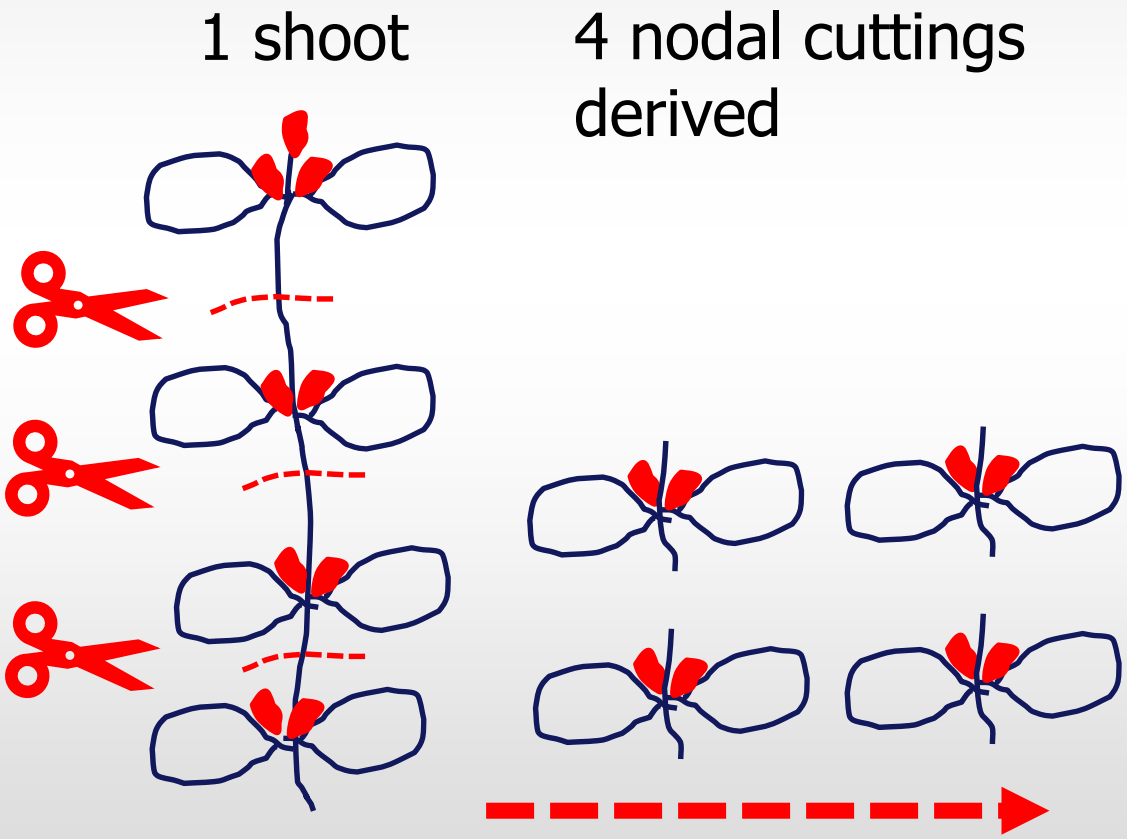

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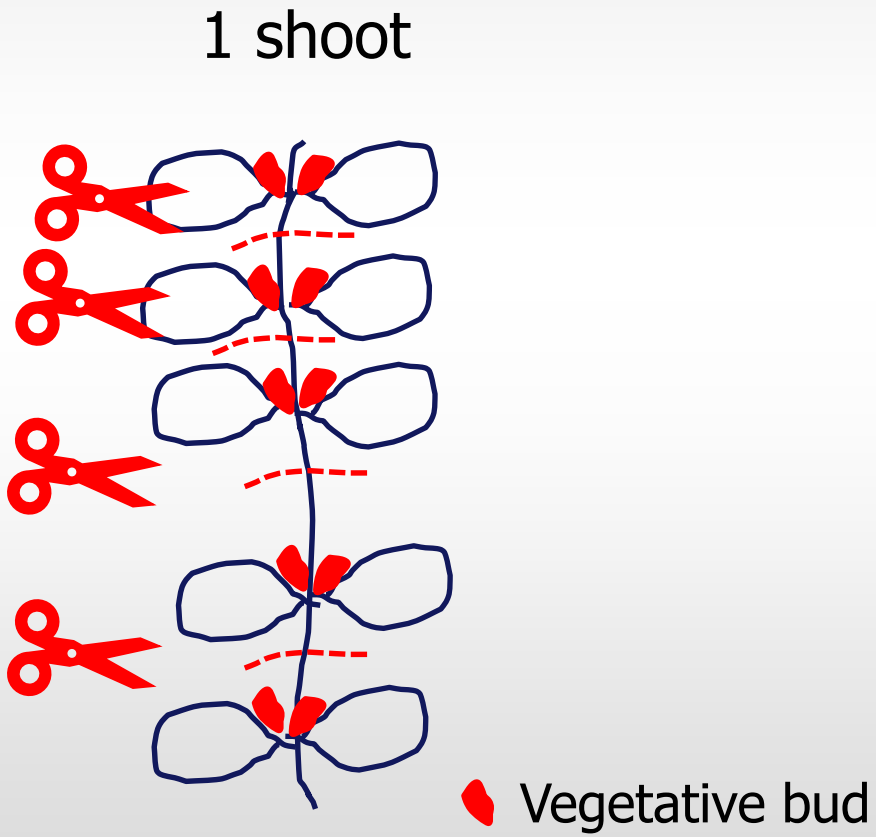

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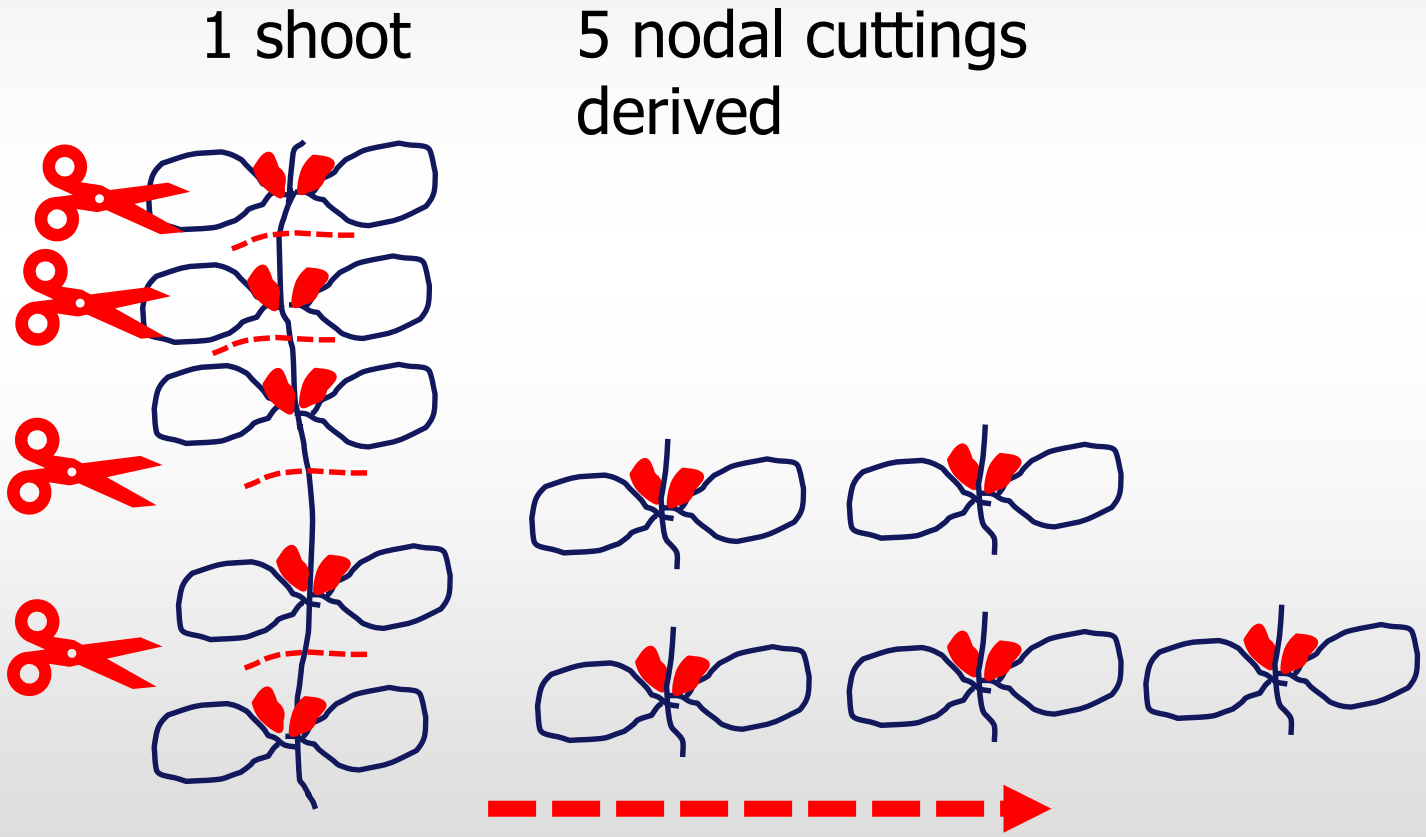

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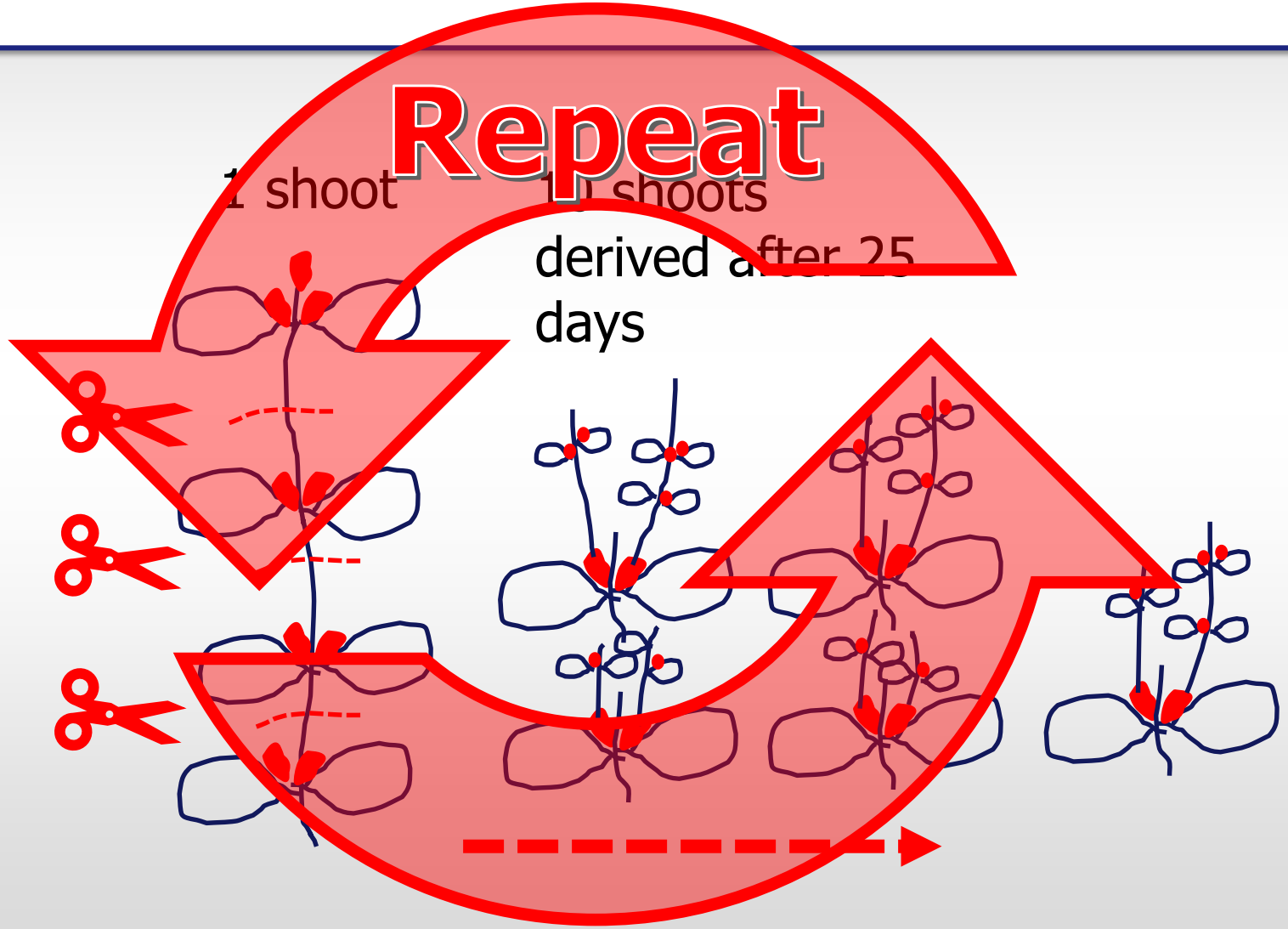


3. Micropropagation: workflows & media

- How productive can micropropagation be?

Day	Sub	MR=4	MR=5	MR=8	MR=10
Day 0	1	1	1	1	1
Day 25	2	4	5	8	10
Day 50	3	16	25	64	100
Day 75	4	64	125	512	1,000
Day 100	5	256	625	4,096	10,000
Day 125	6	1,024	3,125	32,768	100,000
Day 150	7	4,096	15,625	262,144	1,000,000
Day 175	8	16,384	78,125	2,097,152	10,000,000
Day 200	9	65,536	390,625	16,777,216	100,000,000
Day 225	10	262,144	1,953,125	134,217,728	1,000,000,000
Day 250	11	1,048,576	9,765,625	1,073,741,824	10,000,000,000
Day 275	12	4,194,304	48,828,125	8,589,934,592	100,000,000,000
Day 300	13	16,777,216	244,140,625	68,719,476,736	1,000,000,000,000
Day 325	14	67,108,864	1,220,703,125	549,755,813,888	10,000,000,000,000
Day 350	15	268,435,456	6,103,515,625	4,398,046,511,104	100,000,000,000,000

- Very rapid multiplication possible but not realistic or necessary!
- Competent technician could manage 1500-2000 cuttings per day.

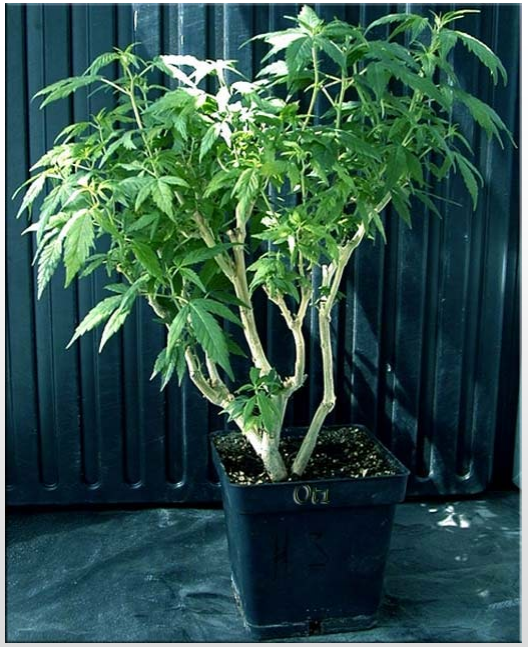
3. Micropropagation: workflows & media

- How much space is required to store *in vitro* stock plants?
- Assume we can hold 10 shoots in a 700 mL tub…

Metric	MR=4	MR=5	MR=8	MR=10
# shoots stored	250	200	125	100
# shoots derived	1,000	1,000	1,000	1,000
SQM required	0.360	0.288	0.180	0.144

For comparison…

- 1,000 cuttings per month from vegetative propagation requires minimum of 2.5-5 SQM and a lot more inputs!



Summary

Micropropagation is:

- well established in horticulture.
- requires optimisation for Cannabis.
- a specialised activity with specialised. facility requirements & applications.
- requires investment.
- suits large scale facilities.