Coping with hyperhydricity, culture decline, and challenging rooting in hemp micropropagation

Jessica Lubell-Brand, PhD.

UCONN
COLLEGE OF AGRICULTURE, HEALTH AND NATURAL RESOURCES

April 2021

HortTechnology

American Society for Horticultural Science Volume 31, Number 2

An In Vitro–Ex Vitro Micropropagation System for Hemp

Jessica D. Lubell-Brand[1], Lauren E. Kurtz[1], and Mark H. Brand[1]

Additional index words. Cannabis sativa, retipping, tissue culture

Summary. Hyperhydricity of shoots initiated in vitro, poor shoot extension, inability of shoot cultures to maintain good growth over an extended time, and unsuccessful ex vitro rooting have limited the development of a commercial scale micropropagation system for hemp (Cannabis sativa). We present a culture initiation method that prevents shoot hyperhydricity using vented-lid vessels with 0.2-mm pores and medium containing agar at 1%(w/v). To optimize shoot multiplication in vitro, a control medium (medium A) and four treatment media (medium B, C, D, and E), with varying inorganic nutrients and vitamins were tested. Control medium A consisted of 1× Murashige and Skoog (MS) with vitamins plus 3%(w/v) sucrose, 0.5 mg·L^{-1} metatopolin, 0.1 mg·L^{-1} gibberellic acid, and 0.8% agar (w/v) at pH 5.7. The four treatment media differed from the control medium as follows: medium B, 2.5× MS with vitamins; medium C, 1× MS with vitamins plus added mesos [calcium chloride (anhydrous), magnesium sulfate (anhydrous), and potassium phosphate (monobasic) nutrients]; medium D, 1× MS with vitamins plus added vitamins; and medium E, 1× MS with vitamins plus added mesos and vitamins. Medium C and medium E produced more microcuttings than the control at 6 weeks after the initial subculture with shoot multiplication media and all other treatments at 9 and 12 weeks. Shoots grown on these two media displayed optimal extension and leaf lamina development; however, they exhibited slight chlorosis by 12 weeks after subculture with shoot multiplication media. In a separate experiment, medium E was supplemented with ammonium nitrate at 0, 500, 1000, or 1500 mg·L^{-1}, and cultures grown with 500 mg·L^{-1} produced the most microcuttings and exhibited the best combination of shoot extension and leaf lamina development. We provide a method of prerooting microshoots in vitro that has resulted in 75% to 100% rooting ex vitro in rockwool. Using 10 recently micropropagated plants, ≈300 retip cuttings (cuttings taken from new shoots from recently micropropagated plants) were harvested over 10 weeks. The average weekly rooting was more than 90%. Retipping can produce nine times as many plants in a similar amount of floor space as stem cuttings derived from traditional stock mother plants. The micropropagation/retipping method proposed can be a more efficient way to generate clonal liner plants for commercial-scale production.

There is increased interest in the production of hemp (*Cannabis sativa*) because of its medicinal properties (Small, 2015). For commercial production purposes, hemp is propagated by seed or stem cuttings to take advantage of superior genotypes (Cervantes, 2015). Many indoor hemp production facilities propagate cultivars by taking stem cuttings from stock mother plants, which they must maintain (Bechtel, 2019). Mother plants are large (10-gal container size) and require a significant amount of grow space to provide enough cuttings to meet production quotas. Growers must maintain mother plants in triplicate, with each replicate grown in a separate area of the facility, to reduce the risk of losing valuable cultivars to sudden disease outbreaks. Mother plants lose vigor because of the serial removal of shoots for cuttings, and they must be replaced every 6 months. Additionally, over time, mother plants accumulate insects and diseases, thus limiting their useful life as donors of cuttings. Overall, this propagation process is labor-intensive and inefficient. Hemp growers are interested in micropropagation as an alternative method of generating clones for commercial production (Rosslee, 2020).

Micropropagation provides unique benefits to growers and has several advantages over traditional plant cloning systems. These include the production of a large number of genetically clonal plants, uniform plants with enhanced vigor, disease-free plants, and preservation of maternal germ lines (Hartmann et al., 2002). Micropropagation also requires substantially fewer mother plants to be maintained compared with traditional stem cutting propagation, and in vitro cultures can be stored for longer in a smaller area than mother plants.

There are few published reports of hemp micropropagation. Wang et al. (2009) evaluated the effects of growth regulator additions to Murashige and Skoog (MS) medium on in vitro shoot multiplication and rooting of hemp cultures started from seed. Using nodal stem segments and MS medium, Lata et al. (2009) similarly tested rates of three growth regulators alone and in combination with gibberellic acid (GA_3) on shoot multiplication. Lata et al. (2016) published a protocol refinement of their previous work (Lata et al., 2009) and introduced the growth regulator meta-topolin (MT), which was found to be superior to thidiazuron (TDZ) for in vitro shoot multiplication. Unfortunately, these published protocols have not translated well to large-scale micropropagation of clones necessary for commercial production. Noted shortcomings of published micropropagation methods

Received for publication 16 Dec. 2020. Accepted for publication 13 Feb. 2021.

Published online 19 March 2021.

[1]Department of Plant Science and Landscape Architecture, University of Connecticut, 1376 Storrs Road, Unit-4067, Storrs, CT 06269

J.D.L.-B. is the corresponding author. E-mail: Jessica.lubell@uconn.edu.

https://doi.org/10.21273/HORTTECH04779-20

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.0929	ft^2	m^2	10.7639
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1	micron(s)	µm	1
28.3495	oz	g	0.0353
1	ppm	mg·L^{-1}	1
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

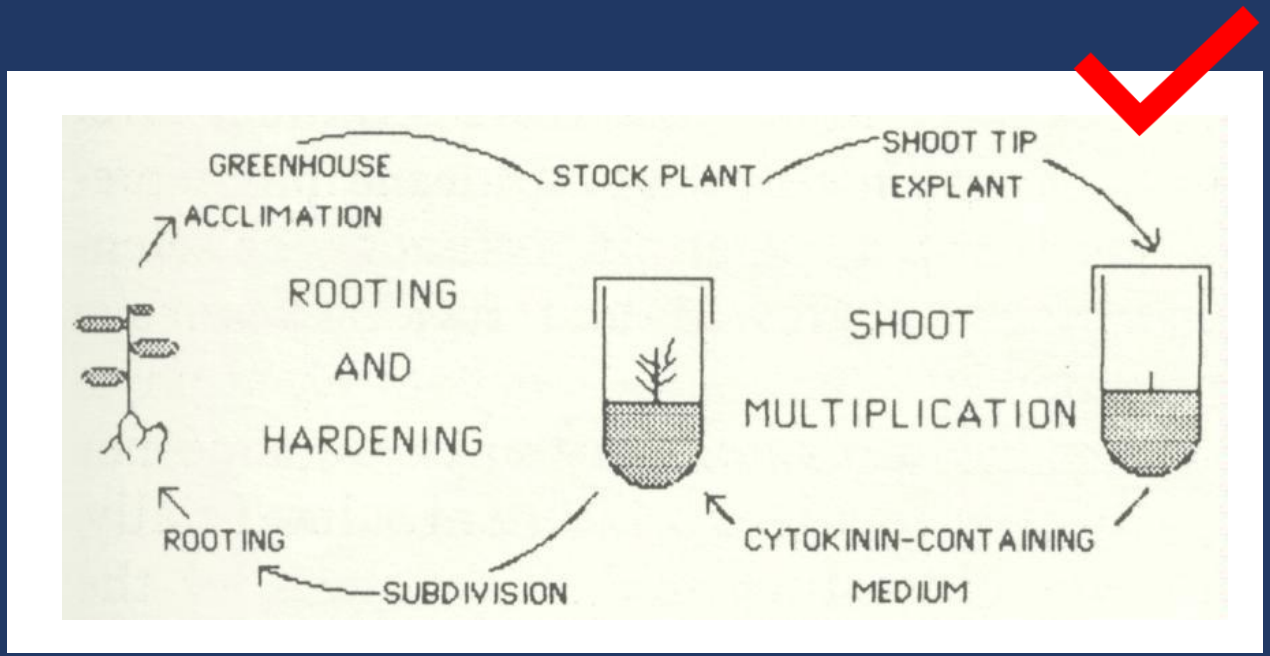
Lauren Kurtz, M.S.
Mark Brand, Ph.D.
UConn Greenhouse Staff

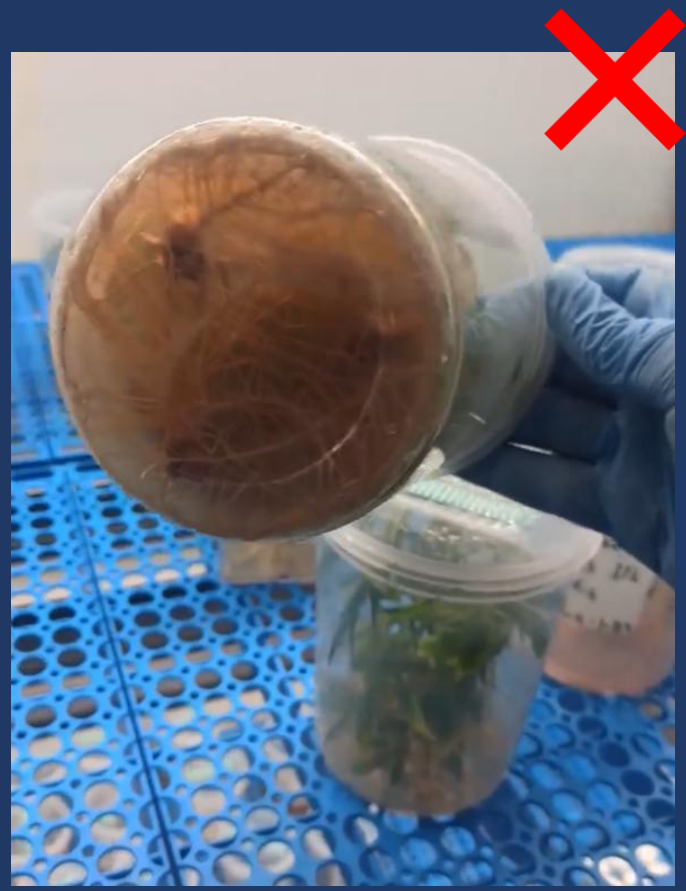
Micropropagation: High throughput in vitro multiplication of plants for production purposes

Tissue culture: growing cells from living tissue in medium (growth of callus, organogenesis, etc.)

3 stages of micropropagation

1. Initiation to in vitro culture
2. Shoot multiplication in vitro
3. Rooting & acclimation



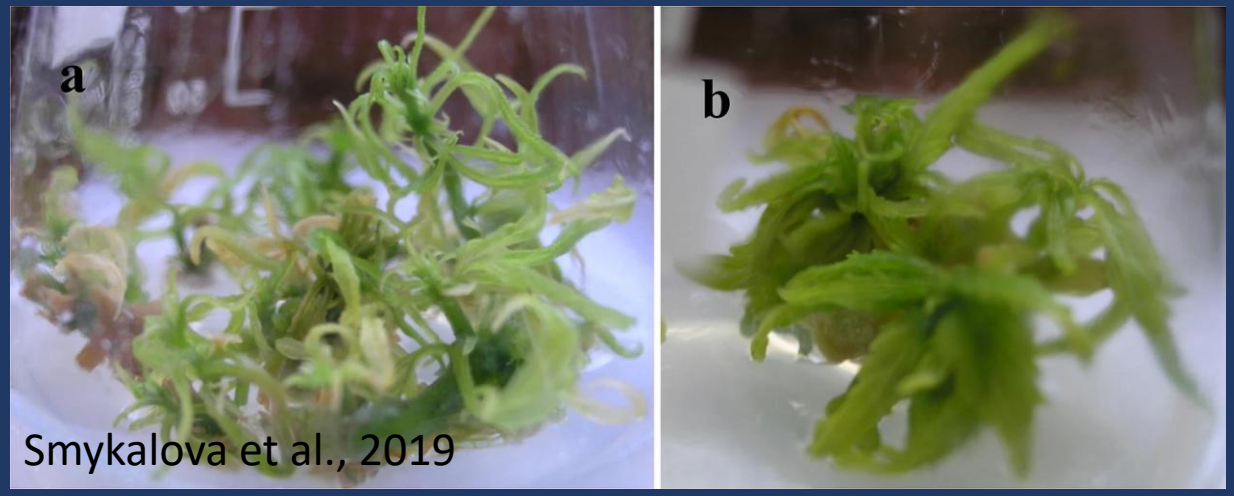


Ongoing problems with hemp at all stages

- Hyperhydricity (stage 1)
- Culture decline (stage 2)
- Ex vitro rooting (stage 3)

1

2



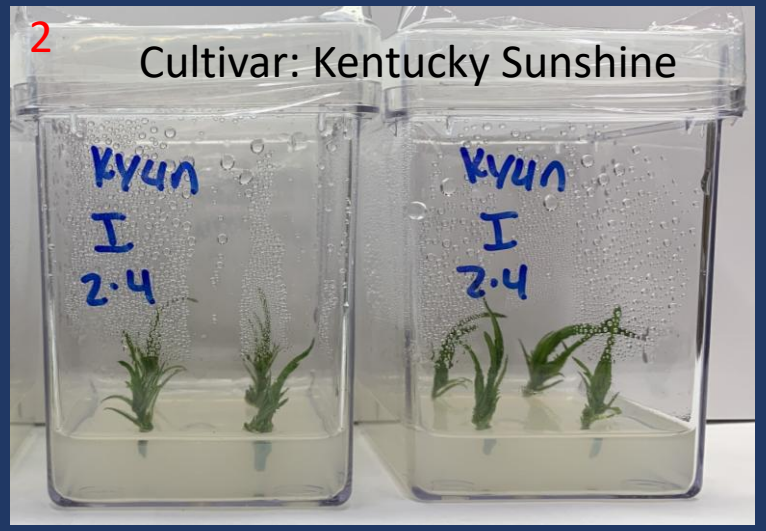
3

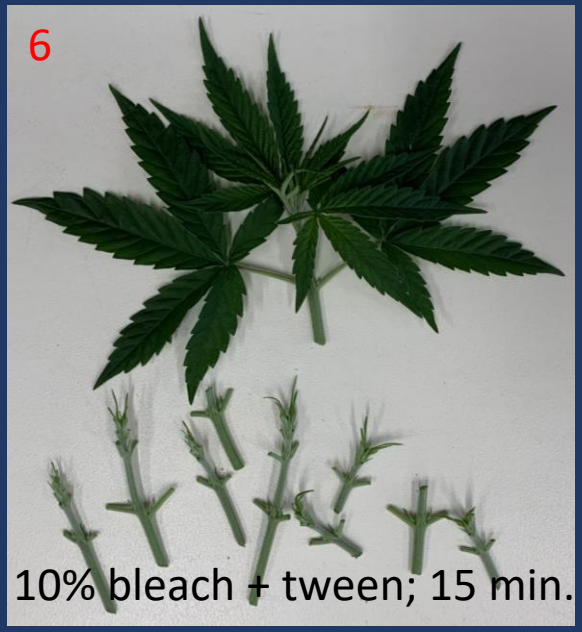
4

5

Initiation to prevent hyperhydricity

- MS, 30 g/L sucrose, 0.5 mg/L metatopolin
- Increased agar at 1%, vented lids, 6 week with 3 week subculture intervals

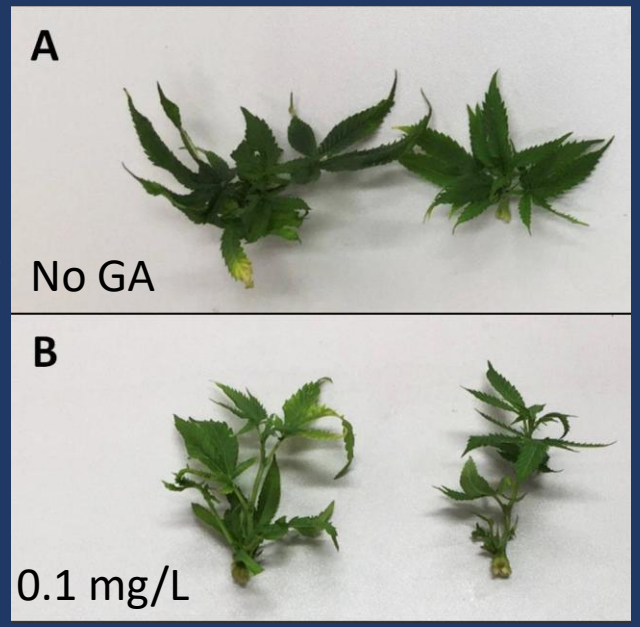




Shoot multiplication

- Reduce agar to 0.8%
- Gibberellic acid at 0.1 mg/L

2



3

1

4

Shoot multiplication

- Increased MS mesos (Ca, Mg, P) and vitamins to 2.5x MS and vitamins

1

2

3

4

Shoot multiplication

- Added ammonium nitrate (N) at 500 mg/L

Wife at 12 week on SM media

Abacus at 6 week on SM media

Without added N

With added N

1

2

3

4

Shoot multiplication

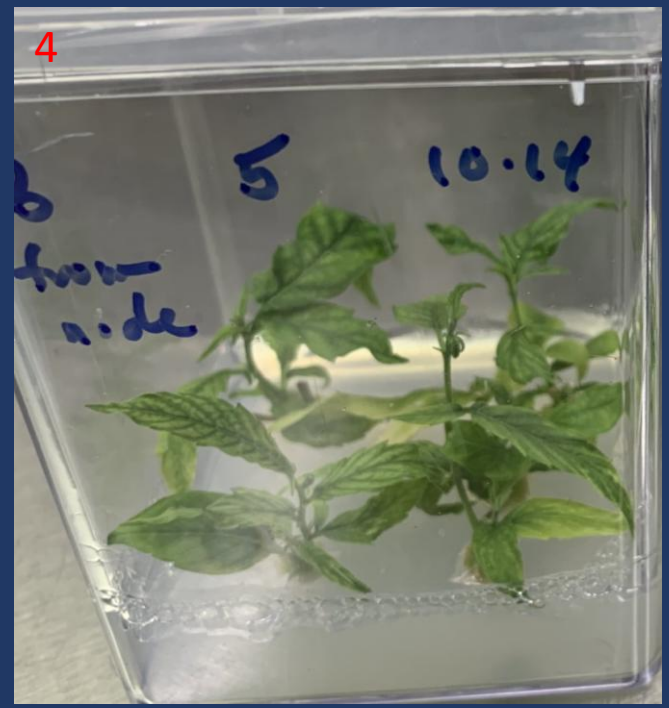
- Subculture tips and 2+ node segments
- Single node segments are slower

1

2

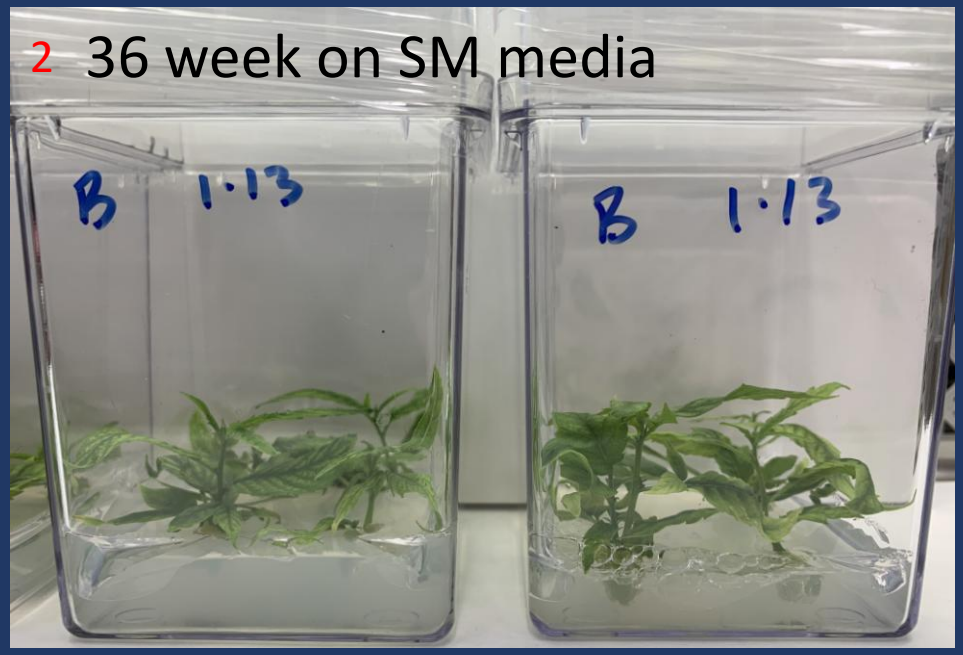
3

4



Shoot multiplication

- 2x multiplication rate



After 13 subcultures and 9 months since initiation (2/4/21)

Shoot multiplication

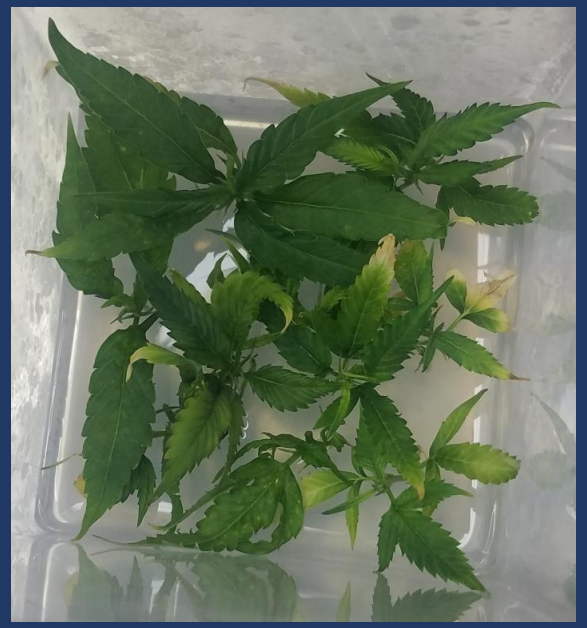
Abacus after 36 weeks on SM media

Abacus one year in culture; 46 weeks on SM media

In vitro Prerooting

- In vitro IBA pulse for 10-14 days
- Next, ex vitro rockwool 75-100% success (~3 wks)

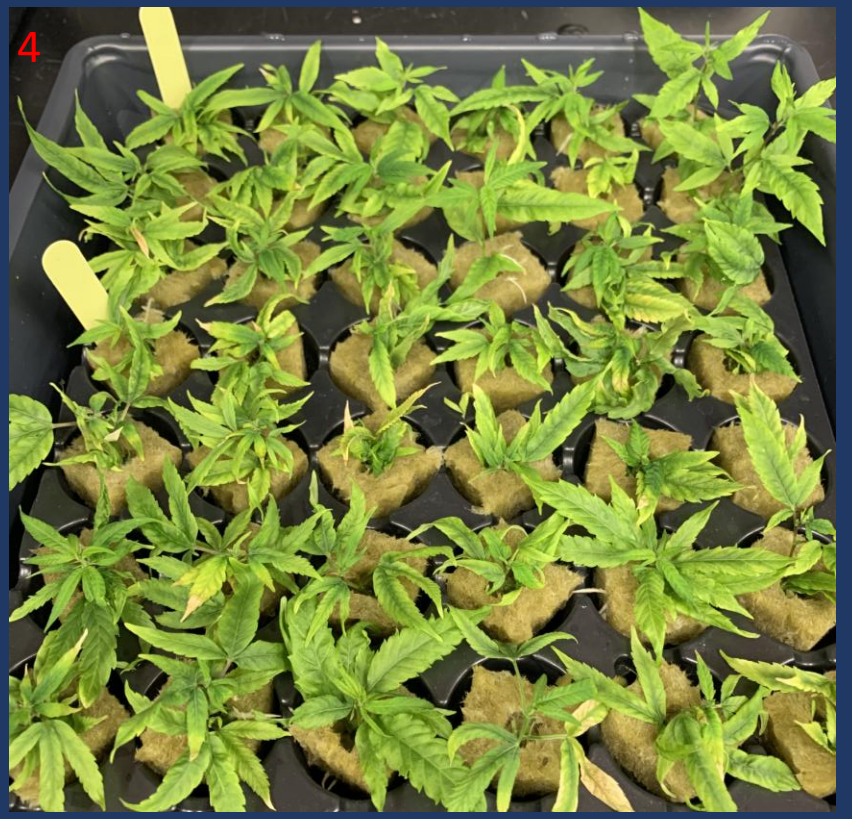
1



2

3

4



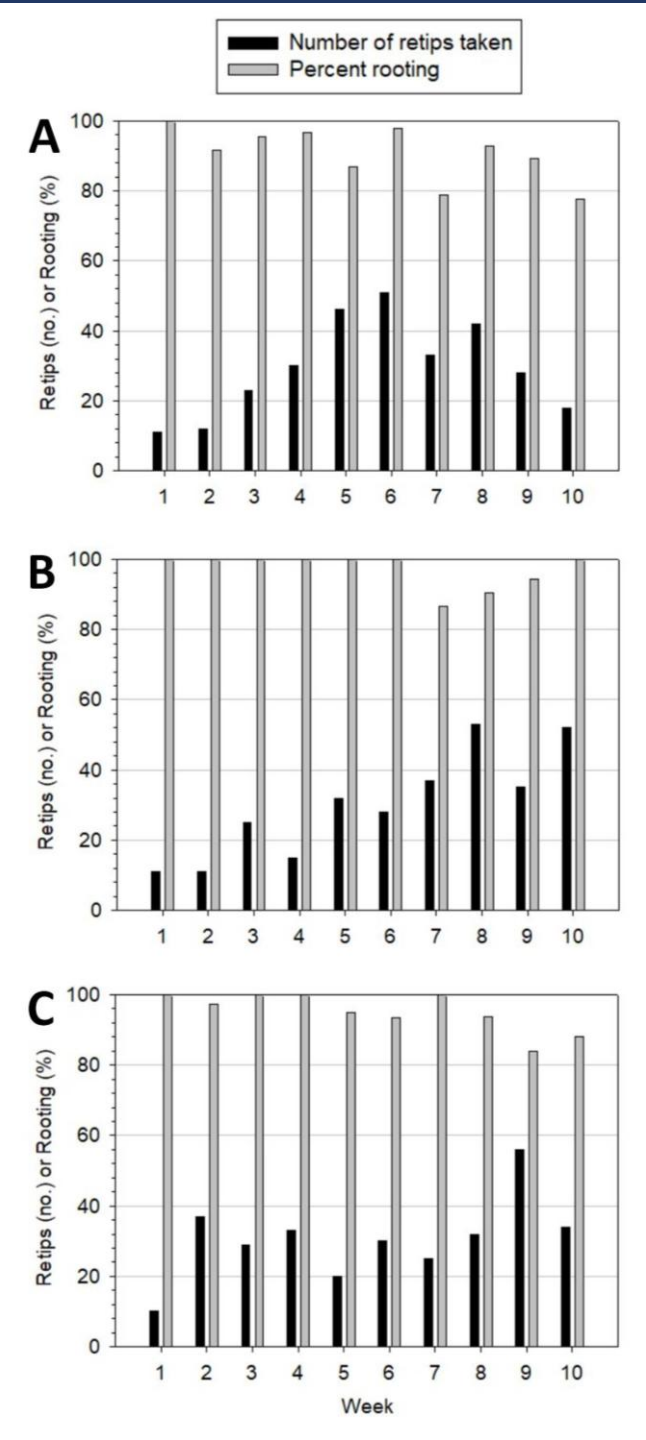
5

Retipping

- Retips: cuttings taken of new shoots from recently propagated plants
- High shoot regeneration allows for serial cuttings
- Enhanced rooting ability >90%

Micropropagation/retipping system

- 9x multiplication rate as traditional stem cuttings from mother plants



Micropropagation R&D

- Optimize shoot multiplication media to improve culture performance
- Rooting timing

Thank you