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

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An In Vitro–Ex Vitro Micropropagation System for Hemp

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Abstract
In vitro micropropagation of hemp (Cannabis sativa) has been successful in developing a micropropagation system. The AE system utilized and evaluated was an ex vitro generation followed by an in vitro propagation system. The in vitro propagation system utilized a combination of media, plant growth regulators, and temperature to produce healthy, disease-free shoots. The ex vitro generation system produced healthy, disease-free shoots that were subsequently transferred to the in vitro propagation system. The in vitro propagation system produced shoots with a high degree of morphological uniformity and a low degree of contamination. The AE system was successful in producing healthy, disease-free shoots that were subsequently transferred to the in vitro propagation system, resulting in a high degree of morphological uniformity and a low degree of contamination.

Keywords: Cannabis sativa, ex vitro, rooting, tissue culture

T here is increased interest in the production of hemp (Cannabis sativa) because of its medical properties (Small, 2015). For commercial production purposes, hemp is propagated by seed or stem cuttings to take advantage of superior genotypes (Carpentier, 2015). Many indoor hemp production facilities propagate cultivars by taking stem cuttings from stock mother plants, which they maintain (Brand, 2019). Mother plants are large (10-gallon container size) and require a significant amount of growing space to provide enough cuttings for the micropropagation system. 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 removal of shoots for cuttings, and they must be replaced every 3 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 disease-free commercial hemp (Brand, 2019).

Micropropagation provides unique benefits to growers and has several advantages over traditional plant clonal systems. These include the production of a large number of genetically identical plants, uniform plants with enhanced vigor, disease-free plants, and preservation of material genotypes (Harbour et al., 2002). Micropropagation also requires substantially fewer mother plants to be maintained compared with traditional stem cutting propagation, and in vitro culture 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 star cuttings in vitro, shoot multiplication, and rooting of hemp cuttings derived from in vitro propagation. Using nodal stem segments and MS medium, Lata et al. (2009) similarly tested rates of growth regulator的事情 alone and in combination with gibberellic acid (GA3) on shoot multiplication. Lata et al. (2016) published a protocol refinement of their previous work (Lata et al., 2009) and introduced the growth regulator actinoplanes (ATP), which was found to be superior to thidiazuron (TDZ) for in vitro shoot multiplication. Unfortunately, these published protocols have not transitioned well to large-scale micropropagation of demersal necessary for commercial production. Noted shortcomings of micropropagation methods

References
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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)

Lata et al., 2009
Smykalova et al., 2019

Lata et al., 2016
3 Rivers Biotech

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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

Cultivar: Kentucky Sunshine

Cultivar: Wife

10% bleach + tween; 15 min.
Shoot multiplication
• Reduce agar to 0.8%
• Gibberellic acid at 0.1 mg/L

Cultivar: Dinamed CBD
Shoot multiplication

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

1. After 3 week on SM media; cultivar Wife
2. After 12 week on SM media
3. After 15 week
4. After 18 week on SM media
Shoot multiplication

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

Without added N

With added N

Wife at 12 week on SM media

Abacus at 6 week on SM media
Shoot multiplication
• Subculture tips and 2+ node segments
• Single node segments are slower
Shoot multiplication

• 2x multiplication rate

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

1 Cultivar triploid Wife; 18 week on SM media

2 36 week on SM media

3 36 week on SM media
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)
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