Improved Foliage Production Using Micropropagation - The Monstera Model

PI: **Wagner Vendrame, Environmental Horticulture**

ABSTRACT

Among foliage plants, Monstera is a genus of tropical evergreens very popular in the nursery industry. Rare and variegated varieties have a high value in the market. Micropropagation allows rapid clonal propagation of such varieties at a larger scale with the production of high quality and uniform plant material. Temporary immersion bioreactors (TIB) represent an advanced micropropagation system allowing increased plant multiplication and reduced costs. In this study, we evaluated the use of TIBs for the micropropagation of variegated Monstera 'Thai Constellation'. Plants grown in the greenhouse were disinfested and offshoots were removed and established in vitro using agar-based culture medium for multiplication. After multiplication in agar-based medium, five in vitro shoots were selected and established per bioreactor using liquid MS culture medium supplemented with 0.5 mg/L NAA and 7.5 mg/L BAP. Four immersion parameters were evaluated: immersion frequency every 1, 1.5 or 2 hours, and immersion duration for 1 or 2 minutes. Contamination was observed causing a delay in the experiment. Preliminary results indicate that immersion every 1.5 hour with a duration of 2 minutes is the best combination of parameters, providing an average of 6-8 shoots per initial explant. Additional bioreactors have been established and the study is under continuous evaluations to improve multiplication rates.

OBJECTIVES AND METHODS

1. To establish clean in vitro cultures of variegated Monstera using agar-based culture medium
2. To evaluate in vitro multiplication of Monstera in liquid culture medium using temporary immersion bioreactors
3. To evaluate temporary immersion parameters in bioreactors, including frequency and duration of immersion to optimize in vitro multiplication of Monstera

Methods

Plants of variegated Monstera 'Thai Constellation' (**Figure 1A**) were obtained from an ornamental nursery in South Florida and transferred to a greenhouse in the Department of Environmental Horticulture, IFAS, University of Florida, located in Gainesville, FL. Plants were maintained in the greenhouse and after 3 weeks, offshoots (**Figure 1B**) were removed from plants and transferred to laboratory to be used as explants. Surface disinfestation was performed using 1% Alconox detergent, after which the explants were submitted to deep disinfestation under laminar flow hood, consisting of 70% ethanol for 1 minute, 2% sodium hypochlorite for 20 minutes performed twice and three rinses in distilled autoclaved water.

Figure 1. Monstera 'Thai Constellation' plant (A). Offshoots used for in vitro studies (B).

Once disinfested, offshoot explants were split horizontally into two halves and established in vitro, with one half per container (baby-food jar) containing Murashige and Skoog (MS) culture medium. Supplemented with 0.5 mg/L NAA (1-naphthaleneacetic acid) and 5.0 mg/L BAP (6-benzylaminopurine). In vitro cultures were monitored weekly for growth and development, and contamination. Subculture to fresh culture medium was performed every 2 to 3 months. After 6 months, explants were transferred to fresh MS medium supplemented with 0.5 mg/L NAA and 7.5 mg/L BAP.

After 3 months, multiple shoots produced from explants were separated into individual shoots and transferred to temporary immersion bioreactors (TIBs) with 5 shoots per bioreactor. Immersion parameters were evaluated, including frequency of immersion (every 1, 1.5, and 2 hours) and immersion duration (1 and 2 minutes). Cultures were monitored weekly for multiplication, growth, and development. After 3 months, in vitro shoots produced in TIBs were transferred to the greenhouse for acclimatization.

RESULTS

Initially, contamination was a major obstacle to the establishment of in vitro cultures, delaying the study for a few months. Once the disinfestation protocol was optimized, clean in vitro cultures were easily established and showed stable growth and development. However, multiplication was slow under the initial agar-based MS medium containing 0.5 mg/L NAA and 5.0 mg/L BAP.

After 6 months, when cultures were transferred to fresh agar-based MS medium with 0.5 mg/L NAA and 7.5 mg/L BAP, multiplication increased with an average of 3-4 in vitro shoots produced per initial explant (**Figure 2A**). This initial multiplication phase under agar-based medium allowed the increase in plant material numbers for subsequent establishment of TIB studies.

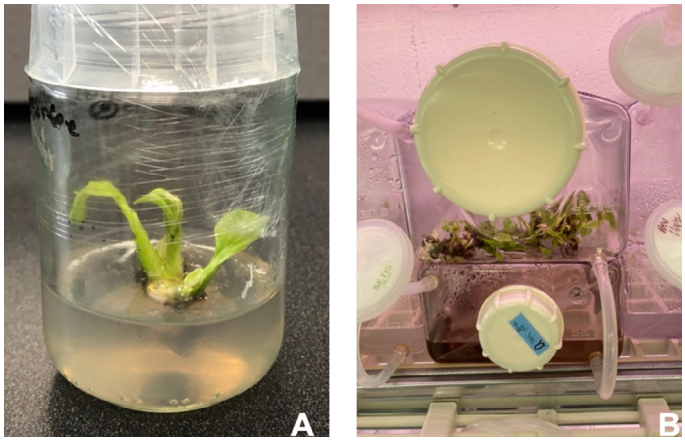

Figure 2. In vitro culture of Monstera in agar-based culture medium (A). Monstera shoots in temporary immersion bioreactors (B)

Subsequent transfers of in vitro shoots to bioreactors were successful and shoots showed stable growth and development (**Figure 2B**). Multiplication of in vitro shoots started at about 2 months after transfers to TIBs, faster as compared to agar-based medium. However, some contamination was observed, resulting from a leakage of air in the bioreactor system, thus causing a delay in the study. Once the problem was identified, it was promptly remediated and new cultures were established, with no signs of contamination.

Our preliminary results show that the best rate of multiplication was obtained under immersion frequency every 1.5 hour with duration of 2 minutes per immersion event in TIBs, with the production of 6-8 shoots per explant, thus doubling the rate of multiplication observed in agar-based systems. As the study is still under evaluations, we predict an increased multiplication rate in TIBs at a range of 3- to 4-fold the original multiplication rate in agar-based systems.

Some of the in vitro shoots produced in TIBs have since been rooted and transferred to greenhouse for acclimatization and further evaluation for survival, growth and development (**Figure 3**). To date, survival has been 100% with normal growth and development and no abnormalities have been observed.

Figure 3. In vitro-derived plantlets of Monstera in the greenhouse (A). Monstera transplanted to larger containers (B).

This study is still being conducted and will include experiments to improve the rates of multiplication by optimizing culture medium composition, such and combinations and concentrations of plant growth regulators, as well as the parameters for immersion frequency and duration.

CONCLUSIONS

Preliminary results from this study suggest that temporary immersion bioreactors can be a feasible system for the micropropagation of Monsteras and could serve as a model for other foliage ornamental crops. The rates of multiplication are higher compared to agar-based systems. In addition, the costs of production are significantly reduced when using bioreactors. The larger vessel size (4 L) compared to agar-based systems (100-200 ml) allows increased space and aeration as compared to agar-based systems, thus resulting in higher number of shoots produced per bioreactor unit. Furthermore, the use of liquid culture medium eliminates the need for agar, one of the most expensive elements of commercial micropropagation systems.

Future studies will incorporate other ornamental foliage plants and the evaluation of biostimulants to promote growth and development of in vitro-derived plants in the greenhouse during acclimatization.