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Standardization of Sucrose Concentration in Micropropagation of Valuable Aquascape Plant *Echinodorus grisebachii*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To standardize the optimum sucrose concentration in shooting and rooting medium in micropropagation of valuable aquascape plant *Echinodorus grisebachii.*
Study Design: CRD.
Place and Duration of Study: Department of Floriculture and Landscaping, College of Agriculture Vellayani 2022-2024.
Methodology: In this study, eight treatments were evaluated separately for their effectiveness in shooting and rooting medium. For the shooting medium, 2 mgL^{-1} BAP (6-Benzylaminopurine) and 3 sucrose concentrations (10, 20,30) gL^{-1} were used in the MS medium and half-strength MS medium. For rooting medium 3.0 mgL^{-1} IBA **(Indole-3-butyric acid),** three concentrations of sucrose as in shooting medium were tried.
Results: The result of the study showed that out of 8 treatments tried for shooting medium MS + 2mgL^{-1} + 30 gL^{-1} sucrose was best resulted in Shoot initiation (100 %), Minimum days to shoot emergence (6.17), Maximum number of shoots (9.83), Longest shoot (10.33) and maximum number of leaves (31). For rooting medium ½ MS + 3.0 mgL^{-1} IBA + 30 gL^{-1} sucrose was best resulted in minimum number of days in root emergence (12.58) and maximum root initiation percentage (99.25).
Conclusion: An effective concentration of sucrose was standardized for *Echinodorus grisebachii*.

Keywords: Micropropagation; Echinodorus greisebachii; aquascaping; ecology; floriculture.

1. INTRODUCTION

Aquascaping is the art of designing and arranging aquatic plants, stones, driftwood, and other materials within an aquarium to create a visually appealing and natural underwater landscape. Originating as a specialized branch of aquarium hobbying, aquascaping combines elements of botany, design, and ecology to replicate natural ecosystems or craft imaginative underwater scenes. Popular styles include the Nature Aquarium, inspired by terrestrial landscapes; the Iwagumi style, characterized by minimalist rock arrangements; and the Dutch style, focusing on vibrant, densely planted layouts. It involves creating aesthetically pleasing creations underwater using plants, rocks, substrates, and sometimes fish or other aquatic creatures. Aquascaping is a combination of art and horticulture to potray alluring and cordial aquatic scenes, often inspired by seascapes (Baby et al., 2024a).

Plants like *Echinodorus*, *Cryptocoryne*, *Anubias*, and *Alternanthera reineckii* are commonly used due to their aesthetic appeal and adaptability to submerged environments. Effective aquascaping requires careful consideration of lighting, CO_2 levels, nutrient supply, and water parameters to support plant growth and maintain ecological balance. Beyond aesthetics, aquascaping promotes biodiversity and creates a tranquil, therapeutic space, making it a popular choice for both hobbyists and interior designers.

Echinodorus grisebachii is a versatile and widely cultivated aquatic plant, native to the freshwater habitats of Central and South America. Known for its lance-shaped, bright green leaves that form a rosette structure, this plant adds elegance and depth to aquascapes. It thrives in moderate to high lighting conditions, preferring nutrient-rich substrates and stable water parameters for optimal growth. Its adaptable nature allows it to perform well in both low-tech aquariums and advanced, CO_2-injected setups, making it a favorite among aquarists.

In aquascaping, *Echinodorus grisebachii* is commonly used as a focal point or a midground plant, especially in Nature Aquarium and Dutch-style layouts. Its lush foliage creates a striking contrast against darker substrates and rocks, enhancing the overall aesthetic of the aquarium. The plant is also known for its rapid growth under favorable conditions, which can help establish balance in newly set up aquariums by reducing nutrient levels and algae growth. Additionally, its ability to grow both submerged and emersed makes it a suitable candidate for paludariums and riparian-style aquariums.

Despite its adaptability, the plant requires regular maintenance to prevent overcrowding, as its vigorous growth can overshadow smaller, delicate species. Trimming older or damaged leaves ensures healthy development and promotes a tidy appearance in the aquarium. With proper care and attention, *Echinodorus grisebachii* not only enhances the visual appeal of aquascapes but also contributes to a balanced aquatic ecosystem, making it a reliable choice for hobbyists and aquascaping enthusiasts.

Tissue culture is preferred in aquascaping plants due to its potential to produce aseptic plantlets, which is an important factor in maintaining an aesthetic aquarium (Baby et al., 2024b). The concentration of sucrose significantly influences the growth and development of aquascaping plants during *in vitro* propagation, impacting shoot proliferation, rooting, and biomass production. Sucrose is an ideal carbon source in tissue culture, providing the necessary energy for key developmental processes, including shoot proliferation and root formation (Yaseen et al., 2013). Sucrose serves as a primary energy source and an osmotic regulator, aiding plants in transitioning from autotrophic to heterotrophic growth conditions. Higher sucrose levels may stimulate the production of pigments and secondary metabolites, enhancing ornamental traits such as vibrant coloration, particularly in decorative plants. However, excessively high concentrations can induce osmotic stress, reducing growth efficiency and tissue health. Thus, the careful optimization of sucrose concentration is essential to achieve desired growth characteristics and maintain the aesthetic and ecological value of aquascaping plants.

2. MATERIALS AND METHODS

The present study, "Standardization of Sucrose Concentration in Micropropagation of valuable aquascape plant *Echinodorus grisebachii***",** was carried out in the Department of Floriculture and Landscaping and Department of Plant Biotechnology, College of Agriculture, Vellayani, during 2022- 2024. The objective of the study was to standardize the optimum sucrose concentration in shooting and rooting medium in micropropagation of valuable aquascape plant *Echinodorus grisebachii*.

2.1 Standardization of Sucrose Concentration for Shooting Medium

To standardize the optimal sucrose concentration for shoot development in tissue-cultured plants, an experiment was conducted using three sucrose concentrations: 10, 20, and 30 gL^{-1}. These concentrations were tested in combination with 2 mgL^{-1} BAP to assess their impact on shoot proliferation which was found to be best according to (Baby et al., 2024c). Eight distinct treatments were developed using full-strength and half-strength Murashige and Skoog (MS) media to evaluate the influence of sucrose and nutritional conditions on shoot growth.

Explants from three plant species were cultured on each treatment medium, with three replicates per treatment for each species. The media were prepared by supplementing MS basal salts with the specified sucrose concentrations and plant growth regulators. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or HCl before autoclaving at 121°C for 15 minutes. After autoclaving, the media were poured into sterile culture vessels under aseptic conditions in a laminar airflow cabinet.

Cultures were incubated in a controlled growth room maintained at 25 ± 2°C with a 16-hour photoperiod provided by cool white fluorescent lamps at an intensity of 50 µmol m^{-2} s^{-1}. Shoot growth parameters, including the number of shoots per explant and average shoot length, were recorded after a 30-day culture period. Data were analyzed to identify the sucrose concentration and medium type that provided the best results for shoot proliferation in each plant species.

2.2 Standardization of Sucrose Concentration for Rooting Medium

To optimize sucrose concentration for root development in tissue-cultured plants, an experiment was designed to test three sucrose levels: 10, 20, and 30 gL^{-1}. These concentrations were combined with 3 mgL^{-1} indole-3-butyric acid (IBA) to assess their impact on rooting which was found to be best according to (Baby et al., 2024c). A total of eight treatments were established, utilizing full-strength and half-strength Murashige and Skoog (MS) media to investigate the combined effects of sucrose and nutrient availability on root formation.

Explants from three plant species were cultured on rooting media enriched with varying sucrose concentrations and IBA. The pH of the media was adjusted to 5.8 using 0.1 N NaOH or

HCl before sterilization in an autoclave at 121°C for 15 minutes. Following sterilization, the media were dispensed into sterile culture vessels under aseptic conditions in a laminar airflow hood.

Cultures were maintained in a controlled growth room. The data were analyzed statistically to identify the sucrose concentration and medium strength that yielded the best rooting results for each plant species.

3. RESULTS AND DISCUSSION

The best sucrose concentration for the shoot induction medium in *Echinodorus grisebachii* was determined to be 30 gL^{-1}. This concentration, when supplemented with 2 mgL^{-1} BAP, significantly promoted shoot initiation, elongation, and leaf production. The increased sucrose concentration provided essential energy and optimized osmotic conditions, which facilitated improved cellular activity and shoot development. The combination of 30 gL^{-1} sucrose and 2 mgL^{-1} bap in the MS medium resulted in the most favorable conditions for shoot proliferation, as evidenced by enhanced growth rates and the production of healthy, robust shoots and leaves. This indicates that sucrose concentration plays a crucial role in supporting the growth of *Echinodorus grisebachii* under tissue culture conditions.

The optimal sucrose concentration of 30 gL^{-1} in the shoot induction medium for *Echinodorus grisebachii* because sucrose serves as a primary carbon source, providing essential energy for cellular metabolism and growth. At this concentration, combined with 2 mgL^{-1} BAP, it creates ideal osmotic conditions that enhance cell division and differentiation, promoting better shoot initiation and elongation. This balance between energy supply and osmotic regulation supports robust cellular activity, resulting in healthier, more vigorous shoots and leaves. Therefore, sucrose concentration directly influences the success of tissue culture by optimizing growth conditions.

The most effective rooting response was achieved with half-strength MS medium supplemented with 3 mgL^{-1} IBA and 30 gL^{-1} sucrose, which optimized root induction. This combination provided a balanced energy source that enhanced root development while also minimizing osmotic stress, ensuring the plants' proper growth and adaptation. The inclusion of 30 gL^{-1} sucrose played a crucial role by supporting the plant's metabolic needs, helping it thrive in a controlled in vitro environment. Additionally, the use of half-strength MS medium with IBA further stimulated root formation, creating ideal conditions for root growth and increasing the success rate of plant propagation (Table 2).

Similar findings were George et al. (2015), where a sucrose concentration of 30 gL^{-1} was effectively utilized in the *in vitro* propagation of *Nymphoides macrosperma*, an endangered aquatic plant. This concentration supported vigorous shoot and root growth, contributing to the successful development and acclimatization of the plantlets.

A sucrose concentration of 30 gL^{-1} is widely recognized for promoting plant growth, although it is important to account for species-specific reactions and potential constraints in carbon metabolism, indicating a need for further studies to fine-tune sucrose levels for different plant species (Kubota et al., 2002; Blanca et al., 1993). In a similar vein, Dogan (2020) investigated the impact of various sucrose concentrations on *Riccia fluitans* l., noting that higher sucrose levels enhanced shoot regeneration, with the greatest regeneration area of 22.34 ± 1.82 cm^2 achieved at 30 gL^{-1}, reflecting a 76.88% increase in regeneration compared to lower concentrations.

Yu et al. (2015) also found that the combination of 30 gL^{-1} sucrose and specific plant growth regulators, such as 6 BA and NAA, was ideal for promoting rapid propagation and rooting in lotus tissue culture. Similarly, Jendy et al. (2019) demonstrated that 30 gL^{-1} sucrose effectively supported the growth and regeneration of *Lilaeopsis brasiliensis*, improving both the quality and quantity of shoots during micropropagation.

Navya et al. (2012) reported that a sucrose concentration of 30 gL^{-1} in MS medium facilitated successful *in vitro* cultivation of various aquarium plants, including *Nymphoides cristata Bacopa caroliniana*, *Rotala rotundifolia*, *Aponogeton ulvaceus* and *Anubias minima*. This sucrose level consistently enhanced both shoot and root development, boosting growth and overall plant health in tissue culture environments.

Table 1. Effect of sucrose concentration for shooting media in *Echinodorus grisebachii*

NO	Treatment Details	Shoot init%	Days to shoot emergence	No of shoot	Shoot length (cm)	No of Leaves
T_1	MS	41.67 (0.70)^{d}	47.43^{a}	0.87^{de}	0.57^{e}	1.70^{g}
T_2	½ MS	46.33 (0.75)^{d}	43.17^{b}	0.17^{e}	0.43^{e}	1.30^{g}
T_3	MS+2 BAP+ 10 gL-^{1} Sucrose	60.00 (0.89)^{c}	36.50^{d}	1.00^{d}	2.20^{d}	6.16^{e}
T_4	MS+2 BAP + 20 gL^{-1} Sucrose	99.33 (1.49)^{ab}	8.43^{f}	3.87^{c}	5.60^{c}	19.50^{b}
T_5	MS+2 BAP + 30 gL^{-1} Sucrose	100.00 (1.52)^{a}	6.17^{g}	9.83^{a}	10.33^{a}	31.0^{a}
T_6	½ MS+2 BAP + 10 gL^{-1} Sucrose	42.33 (0.71)^{d}	39.30^{c}	1.53^{d}	1.47^{de}	3.83^{f}
T_7	½ MS+2 BAP + 20 gL^{-1} Sucrose	96.33 (1.41)^{b}	9.50^{f}	3.27^{c}	5.43^{c}	12.86^{d}
T_8	½ MS+2 BAP + 30 gL^{-1} Sucrose	100.00 (1.52)^{a}	14.25^{e}	8.87^{b}	9.10^{b}	27.30^{b}
	CD (0.05)	0.104	1.904	0.833	1.126	1.619
	CV	5.346	4.299	13.097	14.815	7.219
	SEm (±)	0.035	0.635	0.278	0.376	0.54

Table 2. Effect of sucrose concentration in rooting medium

No	Treatment Details	No of days taken	Root initiation percentage
T_1	MS	25.48^{b}	51.33 (0.80)^{e}
T_2	½ MS	27.42^{a}	43.33 (0.72)^{e}
T_3	MS + 3 IBA + 10 gL^{-1} Sucrose	24.93^{b}	62.67 (0.91)^{d}
T_4	MS + 3 IBA + 20 gL^{-1} Sucrose	22.46^{c}	72.00 (1.01)^{d}
T_5	MS + 3 IBA + 30 gL^{-1} Sucrose	18.00^{d}	92.25 (1.31)^{b}
T_6	½ MS + 3 IBA +10 gL^{-1} Sucrose	21.60^{c}	64.00 (0.93)^{d}
T_7	½ MS + 3 IBA +20 gL^{-1} Sucrose	16.75^{d}	85.33 (1.18)^{c}
T_8	½ MS + 3 IBA + 30 gL^{-1} Sucrose	12.58^{e}	99.25 (1.49)^{a}
	CD (0.05)	1.317	0.112
	CV	3.567	6.201
	SEm (±)	0.439	0.037

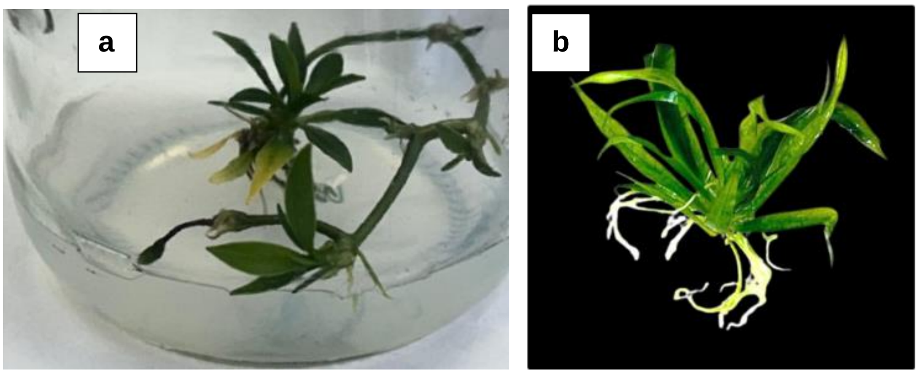

Fig. 1a. Shoot multiplication in *Echinodorus grisebachii* (One month after initiation) T_5 MS+2 BAP + 30 gL^{-1} Sucrose
Fig. 1b. Three weeks after root initiation in *Echinodorus grisebachii* T_8 ½ MS + 3 IBA + 30 gL^{-1} *Sucrose*

4. CONCLUSION

An effective standardization protocol for sucrose concentration was developed to optimize growth in *Echinodorus grisebachii*. For shoot induction, the inclusion of 2 mgL^{-1} BAP and 30 gL^{-1} sucrose in the MS medium significantly enhanced shoot initiation, elongation, and leaf production. For root induction, the most effective treatment involved using half-strength MS medium supplemented with 3 mgL^{-1} IBA and 30 gL^{-1} sucrose, which resulted in optimal root development. This protocol demonstrates the critical role of sucrose concentration in promoting successful in vitro propagation and provides a reliable framework for optimizing sucrose levels at different stages of plant growth.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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