PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.8722

eISSN 2348-1900

RESEARCH ARTICLE

# *In vitro* shoot regeneration potential of water hyacinth (*Eichhornia crassipes* [Mart.] Solms): A study on an invasive aquatic weed

**Thoa Tran Thi Anh[1,2,3*], Kiet Do Thuong[1,2] & Viet Bui Trang[1,2]**

[1]Department of Plant Physiology, University of Sciences, Ho Chi Minh City 70000, Vietnam
[2]Vietnam National University, Ho Chi Minh City 70000, Vietnam
[3] Ho Chi Minh City University of Industry and Trade, Ho Chi Minh City 70000, Vietnam

*Correspondence email - thoatta@huit.edu.vn

Received: 07 April 2025; Accepted: 03 July 2025; Available online: Version 1.0: 29 July 2025

**Cite this article:** Thoa TTA, Kiet DT, Viet BT. *In vitro* shoot regeneration potential of water hyacinth (*Eichhornia crassipes* [Mart.] Solms): A study on an invasive aquatic weed. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.8722

## Abstract

Water hyacinth (*Eichhornia crassipes* [Mart.] Solms) is a fast-growing aquatic plant recognized as a highly invasive weed, posing substantial economic, ecological and social threats. This study evaluates the invasive spread rate of water hyacinth through the plant tissue culture method - culturing the apical and lateral buds separately at different stages to investigate the regenerative ability of *Eichhornia crassipes*. [Mart.] Solms shoots. Additionally, this study evaluated the potential for sexual reproduction from water hyacinth seeds at different stages. After four weeks of culture, a double-layered MS medium (solid agar base with a liquid overlay) under aerobic conditions, supplemented with 0.75 mg/L benzyladenine (BA) and 0.25 mg/L naphthalene acetic acid (NAA), was found to be optimal for *in vitro* growth of water hyacinth buds and seeds. The invasive spread rate of water hyacinth is based on both vegetative propagation from buds and sexual reproduction from seeds. The strongest regeneration from the apical buds and axillary buds with stolon and rootlets reached the highest rate of 60 %. The highest germination reached 40 % from water hyacinth fruit at stage 4 - the stage when the flower turns dark brown.

**Keywords:** BA; *Eichhornia crassipes*; *in vitro* regeneration; NAA; seed germination; tissue culture; vegetative propagation; water hyacinth

## Introduction

Water hyacinth (*Eichhornia crassipes* [Mart.] Solms) is considered the most harmful invasive weed globally (1). The intense spread of this invasive plant has caused substantial socio-economic and ecological damage in many countries, including Vietnam. A study has determined that the invasion area of water hyacinth only needs to reach 10 % of the total lake surface area to cause serious impacts on the aquatic ecosystem (2). In fact, the strong proliferation of water hyacinth is assessed to be increasing rapidly. The rapid spread is attributed to environmental pollution, agricultural runoff, anthropogenic nutrient enrichment and restricted natural water flow. A study reported that the rapid reproductive rate of this plant species reaches a figure of two plants being able to multiply to 1200 plants within 120 days, allowing water hyacinth to rapidly invade water surfaces in a short time (3). This invasion not only spreads in the same area but also to neighbouring areas aided by strong winds and high water levels. However, the determining factors for the reproduction rate of water hyacinth plants are the concentration of nutrients in the water and environmental temperature (4).

Water hyacinths form dense mats intertwined with each other due to their rapid reproductive rate (5). Water hyacinth has two forms of reproduction: asexual reproduction (vegetative propagation) and sexual reproduction. Vegetative propagation contributes significantly to the rapid expansion and spread of water hyacinth into new areas (6). New plants are formed from the elongation of stolons, developing their own root systems and subsequently detaching from the parent plant. The elongation of stolons is the key to the dense growth of water hyacinth, even forming solid mats. Stolons give rise to daughter plants at their nodes, leading to vegetative propagation. The repeated elongation and branching of stolons form dense, interconnected networks of plants, often resulting in floating mats that cover entire water surfaces. The plant hormone auxin is key to promoting cell elongation in stolons. Auxin stimulates the loosening of cell walls, allowing cells to expand (7). Optimal growth of water hyacinth occurs in stagnant or slow-flowing water, under conditions of relatively high humidity, abundant sunlight, a water temperature of 28-30 °C and rich in nutrients such as nitrogen, phosphorus and potassium (8). However, water hyacinths can survive short-term in harsh weather such as frost, survival depends on whether submerged structures escape freezing. After that, limited regrowth can occur from roots or protected shoot bases. Furthermore, in most cases, water hyacinth is often associated with other invasive weeds such as *Pistia*, *Myriophyllum aquum* and *Azolla filiculoides* and water hyacinth consistently dominates (9). Sexual reproduction is also another

form of proliferation in water hyacinths. Their inflorescence contains 8 to 15 flowers and produces 3000-4500 seeds. The seeds can be viable for 5-20 years. The seeds sink after dispersal from the seed coat and germinate within 6 months under low water conditions (10). Seedlings develop into fully leafed and rooted plants within 40 days of germination. Over time, the plants detach from the mud, float freely and then multiply into floating mats. Water hyacinth also spreads through seed dispersal via water and boats, potentially leading to new outbreaks of water hyacinth (11).

Despite numerous management attempts, no long-term control strategy has proven consistently effective. Various methods have been studied and applied, such as chemical, mechanical and biological methods. However, only short-term control of water hyacinth invasion is achieved and its rapid spread quickly resumes thereafter (12). Therefore, continuing fundamental research on this plant species is extremely necessary.

Tissue culture is the growth of sterile plant material on artificial nutrient media under aseptic conditions. *In vitro* plants are essential subjects in studying the growth and development of plants, used to understand the physiological mechanisms of plants. Additionally, *in vitro* plants are a rapid, abundant and clean sample source. Using tissue culture methods to study the reproduction process in plants through rapid clonal propagation and the generation of numerous new plants has also been conducted on various subjects (13). With the applications of plant tissue culture, any particular organ can be targeted and cultured. Furthermore, the elicitors can be used to culture plant tissues, which can enhance the effectiveness of basic research on reproductive processes in plants, particularly in studying vegetative propagation through the apical and lateral buds. The mechanism of biotic elicitation and the applications of biotic elicitors include bacterial, fungal, algal elicitors and other polysaccharides extracted from them (14). Furthermore, seed germination is a stage in the sexual reproduction process. With the application of plant tissue culture, germination *in vitro* can eliminate viruses, facilitate propagation, preserve genetic diversity and provide a large homogeneous sample for research that produces reliable results (15). A recent study has shown that using image processing, Support Vector Machine (SVM) can accurately describe the morphological characteristics of plants such as root morphology, shoot length, generate stem and leaf organs in a short time through the tissue culture method. This is an optimal method for studying plant physiology and is well-evaluated for both its effectiveness and convenience (16). However, in studies on *in vitro* plants, water hyacinth has received little attention due to its untapped economic value as well as the difficulty in *in vitro* cultivation of aquatic plant species. In this study, we focus on describing the vegetative propagation from the apical and axillary buds, as well as the sexual reproduction from seeds of water hyacinth *Eichhornia* crassipes [Mart.] Solms *in vitro*, aiming to evaluate the reproductive rate of water hyacinth through different forms of reproduction. Based on the findings of this assessment, appropriate measures to inhibit reproduction can be proposed to address the current issue of excessive invasion by water hyacinth.

## Material and Methods

### Research materials

#### Research materials for vegetative propagation from the apical and axillary buds

The research materials consisted of buds collected from 3 month-old mother water hyacinth plants raised in the experimental garden. The mother plant is stripped of all leaves and organs is cut separately with 5 different types of explants including (1) apical bud, (2) stem with axillary buds, (3) separate axillary buds, (4) shoots with stolons, (5) shoots having stolons and new roots (Fig. 1).

The apical buds and axillary buds of the mother plant which are arranged in rosettes along the mother stem are cut separately apart and classified into 5 different types, each type is considered a unit of research to evaluate the ability to regenerate buds to create new plants *in vitro* (Fig. 2).

#### Research materials for sexual reproduction from seeds

The research unit is water hyacinth flower. Water hyacinth flowers at the withering stage tend to bend toward the water's surface. Flowers with different colours are called stages 1, 2, 3 and 4. Flowers were anatomized to get fruits and seeds, which were categorized into different stages: Stage 1, 2, 3 and 4 for fruits (fruit at stage 1, fruit at stage 2, fruit at stage 3 and fruit at stage 4) and Stage 1, 2, 3 and 4 for seeds (seed at stage 1, seed at stage 2, seed at stage 3 and seed at stage 4) (Fig. 3).

Cultivation conditions: Lighting of 12 hr/day, light intensity of 3000 lux; temperature of 25 °C ± 2 °C at the Cell Technology Laboratory, Ho Chi Minh City University of Industry and Trade.

### Research methods

#### Investigation of vegetative propagation from apical and axillary buds of *Eichhornia crassipes* [Mart.] Solms under *in vitro* conditions

The research materials included five different types: (1) apical buds, (2) stems with axillary buds, (3) separate axillary buds, (4) shoots with stolons and (5) shoots having stolons and new roots. These materials were disinfected by first washing with soap for 5 min, soaking in water for 3 hr, treating with 70 % ethanol for 5 min and finally disinfecting with 50 % sodium hypochlorite (NaOCl) for 5 min. The explants were cultured on MS basic medium supplemented with 30 g/L sucrose, 0.75 mg/L BA and 0.25 mg/L NAA in two-layer MS system: a solid bottom layer (with agar 8 g/L) and a liquid top layer (without agar). Cultures were maintained under *in vitro* conditions with a light intensity of 3000 lux and a temperature of 25 °C. The experiment consisted of 5 treatments, each with 10 samples. The *in vitro* development of water hyacinth was monitored at 1, 2, 3 and 4 weeks after culture using digital photography, based on observations of live and dead shoots under aseptic, uninfected conditions (infected shoots were removed).

#### Investigation of sexual reproduction from seeds of *Eichhornia crassipes* [Mart.] Solms under *in vitro* conditions

Research materials include 8 different types: Stage 1, 2, 3 and 4 for fruits (fruit bearing seeds at stage 1, 2, 3 and 4) and Stage 1, 2, 3 and 4 for seeds (seeds separated from fruit at stage 1, 2, 3 and 4). The materials were disinfected by washing with soap

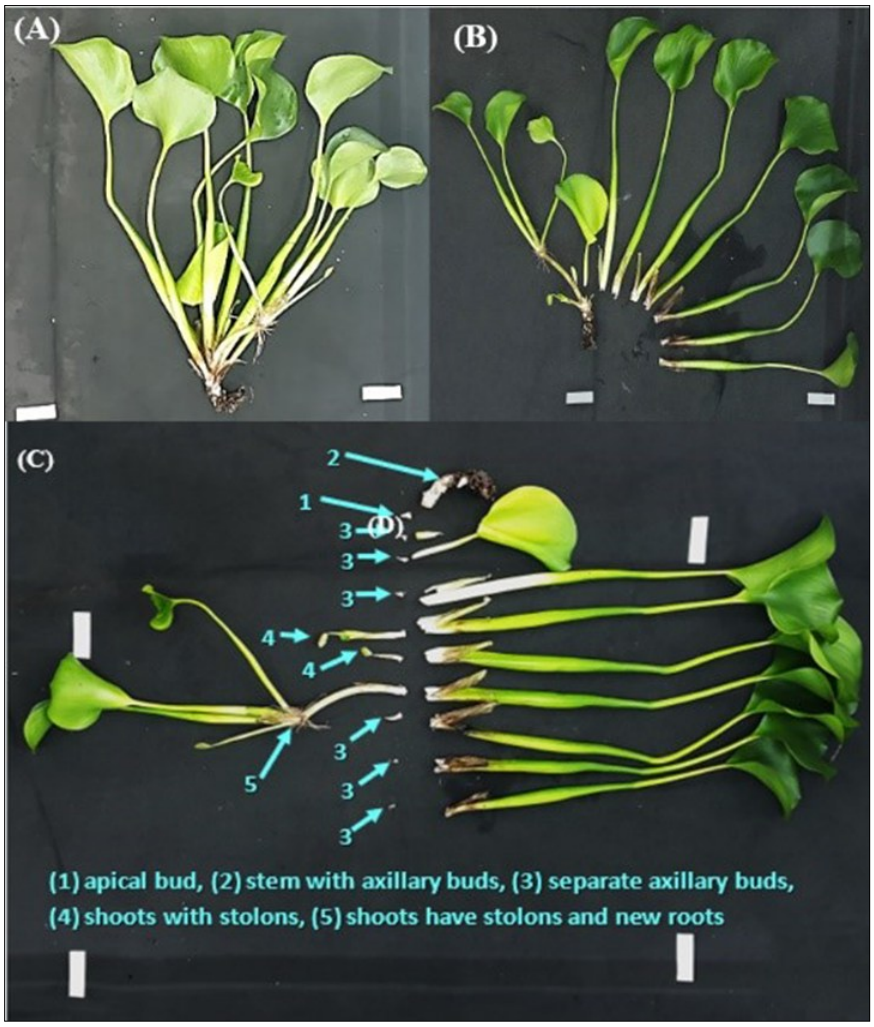


**Fig. 1.** Research materials (A) 3 month-old mother water hyacinth plants (B) The mother plant is stripped of all leaves (C) The mother plant is stripped of all leaves and organs is cut separately with 5 different types.


**Fig. 2.** Research materials (A) apical bud (B) stem with axillary buds (C) separate axillary buds (D) shoots with stolons (E) shoots having stolons and new roots.


**Fig. 3.** Research materials (A) Stage 1, 2, 3 and 4 flowers (B) Stage 1, 2, 3 and 4 fruits (C) Stage 1, 2, 3 and 4 seeds.

for 5 min, soaking in water for 3 hr, treating with 70 % ethanol for 5 min and finally disinfecting with 50 % NaOCl for 3 min. They were then cultured on MS basic medium supplemented with 30 g/L sucrose, 8 g/L agar, 0.75 mg/L BA and 0.25 mg/L NAA. Culturing on the medium in two ways were applied: surface culture and submerged culture in the agar layer. Cultures were maintained under *in vitro* conditions with a light intensity of 3000 lux and a temperature of 25 °C. The experiment consisted of 12 treatments, each with 10 samples. The development of *in vitro* germinated water hyacinth from seeds was monitored at 1, 2, 3 and 4 weeks after culture using digital photography, based on the observation of germinated and non-germinated samples under uninfected conditions (infected samples were removed).

### Statistical Analysis

All experiments were conducted with 10 replicates and the data were recorded and statistically analyzed using Statgraphics Centurion XV software. One-way ANOVA was performed at a significance level of $p < 0.05$.

## Results

### Investigation of vegetative propagation from apical and axillary buds of *Eichhornia crassipes* [Mart.] Solms under *in vitro* conditions

After 4 weeks of culturing the five different explant types, the results showed that (1) apical buds and (5) shoots with stolons and newly formed roots exhibited the highest regeneration capacity, with a survival rate and formation of healthy seedlings reaching 60 %. Explant type (4), shoots with stolons, showed a regeneration rate of 40 %, which was higher than that of (2) stems with axillary buds, which had a 30 % survival rate. In contrast, explant type (3) separate axillary buds have a very low regeneration rate, with 0 % survival recorded in this experiment (Table 1).

The survival rate of the apical bud was 60 %. During the first week of culture, each surviving apical buds developed one new leaf. In the following weeks, stem elongation occurred along with the emergence of 2-3 new leaves arranged in the radial shape. Then the root system develops strongly in the third week and continues to proliferate stems, leaves and roots in the fourth week of culture (Fig. 4).

Stems with axillary buds showed a survival rate of 30 %. In the first week, the buds developed into shoots, with roots from the plant mother still present. By the second week, the young leaves began to emerge from the buds. In the third week, stolon elongation was observed, gradually separating the buds away from the mother stem. The roots of the mother plant helped to grow a new plant and the new plant also forms new roots to create a complete seedling but it is still attached to the mother plant stem through the stolon. During this period, the stolon continued to elongate (Fig. 5).

Separate axillary buds were cultured in MS medium, showing no signs of life and turning black in the fourth week of culture (Fig. 6).

Shoots with stolons were sterilized using ethanol and sodium hypochlorite, then cut into 3 cm pieces and transplanted into a two-layer MS medium. The regeneration rate was 40 %.

**Table 1.** Survival rate and non-induction rate of *in vitro* water hyacinth shoots after 4 weeks of culture

| S.No | Treatment | Survival rate (%) | Non-induction rate (%) |
|---|---|---|---|
| 1 | Apical bud | $60^{d} \pm 0.7$ | $40^{a} \pm 0.4$ |
| 2 | Stem with axillary buds | $30^{b} \pm 0.5$ | $70^{c} \pm 0.3$ |
| 3 | Separate axillary buds | $0^{a} \pm 0.0$ | $100^{d} \pm 0.0$ |
| 4 | Shoots with stolons | $40^{c} \pm 0.4$ | $60^{b} \pm 0.6$ |
| 5 | shoots having stolons and new roots | $60^{d} \pm 0.6$ | $40^{a} \pm 0.4$ |

a, b, c: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

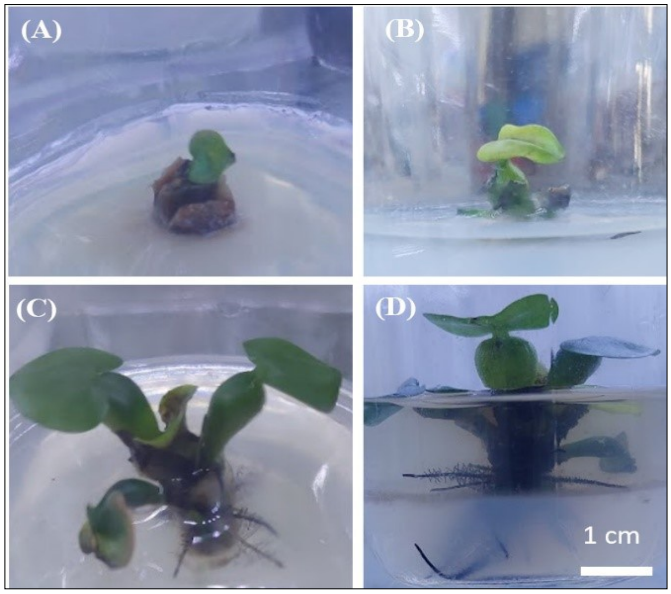


**Fig. 4.** Water hyacinth *in vitro* culturing from apical bud.
(A) 1 week (B) 2 weeks (C) 3 weeks (D) 4 weeks.

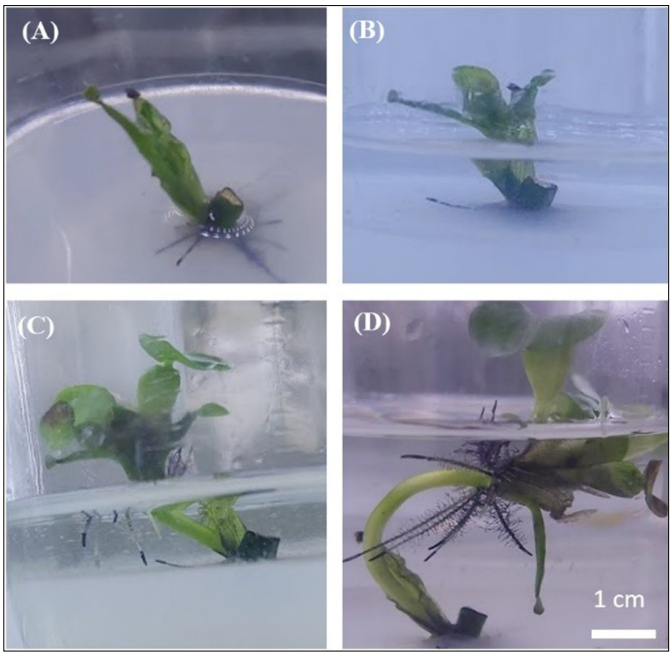


**Fig. 5.** Water hyacinth *in vitro* culturing from stem with axillary buds.
(A) 1 week (B) 2 weeks (C) 3 weeks (D) 4 weeks.

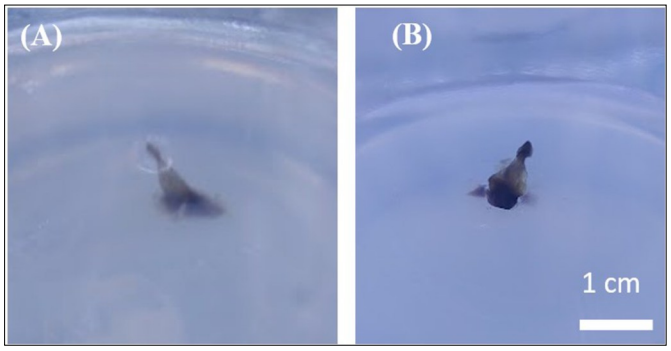


**Fig. 6.** Water hyacinth *in vitro* culturing from separate axillary buds.

(A) 1 week (B) 4 weeks.

In the first week, the shoots remained healthy and green. The stolon was no longer visible and had started to decompose. By the second week, roots had formed, the stolon had fully disappeared and the new leaves emerged. In the third and fourth weeks, the shoots had developed into a complete plantlet with leaves, stems and roots (Fig. 7).

Shoots with stolons and new roots were sterilized using ethanol and sodium hypochlorite. After sterilization, they were cut into 3 cm pieces, the roots were removed and the shoots were transplanted into a two-layer MS medium. The regeneration rate was 60 %. In the first week, the shoots remained healthy and green. The stolon was no longer visible and had decomposed. By the second week, the roots develop healthy, the initial stolon almost disappeared and the new plant produced new leaves. By the third and fourth weeks, fully developed plantlets with leaves, stems and roots were observed (Fig. 8).

## Investigation of sexual reproduction from seeds of *Eichhornia crassipes* [Mart.] Solms under *in vitro* condition

Water hyacinth seeds and fruits at stages 1, 2, 3 and 4 were cultured on MS medium. After 4 weeks of culture, only stage 4 fruits cultured on the surface and stage 3 and stage 4 fruits submerged in agar, showed germination rates of 20 %, 20 % and 40 % respectively. Among them, the highest rate was stage 4 fruit implanted submerged in agar reaching 40 %, seeds after germination did not develop into 100 % seedlings and some sprouts die immediately after germination. This result showed that the percentage of seedlings formed with treatments 8, 11 and 12 was 10 %, 10 % and 30 % respectively (Table 2).

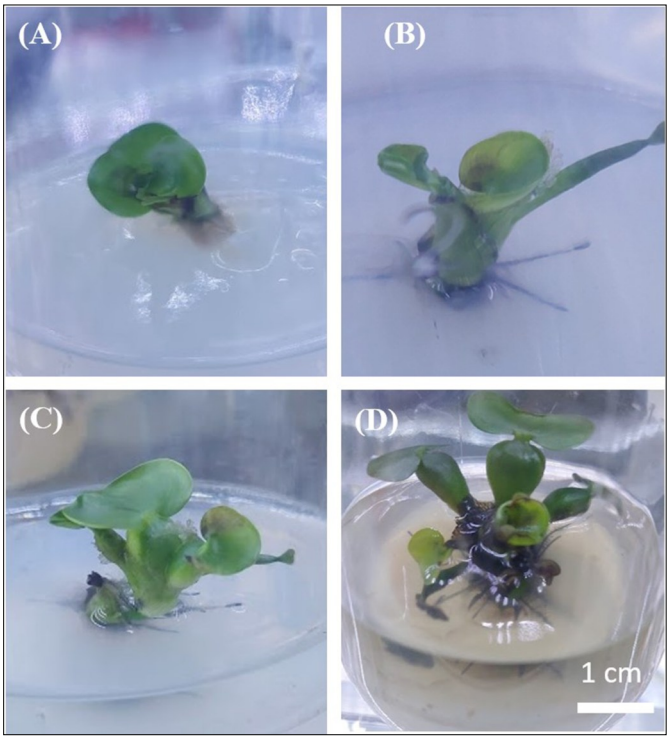


**Fig. 7.** Water hyacinth *in vitro* culturing from shoots with stolons.

(A) 1 week (B) 2 weeks (C) 3 weeks (D) 4 weeks.

Water hyacinth seeds at stage 4 were cultured on MS medium, resulting in a germination rate of 20 %. In the first week after germination, cotyledons were observed. By the second week, the sprouts had developed new leaves. The roots were formed during the third week. A completely healthy seedling was established by the fourth week (Fig. 9).

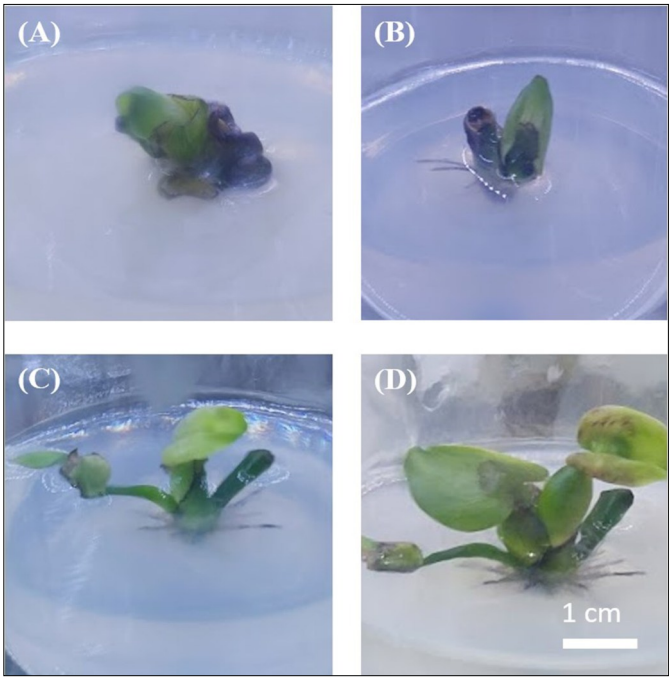


**Fig. 8.** Water hyacinth *in vitro* culturing from shoots having stolons and new roots.

(A) 1 week (B) 2 weeks (C) 3 weeks (D) 4 weeks.

**Table 2.** Germination rate and seedling rate of *Eichhornia crassipes* [Mart.] Solms *in vitro* from seeds after 4 weeks of culture

| S.No | Treament | Germination rate (%) | Seedling rate (%) |
|---|---|---|---|
| 1 | Stage 1 Seed | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 2 | Stage 2 Seed | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 3 | Stage 3 Seed | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 4 | Stage 4 Seed | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 5 | Stage 1 Fruit culturing surface | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 6 | Stage 2 Fruit culturing surface | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 7 | Stage 3 Fruit culturing surface | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 8 | Stage 4 Fruit culturing surface | $20^{b} \pm 0.4$ | $10^{b} \pm 0.5$ |
| 9 | Stage 1 Fruit culturing submerges | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 10 | Stage 2 Fruit culturing submerges | $0^{a} \pm 0.0$ | $0^{a} \pm 0.0$ |
| 11 | Stage 3 Fruit culturing submerges | $20^{b} \pm 0.4$ | $10^{b} \pm 0.5$ |
| 12 | Stage 4 Fruit culturing submerges | $40^{c} \pm 0.5$ | $30^{a} \pm 0.4$ |

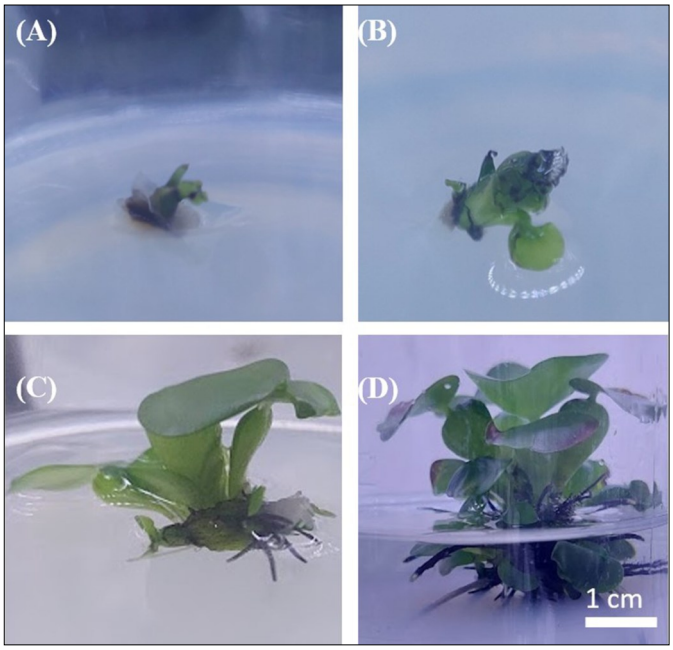


**Fig. 9.** Germination and development into seedlings of stage 4 water hyacinth fruit cultured on agar surface.

(A) 1 week (B) 2 weeks (C) 3 weeks (D) 4 weeks.

Stage 4 water hyacinth seeds cultured submerged in agar on MS medium under *in vitro* conditions had a germination rate of 40 %. In the first week of germination, the sprouts emerged from the agar layer. In the second week, the sprouts formed their first new leaf. By the third week, roots began to form and by the fourth week , a complete seedling with spongy leaf petioles was established (Fig. 10).

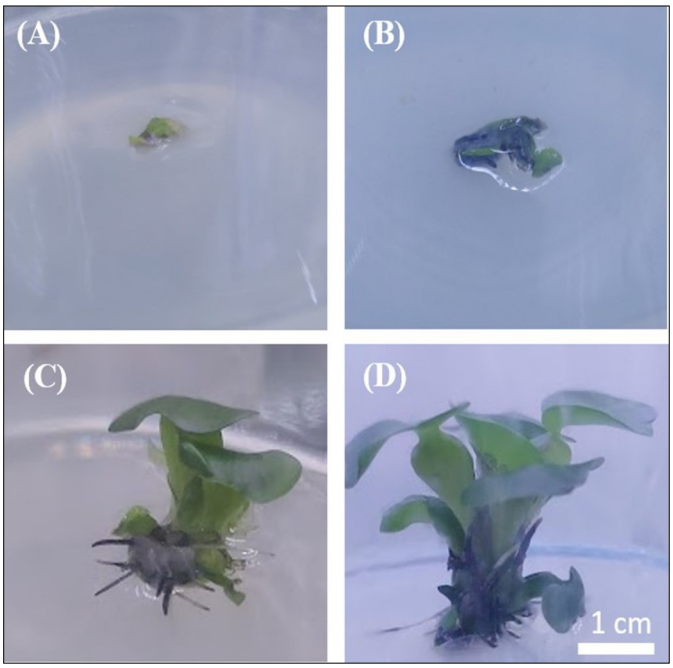


**Fig. 10.** Germination and development into seedlings of stage 4 water hyacinth fruit cultured submerges in agar: (A) 1 week (B) 2 weeks (C) 3 weeks (D) 4 weeks.

After germination, sprouts also die at a high rate, from 25 % to 50 % of the sprouts failing to develop into complete plants and die immediately after first week of germination (Fig. 11).

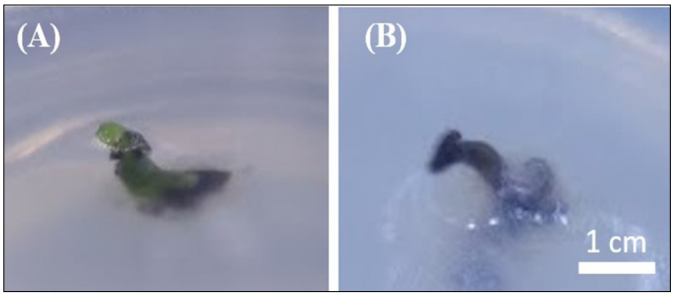


**Fig. 11.** Sprouts death of water hyacinth: (A) 1 week; (B) 2 weeks.

## Discussion

Evaluating the potential for *in vitro* shoot formation in *Eichhornia crassipes* [Mart.] Solms is an important aspect in understanding its reproductive capacity, particularly given its invasive nature and the ecological harm it causes. Despite the natural spread of water hyacinth in Vietnam rivers and canals being described in previous studies (17), scientific evidence demonstrating the strong spreading ability of water hyacinth is detailed only in this study. The results of this research have shown the strong regenerative ability of water hyacinth through vegetative reproduction from apical buds and axillary buds. Moreover, sexual reproduction from flowers, fruits and seeds of water hyacinth also contributes to its spread and dissemination. These results indicate that there are numerous pathways for the regeneration of water hyacinth, despite previous efforts to control it using combined physical, chemical and biological methods (12). Recent studies further confirm that such control measures have had limited effectiveness in halting the invasion of this highly adaptable species.

The main method used to control water hyacinth is mechanical removal. Mechanical removal typically targets the leaves and roots, which constitute the bulk of the plant's biomass. Some water hyacinth removal machines also chop up the plants. With this method, apical buds and axillary buds may remain and they are strong reproductive organs capable of rapid regeneration. This study has demonstrated the high regenerative ability of water hyacinth apical buds and axillary buds. Additionally, water hyacinth is a flowering plant that begins blooming at 3-5 months of age. The flowers open and wither within a single day, followed by fruit and seed development. Mechanical removal is often carried out when water hyacinth has spread and reached the flowering stage. Therefore, after removal, water hyacinth seeds may remain, which is also the reason for its re-invasion afterward. Some recommendations from this study include avoiding chopping up water hyacinths during removal and conducting removal before flowering.

Plant tissue culture is a biotechnological technique widely used in both basic and applied plant research. Tissue culture methods, such as culturing plant organs, can stimulate gene expression pathways to generate specific traits in cultured plant samples, providing high value in research. The current advancements in plant tissue culture research have demonstrated its enormous potential (18). The application of plant tissue culture to culture-separated organs is a strong point of this study. The purpose of the research is to demonstrate the strong regenerative ability of water hyacinth buds. Specifically, culturing apical and axillary

buds at various stages aims to assess the reproductive ability of water hyacinth shoots. Similarly, culturing seeds at various developmental stages highlights the plant's prolific potential for spread through sexual reproduction.

Currently, there is almost no *in vitro* research on water hyacinth. The most recent report on *in vitro* research on water hyacinth is about the effect of phytohormones on the formation of water hyacinth shoots and roots (19). However, the prospects for *in vitro* research are increasingly being emphasized, especially for plant species that are difficult to culture *in vitro* and receive little attention for various reasons. Many emerging technologies are being integrated into plant tissue culture, including the use of electronic grid and cloud computing to simulate the tissue culture process and predict sample productivity prior to experimental work (20). Additionally, artificial intelligence is being applied to model the *in vitro* culture processes of species that are difficult to cultivate, primarily to support research and optimization efforts. An example is *Cannabis* micropropagation; *Cannabis* research is largely an underground effort by some scientists. Along with the lack of deep understanding of *Cannabis in vitro* techniques, which has limited the biological technology utility. In fact, *Cannabis* is known to be difficult to regenerate into complete plants under *in vitro* conditions (21, 22). *In vitro* modeling using artificial intelligence is a future direction that can be applied to water hyacinth research on its spread and control strategies.

In this study, the results show that the reproduction rate of water hyacinth from buds and seeds is also limited by environmental factors such as nutrition, light, temperature and humidity in the culture room. The highest reproductive rate from water hyacinth buds is 60 % and the highest from seeds is 40 %. In the tissue culture method, many factors limit the research process. A recent study also reported that shoot apical necrosis is a physiological disorder that can arise in seedlings or *in vitro* shoots leading to *in vitro* shoot death. This condition, which can spread basipetally and affect the emergence of axillary shoots from buds lower down the stem, is due to the cessation of apical dominance. Shoot tip necrosis (STN) can occur at both shoot multiplication and rooting stages. One of the most common factors that cause STN is nutrient deficiency or imbalance. Moreover, the presence or absence of plant growth regulators (auxins or cytokinins) at specific developmental stages can influence shoot tip necrosis (23). Although *in vitro* plant regeneration has been studied for over 50 years, several mechanistic aspects remain poorly understood. The path associated with the reprogramming of explants in the fully functioning regenerants includes a series of processes that may result in the appearance of morphological, physiological, biochemical and ultimately, genetic and epigenetic changes. All these changes occur during the tissue culture stage and appear in regenerants as tissue culture-induced variation. These variations can then be inherited by generative progeny as somaclonal variation. The cause may be oxidative stress, which can arise during explant preparation and throughout tissue culture due to nutrient components and environmental factors (24). The most recent report also concluded that the success of plant tissue culture is dependent on several factors such as available nutrients, endogenous auxin synthesis, organic compounds and environmental conditions (25). Additionally, successful root formation is a crucial factor for achieving effective tissue culture. The results of this study also indicate that most *in vitro* seedlings grow healthily after 4 weeks of culture, primarily due to root formation occurring during the first week or second week. Many *in vitro* studies have also demonstrated the importance of root formation in plant research. For example, *in vitro* root systems serve as useful tools for learning the biosynthesis of valuable plant compounds, with proper nutritional balance being essential for the development of healthy roots (26).

The results of this research suggest that cutting nutrients can be made to limit the strong spread of water hyacinth because limiting nutrient availability is a factor that reduces the reproductive rate.

Research on plants always opens up results that serve economic, social and cultural life. Currently, new achievements in plant research are also of interest such as the breeding and development of research crops, with an extensive scope for molecular research, especially changes in the genome that are produced by plant tissue culture (27). Genome editing is a new strategy to improve plant characteristics (28). The understanding of plant metabolism is very important because it affects the nutritional value of plants as well as studying the stress resistance of plants (29, 30). Research on plants consistently contributes to addressing the economic, social and cultural challenges faced by humanity.

## Conclusion

After four weeks of culture, a two-layer MS medium comprising a solid agar base and liquid upper layer under aerobic conditions and supplemented with 0.75 mg/L BA and 0.25 mg/L NAA was found to be suitable for the *in vitro* development of water hyacinth buds and seeds. The results confirmed that the reproductive rate of water hyacinth is based on both vegetative reproduction from buds and sexual reproduction from seeds. The highest regeneration rate, 60 %, was observed in apical and axillary buds with stolons and newly formed roots. The highest seed germination rate, 40 % was recorded from stage 4 fruits corresponding to the stage when the flower turns dark brown. These findings provide a scientific basis to evaluate the strong invasive spreadability of *Eichhornia crassipes* [Mart.] Solms, an invasive aquatic weed.

## Authors' contributions

TTTA conducted the experiments and wrote the manuscript. KDT and VBT supervised the work and contributed to manuscript editing. All authors have read and approved the final version of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

## References


1. Nandiyanto AB, Fiandini M, Al Husaeni DN. Research trends from the scopus database using keyword water hyacinth and ecosystem: A bibliometric literature review. ASEAN Journal of Science and Engineering. 2024;4(1):33–48. https://doi.org/10.17509/ajse.v4i1
2. Villamagna AM, Murphy BR. Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): A review. Freshwater Biology. 2010;55(2):282–98. https://doi.org/10.1111/j.1365-2427.2009.02294.x
3. Ajithram A, Jappes JW, Brintha NC. Water hyacinth (*Eichhornia crassipes*) natural composite extraction methods and properties - A review. Materials Today: Proceedings. 2021;45:1626–32. https://doi.org/10.1016/j.matpr.2020.08.472
4. Wilson RJ, Gutiérrez D, Gutiérrez J, Martínez D, Agudo R, Monserrat VJ. Changes to the elevational limits and extent of species ranges associated with climate change. Ecology Letters. 2005;8(11):1138–46. https://doi.org/10.1111/j.1461-0248.2005.00824.x
5. Mitchell DS. African aquatic weeds and their management. In: Denny P, editor. The Ecology and Management of African Wetland Vegetation: A Botanical Account of African Swamps and Shallow Waterbodies. Dordrecht, Netherlands: Springer; 1985. p. 177–202.
6. Barrett SC, Forno IW. Style morph distribution in new world populations of *Eichhornia crassipes* [Mart.] Solms -Laubach (water hyacinth). Aquatic Botany. 1982;13:299–306. https://doi.org/10.1016/0304-3770(82)90065-1
7. Vaz LR, Borges AC, Ribeiro DM. Exogenous auxin and gibberellin on fluoride phytoremediation by *Eichhornia crassipes*. Plants. 2023;12(8):1624. https://doi.org/10.3390/plants12081624
8. Reddy KR, Agami M, d'Angelo EM, Tucker JC. Influence of potassium supply on growth and nutrient storage by water hyacinth. Bioresource Technology. 1991;37(1):79–84. https://doi.org/10.1016/0960-8524(91)90114-Y
9. Gebregiorgis FY. Management of water hyacinth (*Eichhornia crassipes* [Mart.] Solms) using bioagents in the Rift Valley of Ethiopia. PhD [Dissertation]. Wageningen University; 2017.
10. Wright AD, Purcell MF. *Eichhornia crassipes* [Mart.] Solm-Laubach. In: Groves RH, Shepherd RC, Richardson RG, editors. The Biology of Australian Weeds. Melbourne; 1995. p. 111–21.
11. Gopal B. Water hyacinth. Elsevier Science Publishers; 1987.
12. Karouach F, Ben Bakrim W, Ezzariai A, Sobeh M, Kibret M, Yasri A, et al. A comprehensive evaluation of the existing approaches for controlling and managing the proliferation of water hyacinth (*Eichhornia crassipes*). Frontiers in Environmental Science. 2022;9:767871. https://doi.org/10.3389/fenvs.2021.767871
13. Adhikary D, Kulkarni M, El-Mezawy A, Mobini S, Elhiti M, Gjuric R, et al. Medical *Cannabis* and industrial hemp tissue culture: Present status and future potential. Frontiers in Plant Science. 2021;12:627240. https://doi.org/10.3389/fpls.2021.627240
14. Bhaskar R, Xavier LS, Udayakumaran G, Kumar DS, Venkatesh R, Nagella P. Biotic elicitors: A boon for the *in vitro* production of plant secondary metabolites. Plant Cell, Tissue and Organ Culture. 2022;149(1–2):7–24. https://doi.org/10.1007/s11240-021-02131-1
15. Delgado-Paredes GE, Vásquez-Díaz C, Esquerre-Ibañez B, Bazán-Sernaqué P, Rojas-Idrogo C. *In vitro* tissue culture in plants propagation and germplasm conservation of economically important species in Peru. Scientia Agropecuaria. 2021;12(3):337–49. http://dx.doi.org/10.17268/sci.agropecu.2021.037
16. Aliabad FA, Habbab E, Bello AM. Evaluation of the ability to measure morphological structures of plants obtained from tissue culture applying image processing techniques. Research Square.2023. https://doi.org/10.21203/rs.3.rs-3153365/v1
17. Thoa TT, Viet BT, Kiet DT. Survey of factors (sodium chloride, acid acetic, shadow, cutting) affect to the spreading of water hyacinth *Eichhornia crassipes* [Mart.] Solms. International Invention of Scientific Journal. 2021;5(05):1–13. http://doi.org/10.5281/zenodo.7813294
18. Wawrosch C, Zotchev SB. Production of bioactive plant secondary metabolites through *in vitro* technologies status and outlook. Applied Microbiology and Biotechnology. 2021;105(18): 6649–68. https://doi.org/10.1007/s00253-021-11539-w
19. Thoa TT, Viet BT, Kiet DT. Effect of phytohormones on *in vitro* bud and root formation of the water hyacinth, *Eichhornia crassipes*. Bioscience Biotechnology Research Communications. 2022;15(4):490–5. http://dx.doi.org/10.21786/bbrc/15.4.3
20. Udanor CN, Akaneme F, Ukekwe E. Deploying plant tissue culture simulation use case for e-infrastructures. International Journal of Advance Research, Ideas and Innovations in Technology. 2021;7 (3):1475–80.
21. Malabadi RB, Nethravathi TL, Kolkar KP, Chalannavar RK, Mudigoudra BS, Lavanya L, et al. *Cannabis sativa*: applications of artificial intelligence and plant tissue culture for micropropagation. International Journal of Research and Innovations in Applied Science. 2023;8(6):117–42. https://doi.org/10.51584/IJRIAS.2023.8614
22. Monthony AS, Page SR, Hesami M, Jones AM. The past, present and future of *Cannabis sativa* tissue culture. Plants. 2021;10 (1):185. https://doi.org/10.3390/plants 10010185
23. Teixeira da Silva JA, Nezami-Alanagh E, Barreal ME, Kher MM, Wicaksono A, Gulyás A, et al. Shoot tip necrosis of *in vitro* plant cultures: a reappraisal of possible causes and solutions. Planta. 2020;252:1–35. https://doi.org/10.1007/s00425-020-03449-4
24. Bednarek PT, Orłowska R. Plant tissue culture environment as a switch-key of (epi) genetic changes. Plant Cell, Tissue and Organ Culture. 2020;140(2):245–57. https://doi.org/10.1007/s11240-019-01724-1
25. Pasternak TP, Steinmacher D. Plant growth regulation in cell and tissue culture *in vitro*. Plants. 2024;13(2):327. https://doi.org/10.3390/plants13020327
26. Shi M, Liao P, Nile SH, Georgiev MI, Kai G. Biotechnological exploration of transformed root culture for value-added products. Trends in Biotechnology. 2021;39(2):137–49. https://doi.org/10.1016/j.tibtech.2020.06.012
27. Mehbub H, Akter A, Akter MA, Mandal MS, Hoque MA, Tuleja M, et al. Tissue culture in ornamentals: Cultivation factors, propagation techniques and its application. Plants. 2022;11(23):3208. https://doi.org/10.3390/plants11233208
28. Li T, Hu J, Sun Y, Li B, Zhang D, Li W, et al. Highly efficient heritable genome editing in wheat using an RNA virus and bypassing tissue culture. Molecular Plant. 2021;14(11):1787–98. https://doi.org/10.1016/j.molp.2021.07.010
29. Strobbe S, Verstraete J, Fitzpatrick TB, Stove C, Van Der Straeten D. A protocol for a turbidimetric assay using a *Saccharomyces cerevisiae* thiamin biosynthesis mutant to estimate total vitamin B1 content in plant tissue samples. Plant Methods. 2023;19 (1):144. https://doi.org/10.1186/s13007-023-01117-8
30. Morinaka H, Coleman D, Sugimoto K, Iwase A. Molecular mechanisms of plant regeneration from differentiated cells: approaches from historical tissue culture systems. Plant and Cell Physiology. 2023;64(3):297–304. https://doi.org/10.1093/pcp/pcac172

## Additional information

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

**Reprints & permissions information** is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

**Publisher's Note**: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Indexing**: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc

See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting



**Publisher information:** Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.