# IN VITRO CULTURE OF WATER HYACINTH (*EICHHORNIA CRASSIPES*)

**Tanvir Hussain**[1]

## Abstract

The current study was undertaken in 2001 to assess the in-vitro culture potential of water hyacinth by taking different plant-parts (root, stem, leaf, anther, stolon, ovules, embryos/embryos with stem) as explants in different culture media i.e. Murashige & Skooge (MS) medium, Gamborgs B5-salt (B5)and Basal nutrient medium (BNM) alone and in combination with Auxins and Cytokinins. It was found that only the axillary buds gave good response to all media. For callus initiation, same ex-plants and media (alone and in combination with callus initiators) were tried but callus formation was not observed. Aseptically grown young plants were then transferred to hydroponic conditions for maturity. For this purpose MS (Full-strength, 1/2, ¼), B.N.M and Hoogland's solution were used. It was researched out that ¼ MS (pH 5.8) and B.N.M. (pH 5.8) gave excellent results. The study concluded that water hyacinth can be cultured and established in-situ by using usual techniques and methodologies of tissue culture.

## Introduction

Water Hyacinth (*Eichhornia crassipes*) (Mart.) Solma in D.C. belonging to family Pontederiaceae is a prolific free-floating aquatic, 30-50 cm high with short stem weed found in tropical and sub-tropical areas of the world (Mahmood *et al.*, 2005). It is native to Brazil, introduced and naturalized in many tropical countries. In Pakistan it is very common in the Punjab especially in the Gujranwala district and some where in other districts too, inhabiting vast marshy areas, propagating by stolons very fast. It covers vast areas of water forming impenetrable growth (Shahina and Ghazanfar,1977).It is difficult to eradicate due to its fast growth and has become a troublesome weed lead to serious problems in navigation, irrigation and power generation but its role in Phytoremediation of pollutants, production of Bio-gas/compost and manufacture of Furniture (after twisted or braided fiber ) has boomed up in the last few decades (Kojima, 1986; Anushree, 2006).Water hyacinth is an excellent candidate for a biological filtration system for a number of reasons. It possesses an extensive root system which allows them to feed directly from the aqueous medium, extracting chemicals and nutrients rapidly and efficiently (Walverton, 1986).

Another feature is the plant's tremendously high growth rate i.e. capable of producing 17.5 metric tons of wet biomass per hectare per day under ideal growing condition (Walverton, 1979). Furthermore, its fiber, pulp, cellulose and phylloshere extracts are being used for different Agricultural, industrial and experimental purposes (Anon, 1976; Bates and Hentges, 1976).

In this study an attempt was made to find the in-vitro culture potential of water hyacinth and its establishment in hydroponic environment, so they may be helpful for further research-work like uptake of chemicals (pollutants) on Lab. scale under controlled condition.

1 Assistant Wood Technologist, Pakistan Forest Institute Peshawar

## Materials and Methods

### Source of Plants

For tissue culture purposes, plants were brought from Shah Pure-Multan Road, Lahore and Botanical Garden of Punjab University, Lahore.

### Selection of Ex-plants

Different plant portions were chosen as explants i.e. different leaf, stem, root, and stolon (runner) portions, another, ovules, young embryos, stems containing young embryos.

### Sterilization of explants

To sterilize the explants, first washed with autoclaved distilled water, then were passed through different concentrations of hypochlorous acid (sun-bleach) for different time duration (given in Table 1) followed by 70% ethanol and three times autoclaved distilled water washing.

Table 1. Sterilization of ex. Plants with different conc. of hypochlorous acid for various time durations

| Ex. Plants | Conc. of hypochlorous acid (%) | Time duration (min.) | Purpose of culture |
|---|---|---|---|
| Root (tips, middle, initial from stem) | 10 | 5 | Callus formation |
| Leave (margin, middle, near petiole) | 50 | 5 | = |
| Stem (different portions) | 50 | 10 | = |
| Anthers, ovoules, and young embryos | 5 | 3 | = |
| Stolon(runner) (different portions) | 50 | 10 | = |
| Stems containing young embryos | 50-70 | 5-15 | Callus formation/ rooting and shooting |

### Nutrient Media

Following media for above-mentioned explants were used.

1. Murashige & skooge (MS) medium (1962).
2. Gamborg, s (1968) B5 salt (B5).
3. Basal nutrient medium (BNM) (Pandy, 1975).

The media were used as original formulation or in modified form to fulfill the needs of experiments. The basal media were supplemented with various concentration of different growth regulators i.e. Naphthalene acetic acid (NAA), 6-benzylamino purine (BAP), Dichlorophenoxy acetic acid (2, 4-D), Indole-3-butyric acid (IBA).

### Establishment of Hydroponic conditions

The young plants grown under aseptic conditions were then shifted to hydroponic environment by using MS, Basal nutrient medium and Hoogland's solution (1938) for further growth under controlled conditions.

## Results

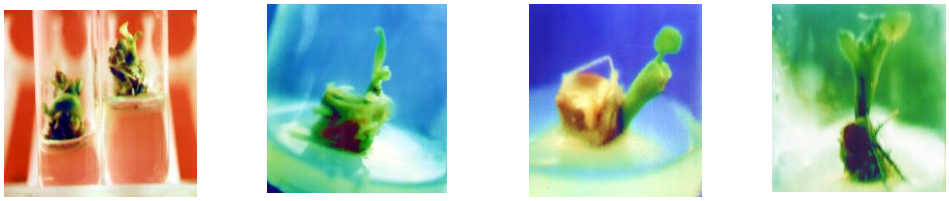
### Manipulation of water hyacinth through tissue culture

In order to manipulate water hyacinth through tissue culture (to grow under aseptic conditions) different plant portions under different media, alone or in combination with auxins and cytokenins were tried. It was found that only axillary buds gave good response to all media and so these were selected for callus formation. For this purpose MS, B5, BNM alone or in combination with hormones were used but callus was not observed. Results obtained by using the media are as follows.

Table 2. Response of Axillary buds to different media (alone or in combination with growth regulators)

| Explants | Media used | Growth Regulators | Callus initiation | Rooting / Shooting response |
|---|---|---|---|---|
| Axillary buds | MS | Original formulation | - | R/S |
| = | MS | Kinetin (0.1mg/L) | - | R/S* |
| = | MS | 1BA+Kinetin (2mg/L, 2mg/L) | - | R*/S* |
| = | MS | NAA + BAP (0.05 mg/L, 0.5 mg/L) | - | R/S |
| = | MS | 2,4-D (0.1mg/L) | - | S |
| = | B.N.M | Original formulation | - | S |
| = | B.N.M | IBA (2mg/L) | - | R/S |
| = | B.N.M | NAA + BAP (0.1 mg/L, 0.5 mg/L) | - | R/S |
| = | B-5 | Original formulation | - | S |
| = | B-5 | NAA + BAP (0.1 mg/L, 0.5 mg/L) | - | S* |
| = | B-5 | BA + Kinetin (0.1 mg/L, 0.1 mg/L) | - | S/R |

S = Shooting
R = Rooting
R* = Excellent Rooting
S* = Excellent shooting
R/S = both rooting and shooting



(a) (b) (c) (d)

Fig.1. Micro-propagation of water hyacinth. (a-c) caulogenesis from stem (d) plantlet formation

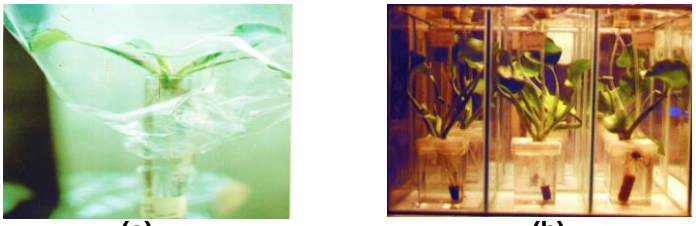
**Hydroponic conditions**

After aseptic growth, young plants were transferred to hydroponic conditions. For this purpose, MS, Basal nutrient medium and Hoogland solution with different pH ranges were attempted. It was found that ¼ MS (pH 5.8) and Basal nutrient medium (pH 5.8) gave excellent result for the establishment of natural hydroponic conditions upto maturity (1-2 months). Hoogland's solution also gave response but plants for long duration showed no good results as shown in the following table.

Table 2. Response of different media under hydroponic conditions

| Nutrient solutions | pH ranges | Response | Best response pH ranges |
|---|---|---|---|
| MS (Full strength) | 5 – 8 | – | – |
| ½ MS | 5 – 8 | + | 5.8 |
| ¼ MS | 5 – 8 | ** | 5.8 |
| B. N. M. | 5 – 8 | ** | 5.8 |
| Hoogland's solution | 5 – 8 | + | 5.8 |
| B5 | 5 – 8 | – | – |

– = No response
+ = Show response
** = Excellent response



(a) (b)

Fig. 2. Plants under Hydroponic environment (a) Aseptically grown young plant (b) Aseptically obtained mature plants under aseptic condition

## Discussion

In order to raise these plants through tissue culture, different plants portions were attempted. For callus initiation, every part of the plant (leaf, stem, root, stolon) as explants were used but no callus formation was observed. After a hard struggle, axillary buds alone and with base of main stem gave good response. By obtaining these responses, axillary buds were tried for callus initiation. During this, MS, B-5, BNM, alone and with combination of growth regulators (2, 4-D, NAA, BAP, IBA etc) were used but callus formation did not take place. In one experiment comlet formation happened but further callus initiation was not observed. During aseptic conditions, it was observed that axillary buds show rooting and shooting when MS (full-strength, IBA + kinetin, NAA+BAP) B-5 (B.A + Kinetin) and B.N.M (NAA + BAP) were used. In case of MS (2, 4-D) B.N.M (Full strength) B-5 (Full strength) only shooting happened. For Hydroponic trials, ¼ MS (pH 5.8) and B.N.M (pH 5.8) were found most suitable.

## Conclusion and recommendation

The results of this study showed that water hyacinth can be cultured on lab-scale by using the usual techniques and methodologies of tissue culture. Furthermore it can be suggested for inexpensive and effective water filtration system for uptake and utilization of pollutants on lab scale experiments.

## References

Anon, 1976. Agency for International Development. Making aquatic weeds useful, some perspectives for developing countries. National Tech. Inf. Ser. Washington D.C., No.PB161-255.

Anushree, M., 2006 Environmental challenge vis a vis opportunity: the case of water hyacinth. J. Environ. 33:122-138.

Bates, R. P. and J. F. Hentges, 1976. Aquatic weeds-eradicate or cultivate. *Econ. Bot*., 30:39-50.

Gamborg, O. L., R. A. Miller and K. Ojimma, 1968. Nutrient requirements of suspension culture of Soybean root cells. Expt. cell Res. 50:148-151.

Hoogland, D. and D. I. Arnon, 1938.The water culture method for growing Plants without solid. Univ. California Agri. Expersta. Circular #347.

Kojima, T., 1986. Generation of methane gas from water hyacinth (*Eichhornia crassipes*), production of methane gas from combination of water hyacinth and fowl droppings. Bulletin of the Faculty of Agriculture, Saga University, Japan, 61:1-8.

Mahmood, Q., P. Zheng, M. R. Siddiqi, E. Islam, M. R. Azim and Y. Hayat, 2005. Anatomical studies on water hyacinth *(Eichhornia crassipes* (Mart.) Solms) under the influence of textile wastewater. J. Zhejiang Uni. Sci.10:991-998.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Pandy, S. N., 1975. Response of *Chlorella vulgaris* Beig. To selenium oxide. Pl. Sc. 7:88-90.

Shahina and Ghazanfar, 1977. Flora of West Pakistan. No.114 Pontederiacaeae, National Herbarium, Agricultural Research Council, Islamabad.

Walverton, B., C. C. Rebecca and McDonald, 1979. Water Hyacinth (*Eichhornia crassipes*) and harvesting study. Economic Botany: 33:1-10.

Walverton, B. C., 1986. Aquaculture Plants and waste water treatment conference on research and application of aquatic plants for water treatment and resource recovery, Orlando, Florida.