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Basic Aspects of *Hibiscus* Plant Tissue Culture and its Commercial

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ABSTRACT

At present time Global warming is a major problem on earth. Biotechnology is the use of biology with Technology to make new hybrid variety of plants to solve the problem of global environment. Biotechnology is most useful technique in genetic engineering. This biotechnology makes new genetic variety or a new genome of plant to protect entire ecosystem and environment. Present study deals with Hibiscus Plant tissue culture to commercial production of disease free plants from a single cell or a tissue. There are more than 200 different species of Hibiscus plant in the world which is produced by plant tissue culture. In the formation of plant from tissue culture Hibiscus rosa-sinensis. choose a species to produce many plants but here Hibiscus plantlet face a major problem in transplantation in soil. The transplantation and hardening in soil is unsuccessful due to soil parameters. There are a major problem to plantlet for support the environment of greenhouse due to pH of soil, Humidity, manor and soil nutrition elements.

Keywords: *Global warming, Biotechnology, Hibiscus, Plantlet, Environment.*

INTRODUCTION

Hibiscus is a genus of flowering plants belongs to Malvaceae family. *Hibiscus* are water-loving plants. They have large leaves and blooms are big and full of moisture themselves. In the tropical areas where *Hibiscus* originated mostly islands or coastal areas and the rainfall is good. Most of *Hibiscus* not growing in the tropical areas, so it need to simulate as much as possible the natural conditions where *Hibiscus* originally developed. Plant tissue culture was a new methods of plant breeding that developed around the 1950s. Since the conventional breeding techniques could not fulfil the required demand of crops, tissue culture came around as a grand leap in breeding practices. It makes use of parts of a plant to generate multiple copies of the plant in a very short duration. The technique exploits the property of totipotency of plant cell which means that any cell from any part of the plant can be used to generate a whole new plant.

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Plant tissue culture is most useful method which is used to produce disease free resistant plants from a single cell or tissue. A Plant which is produced from a cell by plant tissue culture is identical with mother plant and all character also same with it. A successful plant tissue culture is not easy but in well developed biotech laboratory it is possible and successful.

Objectives of *Hibiscus* plant tissue culture:-

1. Commercial production of hibiscus plant spp.
2. Formation of disease Resistant plant
3. Use a single cell or a tissue for produce many plants
4. Successful formation of plantlet
5. Successful transplantation in greenhouse
6. Successful hardening in soil
7. Commercial production of hibiscus spp.
8. Protection of environment

MATERIALS AND METHODS

(1). Plant material

plants grown in a greenhouse under the controlled condition before three months. After three months select shoot apex or meristematic tissue as explant for plant tissue culture.

(2). Culture media

There are many culture media to prepare a plant from tissue culture like – M.S. (Murashige and Skoog, 1962) Medium , Agar Agar medium , B.S. Medium , B5 Medium and white's Medium. To prepare appropriate culture medium use all nutrition element which is naturally used by plants. Most appropriate culture mediumuse in plant tissue culture is M.S. medium. To prepare M.S. (Murashige and Skoog, 1962) medium there are Five different stock solution of MS basal medium is macronutrient, micronutrient, iron source, E.D.T.A.(Ethyline Diamine Triacetic acid), vitamins and amino acids concentration were used. To prepare M.S. mediun concentration of all nutrient element ten times pure in concentration.

To prepare M.S. Medium use four Stock Solutions:-

(1). Stock Solution 'A' – Macronutrient Element:-

CHEMICALS	IN 100 ml
Mg SO_4. $7H_2O$	0.74 gm
K_2PO_4	0.34 gm
KNO_3	3.8 gm
NH_4NO_3	3.3 gm
$CaCl_2$. $2H_2O$	0.88 gm

(2). Stock Solution 'B' – Micronutrient:-

CHEMICALS	IN 100 ml
H_2BO3	0.124 gm
$MnSO_4$. H_2O	0.005 gm
ZnSO4. 7H2O	0.172 gm
NO_2MoO_4. $2H_2O$	0.005 gm
$CuSO_4$. $5H_2O$	0.0005 gm
$CaCl_2$. $6H_2O$	0.002 gm
KI	0.0166 gm

(3). Stock Solution 'C': – Vitamins and Amino acids:-

CHEMICALS	IN 100 ml
NICITINIC ACID	0.01 gm
THIAMINE (B1) VITAMIN	0.002 gm
PYRIDOXIN HCL	0.01 gm
GLYCINE AMINO ACID	0.04 gm

(4). Stock Solution 'C': – Iron and E.D.T.A.:-

CHEMICALS	IN 100 ml
$FeSO_4$. $7H_2O$	0.0556 gm
Na_2 E.D.T.A.	0.0746 gm

FORMATION OF PLANTLETS FROM EXPLANT:–

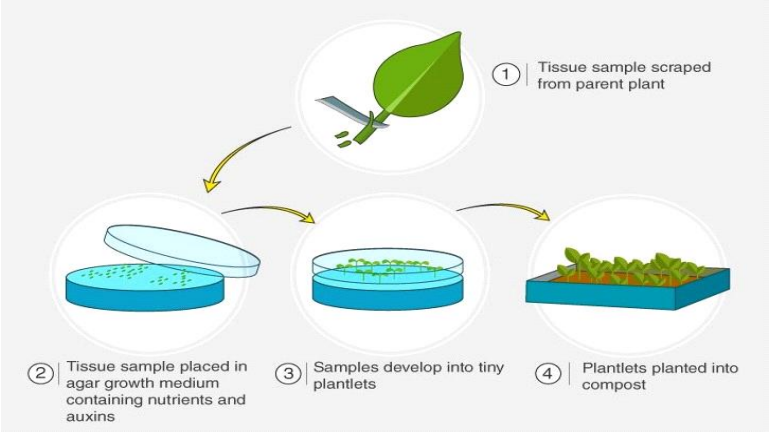

Figure 1 : hibiscus plant tissue culture

Formation of plantlets from explant to prepare appropriate culture medium and than establish explant on culture medium. For culture medium there are Five different stock solution in the basal medium like - macronutrient, micronutrient, iron source, E.D.T.A. (Ethyline Diamine Triacetic acid), vitamins and amino acids were used.

Acclimatization and hardening-off of Regenerated plant:-

There are many ways to regenerate complete plant from plantlets. At first test soil parameter of greenhouse and hardening area because of the soil parameter plantlet is unable to grow. There are a major problem to plantlet for do not support soil pH, Humidity, manor and soil nutrition elements. Then transplant all plantlets in greenhouse. After transplantation check growth every week in all *Hibiscus* plants.

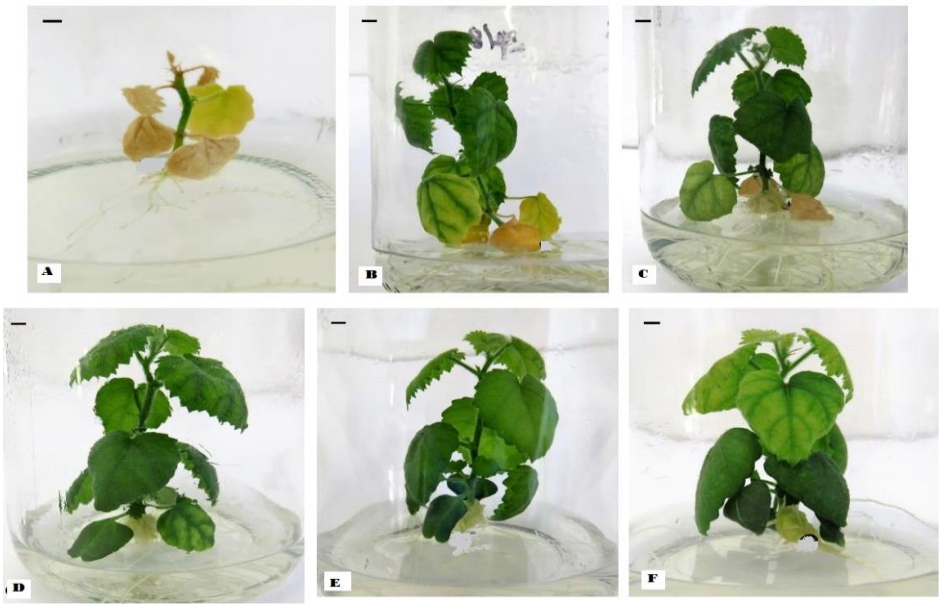

Figure.2 - *Hibiscus rosa-sinensis* plant tissue culture

Experimental design:--

There are two methods for tissue culture:-

- Callus culture
- Suspension culture

At present both methods are important and useful for plant tissue culture.

Transfer of plant material to tissue culture medium:-

Use the sterile gloves and equipment for all of these steps.

1. Place the plant material still in the chlorox bleach sterilizing container, the containers of sterile water, the sterilized forceps and blades, some sterile paper towel to use as a cutting surface and enough tubes containing sterile medium into the sterile aquarium. The outside surfaces of the containers, the capped tubes and the aluminum wrapped supplies should be briefly sprayed with 70% alcohol before moving them into the chamber.

2. The gloves can be sprayed with a 70% alcohol solution and hands rubbed together to spread the alcohol just prior to placing hands into the chamber. Once students have gloves on and sprayed they must not touch anything that is outside of the sterile chamber.

3. Carefully open the container with the plant material and pour in enough sterile water to half fill the container. Replace the lid and gently shake the container to wash tissue pieces (explants) thoroughly for 2-3 minutes to remove the bleach. Pour off the water and repeat the washing process 3 more times.

4. Remove the sterilized plant material from the sterile water, place on the paper towel or sterile petri dish. Cut the cauliflower into smaller pieces about 2 to 3 mm across. If using rose cut a piece of stem about 10 mm in length with an attached bud. The African violet leaf can be cut into small squares about 1-1.5 cm across. Be sure to avoid any tissue that has been damaged by the bleach, which is apparent by its' pale color.

5. Take a prepared section of plant material in sterile forceps and place into the medium in the polycarbonate tube. Cauliflower pieces should be partly submerged in the medium, flower bud facing up. Rose or other cuttings should be placed so that the shoots are level with the medium surface. The African violet leaf pieces should be laid directly onto the medium surface.

6. Replace the cap tightly on the tube.

Figure 3: The small explant develops callus which then produces shoots a few weeks after being placed into tissue culture media

Data collection and statistical analysis

The performance of plantlet derived from the callus cultures was evaluated with respect to growth, survival and morphology for a period of up to five weeks in order to select the best culture medium for *in vitro* plant tissue culture growth. whilst the callus remained in the culture bottles (non-destructive method). The callus was recorded after five weeks. Visible qualitative changes in the morphological appearance of callus developed on the different strengths of MS media and cultures were also monitored and photographed (Figure. 3 - *Hibiscus rosa-sinensis* plant tissue culture A, B, C, D, E and F) weekly for five weeks. The number of nodes on the main stem of the shoot was counted weekly over a period of five weeks.

Table1. Concentration of Cytokinin (for shoot) and Auxin (for Root) on prolifration of callus and shoot:-

S.N.	HORMONE $CONC^N$ (CYTO.) (mg/l)	HORMONE $CONC^N$ (AUX.) (mg/l)	RESPONSE (%)	NO. OF SHOOT/ EXPLANT (mean ± SD)	SHOOT LENGTH (in cm) (mean ± SD)
1	1.0	0.5	20	0.80 ± 0.41	0.52 ± 0.041
2	2.0	1.0	35	3.80 ± 0.71	2.60 ± 0.49
3	3.0	1.5	70	2.80 ± 1.45	1.38 ± 0.35
4	4.0	2.0	50	1.80 ± 0.41	0.88 ± 0.28
5	5.0	2.5	80	0.80 ± 0.38	0.81 ± 0.21
6	6.0	3.0	60	0.70 ± 0.21	0.70 ± 0.16
7	7.0	4.0	35	0.50 ± 0.15	0.58 ± 0.11

Table2. Effect of different Hormones on Callus proliferation and Morphology:-

HORMONE $CONC^N$ (mg/l)	CALLUS GROWTH AFTER 7 WEEK	CALLUS SCORE	COLOUR OF CALLUS	MORPHOLOGY OF CALLUS
2,4 D + Kn				
0.25 + 0.1	3.4	Intense	Green	Nodular
0.25 + 0.2	2.8	Meager	Dark green	Compact
0.25 + 0.3	4.1	moderate	Moderate green	Nodular
2,4 D + BAP				
0.25 + 0.1	4.5	moderate	White green	Friable
0.25 + 0.2	2.9	Intense	Green	Compact

RESULTS AND DISCUSSION

Callus were successfully acclimatized over a period of 2-3 weeks in moist vermiculite under controlled environmental conditions and exhibited 90% survival. Further hardening-off of callus in a greenhouse (uncontrolled environment) resulted in well-established mature plants (90% survival) which proved suitable for transplanting to an open environment. Mature plants flowered 2-3 months after the transplant and showed phenotypic similarities with naturally grown plants.

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CONCLUSIONS

- Aseptic *in vitro* callus cultures (MS medium) of *H. rosa-sinensis* were successfully established.
- Callus germination was most successful on MS media. However, these callus cultures proved unsustainable for callus establishment, which was best supported on full strength MS medium. *In vitro* callus propagation of *H. rosa-sinensis* could be beneficial for faster commercial production of plantlets and plants.

- all year round in a limited space with low maintenance of plant material.
- *In vitro* produced callus can also serve as an aseptic explant source for *in vitro* shoot culture and for *ex vitro* establishment of plants.
- Formation of plantlet from tissue culture
- Successful plantation of plantlet.
- Successful hardening.
- Commercial production of *H. rosa-sinensis*

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