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List of Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
AC	Activated charcoal
BA	Benzyl adenine purine
BAP	Benzyl adenine purine
C. borivilianum	Chlorophytum borivilianum
IAA	Indole acetic acid
IBA	Indole butyric acid
MS	Murashige and Skoog
NAA	Naphthalene acetic acid

1. Abstract

Chlorophytum borivilianum has been known for the medicinal uses of its roots. Roots contain many constituents which can helps in curing diseases like cancer, male sterility, diabetes, physical weakness, post natal problems etc. but the species is endangered due to lack of propagating conditions in nature. Its roots are valuable in pharmaceutical industries as well as it can also leads to economic growth because to its high demand in the world market. Plant tissue culture is the biotechnological area that can promise its large scale propagation and helps to meet the ever growing demand. We have tried full strength MS media with different PGRs in both solid and liquid media. Activated charcoal was used as a supplement in the media. It has found that full strength liquid MS media with charcoal added gives out the best results as the rooting media in a short time period as compared to other media composition. The liquid media with 250 mg/l activated charcoal containing 0.8 mg/l NAA and 1 mg/l IAA shows the best result for rooting. While in shooting MS media containing 3 mg/l BAP without AC gives the best result. However solid media containing AC show no appreciable result.

2. Introduction

Most of the drug industries depend upon plants to extract important pharmaceutical compounds. In recent years, there has been an increased interest of researchers in the *in vitro* culture techniques which offer a viable tool for mass propagation and conservation of rare, endangered and threatened medicinal plants.

Chlorophytum borivilianum is commonly known as *Safed musli* is a medicinal plant which belongs to the family *Liliacea.* Its roots are valuable as it contain medicinal properties.

The genus *Chlorophytum* is having more than 215 species (Li *et al.*, 1990). The roots are widely used in the Indian system of medicine for the treatment of weakness, impotency, and sterility. It is used to enhance male potency and can also be used for cardiac and brain treatment. It has been also used in various diseases like diabetes, piles and used as anti-pyretic.

Chlorophytum borivilianum can be found in the tropics and subtropics. In India, it is found in Southern Rajasthan, Northern Gujarat and Western Madhya Pradesh (Maiti *et al*., 2005). In the "Red data book of Indian plants" the survey conducted for medicinal plant by the Botanical Survey of India, it is listed as an endangered species (Nayar *et al*., 1988).

The plant is having great economic importance in India as its dried roots are sold in the market at a price of per kilogram for Rs. 1500. Due to its slow growth rate in the conventional vegetative propagation methods, it cannot meet the present demand. The annual demand of its root in the India was estimated to nearly 500 tones (Bordia *et al*., 1995).

Obstacles in production of *Chlorophytum borivilianum*:-

Safed musli is a rhizomatous herb that usually reproduces vegetatively in nature by shoot buds from root tubers in the soil and sexually by seeds.

- Seeds of *Chlorophytum borivilianum* have poor germination percentage (11–24%), low viability and long dormancy period (Jat *et al*., 1990).
- *C. borivilianum* has a very short life cycle of about 90-100 days.
- The propagation of *Safed musli* through seeds and tubers may take several months by conventional process. (Maithi *et al*., 2005)
- With the low rate of seed germination (11–24%), the plants are traditionally propagated through tubers but due to large-scale demand of its wild material and

insufficient attempts to allow its replenishment, *C. borivilianum* is facing problem of being an endangered species.

- Conventional micropropagation requires huge labour which has often limited its commercial uses.

To meet the present demand and to protect the species from going endangered, work is going on in plant tissue culture technique. Root induction in this species is difficult as well as important. To induce rooting from shoots solid and liquid MS media containing different combinations of auxins with supplements are used.

3. Review

Safed musli is the botanical herb name for the plant *Chlorophytum borivilianum*. It is an annual herb which is having 3 different parts namely tubers, crown leaf and flower. *Safed musli* has use in the *Ayurvedic* medicinal system. It is a medicinal plant that has become worldwide valued for the properties of its roots. It has been reported that there are thirteen species of *Chlorophytum* in India (Kothari *et al*., 2006).

These species differ in appearance and it is commonly known as *Safed musli* in the Indian drug market. This genus has reported to have 300 species, which are spread out in many parts of the world. About 85% of these species are found in the Africa.

Among all of these species, *Chlorophytum borivilianum* is known to yield the highest saponin content. Other important species are *C. arundinaceum*, *C. tuberosum*, *C. laxum*, and *C. breviscapum.* But it is at the urge of facing extinction in a few years due to its ample usefulness in the medicinal area and also due to lack of propagating via seeds and tubers.

For commercial purposes plant tissue culture can produce ample amount of plant material in a small period of time (Tandon *et al*., 1995).

Plant tissue culture area in biotechnology had an efficient effect on agricultural field. It is the best way to propagate a large number of plants in short period of time (Thakur *et al*., 2006). Explant used can be a single cell, piece of plant or tissue that must be able produce a suitable amount of new plants (Kirtikar *et al*., 1986).

Because plant tissue culture requires a minimum amount of plant material to start with; significant saving can be done by reducing money for the growing facilities (Tandon *et al*., 1995).

2.1 Components of *Chlorophytum borivilianum* roots:-

Chlorophytum borivilianum have tuberous roots which are a source of steroidal saponins (neohecogenin, neotigogenin, stigmasterol, tokorogenin). Saponins are steroid and triterpenoid glycosides that display various biological activities.

These steroids possess properties like

- Immunomodulatory,
- Adaptogenic,
- Antipyretic,
- Diuretic,
- Aphrodisiac,

- Hemostatic and
- Anti-tumour

Saponins consist of a sugar part such as glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose that are glycosidically linked to a hydrophobic aglycone (sapogenin). In nature sapogenin are triterpenoid or steroid. (Kaushik N, 2005)

The roots are reported to contain 42% of carbohydrates, 8–9% of proteins, 3–4% fibres, and 2–17%) of saponins. (Bordia *et al.*, 1995)

2.2 Plant characteristics

Chlorophytum borivilianum is a small herb whose leaves are radical in shape which appeared above the ground. Naturally it starts growing in summer and rain season. Roots are having cylindrical structure and are usually 5 to 20 in number. Its radical leaves are 6 -13 in number that overlap at the base. These are usually sessile in nature. The leaves are horizontal having smooth surfaces. Flowers of *Chlorophytum borivilianum* are small, white that usually arranged in clusters. Each cluster consists of 3 flowers. The flower clusters are 1.0 to 10.5 cm long and are dense. Its white pedicels are 5-10 mm long. Fruits are green or yellow in color and equal in length and breadth. Seeds are endospermic in nature and are of onion structure. These are black in color and angular in shape.

Major constituents *of Safed musli* are carbohydrates 42%, protein 80% to 90%, fibres 3% to 4%, saponins 2% to 17% and alkaloids 15% to 25%. (Mishra *et al*., 2005)

A number of health tonics (sexual tonics) are prepared from *Safed musli*. *Chlorophytum borivilianum* have biochemical constituents like steroidal sapogenins having adoptogenic and aphrodisiac properties. (Patwardhan *et al*., 2005)

The plant tissue culture produces high quality of the planting material that is propagated from vegetative parts. It has many applications in the social area such as it has created global commercialism of plant that can further benefits the farmers, improve employment and benefit several patients across the world. However, there are still major functioning areas to produce quality graded medicinal plants like *Chlorophytum borivilianum*. The further advantage of tissue culture technology lies in the production of high quality planting material that can be multiplied in all the seasons under disease-free conditions.

This species can be prevailed by both methods vegetative propagation of tubers and by sexual propagation of seeds. It has a low rate of seed germination in nature (25%). Further, seed-

raised species can show variation due to its out crossing nature. It is uneconomic to propagate through tubers as it constitutes the commercial product. It can lead to deficiency of the plant material.

Due to these reasons plant tissue culture provides a potential method for rapid multiplication of this species via meristems culture or tip culture. In conventional cultivation, plantation is possible only once a year (June or July). (Halpern *et al*., 1953)

Micropropagation of medicinal plants using tissue culture technique has already been conducted but most of them have reported on micropropagation using the auxiliary shoot obtained from the shoot tip as an explant.

In vitro regeneration of medicinal plants has also been found by many authors.

Micropropagation offers a way out to achieve plant material that is free of diseases and pest. Genetic integrity of plants can be maintained by following proper protocol for the commercial propagation of *Chlorophytum borivilianum*. In vitro propagation of can lead to genetic alteration because of culture stress. (Damre *et al*., 2003)

2.3 Climatic Requirements

As *Safed musli* grows naturally in the most part of the central region of India, the normal climate of the central region suits the crop most and it can grow successfully in the wide range of the temperature and rainfall. Sandy loam soil with proper drainage system facilitates its growth. (Thakur *et al*., 2006)

2.4 Reproductive characteristics

Conventionally *Safed musli* is grown in field by cutting roots along with the leaf base or can be propagated by germinating seeds in species (Mishra *et al.,* 2005).

Safed musli is an extremely endangered species, for reproducing the plant vegetatively can lead to loss of a suitable amount of plant material. *Chlorophytum borivilianum* can be seen in the rainy season in the southern, western northern and eastern belt of India. (Mishra *et al*., 2005)

The plant material / seeds of these species will be available in July/August. *Chlorophytum borivilianum* can be collected in September.

2.5 *In vitro* propagation of *Chlorophytum borivilianum*

Chlorophytum borivilianum being an endangered species is known for its dried fasciculate storage in roots (Nayar *et al*., 1988).

C. borivilianum has a short life cycle so it should be grown or propagate regularly throughout the year. The tuberous root of this plant can either be, sold in the market for economic gains or saved for commercial cultivation year after year. This has created a severe shortage of planting material for large-scale cultivation.

Micropropagation technology is used for commercial cultivation of many medicinal, horticultural, ornamental, and forest plants.

Micropropagation of *C. borivilianum* has achieved on MS (Murashige and Skoog, 1962) medium supplemented with benzyladenine (BA) when shoot bases are used as an explants (Purohit *et al*., 1994).

Balaraju *et al*., 2009 found that the highest frequency of rooting in *Swertia chirata* was obtained in half-strength MS medium supplemented with NAA.

2.6 Micropropagation of *Safed musli*:-

The method of micropropagation goes through several stages-

1. Establishment
2. Multiplication
3. Pretransplantation
4. Finally transfer to culture.

Finally the plant is removed from the media and transferred to soil for its further growth known as acclimatization. Sterilization is the basic step to initiate *in* vitro propagation.

2.6.1 Sterilization

The first step in any successful tissue culture technique should be the selection of suitable explants followed by its complete disinfecting. Disinfecting the surface generally requires sterilization with one or more disinfecting agents after that carried by thorough washing (Torres *et al.*, 1989).

Most widely used disinfectants in plant tissue culture includes sodium hypochlorite (0.5% to 5.25%), calcium hypochlorite (9% to 10%), ethyl alcohol (75% to 95%) and chemicals like bromine water (1% to 2%), hydrogen peroxide (0.1% to 1.0%) and silver nitrate (1%). Triton-X or Tween-80 surfactant can also be added to enhance the effectiveness of these disinfectants. It breaks the surface tension between the plant tissue and the water molecules. The explants must be rinsed 2 to 3 times in distilled water after disinfecting and sterilizing to remove the effect of the disinfectants. (George *et al*., 1993; Razdan *et al*., 1993).

The addition of both ampicillin and bavistin to the medium was found suitable for efficiently removing remaining the bacteria and fungi contaminations (Nair *et al.*, 1994)

2.6.2 Explants

Several species of *Chlorophytum*, are endangered because of its over exploitation for the valuable tuberous roots which are commonly used as an ingredient in many tonics and aphrodisiacs.

A method was developed for the rapid multiplication of *C. borivilianum* using stem discs as explants by Suri *et al.*, 1999.

The four explants of *C. borivilianum* can be used for propagation are seed, roots, shoot base and leaf. They were tried with different combinations and concentrations of the hormones and vitamins for root, shoot and callus regeneration. The best explant was leaf base as reported by Gaikwad *et al*.,, 2003 and using root as the explant gave the poorest result.

Further research by Pudake and Dhumale, 2003 shows shoot base and stem disc explants gave the best results for large scale propagation of *C. borivilianum.*

2.6.3 Shoot induction

Dave *et al*., 2003 found the best shooting media for multiplication agar containing Murashige and Skoog medium having 22.2mM of 6-benzylaminopurine (BA) and 3% of sucrose.

Balaraju *et al*., 2009 showed the in vitro shoot production using Murashige and Skoog (MS) medium with different hormones such as 6-benzylaminopurine (BA), Kinetin and TDZ. 1.0 mg/l 6-benzylaminopurine (BA) and 0.1 mg/l Kinetin gives highest number of multiple shoots.

Mehdi Farshad Ashraf *et al*., 2014 found that BAP only in MS media is effective on shoot multiplication, while Kinetin alone is effective on shoot elongation if compared to the control as MS basal medium without any PGR. The combination of both BAP and kinetin leads to enhancement of both shoot number and shoot length.

Raun *et al.,* (2001) studied propagation of *Lilium* using different hormones such as BAP, 2, 4-D, NAA, kinetin, adenine and gibberellic acid cultured on MS medium.

Shimasaki *et al*., 2000 found that BAP and TDZ have beneficial effect on the survival of *Gloriosa superba* and are effective on the shoot multiplication.

Rui *et al*., 1998 examined that in *tulip* the callus formation was best in MS medium supplemented with 2 mg/l of BAP and 0.2 % active carbon when kept in darkness for 5-10 days (Tefera *et al*., 1998).

Multiple shoots were obtained with nodal segments in MS medium supplemented with kinetin when concentration was between 0.5 mg/l to 2.0 mg/l (Sharma *et al*., 1991).

Sen *et al*., 1991 achieved shoot multiplication in 30 days when propagated *in vitro* from shoot tip explants *Coleus forskohlii* with 2 mg/l of BAP.

Mukherjee *et al*., 1996 used shoot tips of *Coleus forskohlii* cultured on MS basal medium with 3% of sucrose. The shoot tips were further proliferated on MS medium with 1 mg/l kinetin.

Uchendu *et al*., 2011 found improvement in the growth of NAG when single-node are cultured in liquid medium containing 2.5 mg/l of kinetin, 0.5 mg/l of NAA, and 50 mg/l of activated charcoal.

2.6.4 Rooting

Tefera *et al*., 1998 has found that 1 mg/l IAA in media can induce rooting effectively.

Quraishi *et al*., 2004 reported the micropropagation of neem (*Azadirachta indica*) using culture of buds from branches of the neem tree. For rooting microshoots are used in half-strength DKW medium with IBA or NAA.

Makunga *et al*., 2006 found that the cultures of *Thapsia garganica* grown on half strength MS media when supplemented with BAP gives best result in combination with IBA.

Kusampudi *et al*., 2010 found in *Ocimum sanctum* that when friable callus is transferred to the liquid MS medium supplemented with NAA and BAP under continuous agitation roots were obtained.

Purohit *et al*., 1994 found that ¾MS media when subjected with BAP and IBA all the shoots are produces roots. Also it is found that roots are produced when subjected to low concentration of cytokinins in the media. Shoot bases were taken as explant in this case.

Suri *et al*., 1999 found that rooting in *Chlorophytum borivilianum* was also found using B5 medium containing IBA with sucrose and glucose using stem as the explant. Tuberous root are also found.

Pudake *et al*., 2003 examined different combination of auxins and cytokinins for multiplication and rooting in *Chlorophytum borivilianum*. IBA and NAA were used and it was found that IBA gives out the best result for rooting.

Regenerated shoots were transferred to MS medium containing various auxins (0.5 mg/l NAA, 1.0 mg/l IBA and 1.0 mg/l IAA) either alone or in combination and rooting was seen. (Kemat *et al*., 2010).

Main advantages of micropropagation

1. Micropropagation leads to production of plants clone to each other. It is known for producing healthy plants without any disease.
2. It save time and propagate plant that are difficult to grow conventionally in nature.
3. A large number of plants can be grown on small area.
4. Production is higher than conventional method.

2.7 Liquid media culture

In conventional plant tissue culture systems solidified media is utilized that involve agar or other gelling agents that functions as a support (Savio *et al*., 2012).

The liquid culture systems offer improvements over solid media as follows:-

- Lower media costs
- Better growth of plants
- Sterilization by microfiltration
- Easier acclimatization
- Uniform culturing conditions
- Renewal of the media without changing the container
- Ease of cleaning containers

Higher multiplication rates in liquid media compared to cultures on agar gelled media were reported for many species.

Sarethy *et al*., 2014 reported that liquid media of *S. nigrum* is better suited for in vitro propagation as compared to solid as it in short interval subculture it have high multiplication rate of was seen.

Ziv *et al*., has reported that in liquid media the shoot cultures maintain their genetic identity and easier mechanical separation. The automation can be achieved as compared to those grown on conventional agar based media.

Many recent studies have evaluated liquid systems are suitable for the proliferation of adventitious roots and shoots of *Hypericum perforatum* (Cui *et al*., 2010, Savio *et al*., 2012)

Also it has goof effect on shoots of *Catharanthus roseus* as found by Pati *et al*., 2011.

Gangopadhyay *et al*., 2001 reported that higher number of roots with root hairs was recorded in liquid medium with coir compared to conventional solid media.

Nokwanda *et al*., 2006 found that *Thapsia garganica* grown on MS were best rooted in ½MS liquid medium supplemented with IBA.

Uchendu *et al*., 2011 found North American ginseng from nodal explants used in the bioreactor was 2.5 mg/l of kinetin, 0.5 mg/ l of NAA, and 50 mg/l of activated charcoal in liquid culture medium gives the best output.

The *Rosa chinensis* shoots cultured in liquid medium were better than those cultured on two-phase medium or alone in solid medium (Chu *et al.,* 1993).

Drawbacks of liquid culture

- Liquid culture shows hyperhydricity (Ziv *et al*., 2000; Staden *et al*., 2011) that leads to undesired morphology of shoots.
- Shoots and leaves are translucent, succulent with excessive tissue hydration, show chlorophyll deficiency and exhibit fasciation (Ziv *et al*., 2004) possibly as a result of water availability and, to some extent, concentration and balance of auxins/cytokinins (Wu *et al*., 2009).
- It is limited by low oxygen content (Smith *et al*., 1995).

2.8 Activated charcoal

Charcoal is any form of carbon compounds that are known to have high adsorptive capacity for solids, gases and vapors. It is formed by the destructive distillation of woods, coal, bones, nut shells, organic materials like vegetable waste and other carbaneous compounds.

Activated charcoal helps in darkening the medium and to stimulate the soil conditions. In plant tissue culture it has been reported to improve various phenomenons like rooting of micro-propagated tissues, somatic embryogenesis, adventitious shoot production, shoot growth and organogenesis.

There are different forms of active carbon used for different purposes intends. Charcoals activated for the adsorption of gases are harder and denser than those used for purification of liquids. They contain small pores.

The effects of activated charcoal could be attributed to

(a) Providing a dark environment in the medium;

(b) Adsorption of certain inhibitory substances in culture, produced by either media or explants;

(c) Adsorption of plant growth regulators and other organic compounds

(d) Release of substances naturally present in or adsorbed by activated charcoal, which are beneficial to growth of *in vitro* culture.

Properties of activated charcoal

- The activated charcoal has a high effiency for moderately polar organics than a polar or highly polar organics.
- Its surface area is very large.
- Adsorption of unsaturated products is high like oleificnic compounds (Ernst *et al*., 1990)
- Due to these properties aromatic compounds like phenolic compounds, auxins, cytokinins and hormones has high adsorption affinity for AC.
- Polar substances like glucose, sucrose etc does not show adsoption affinity for AC (Pan *et al*., 1998; Staden 1998).

Use of charcoal in *in vitro* culture

- Charcoal provides darkness in the *in vitro* culture. The properties of light have affect on tissue cultures, and influences their growth and development.
- Adsorption of undesirable or inhibitory substances in *in vitro* cultures
- During the procedure of transfer of explants to fresh medium the increase in number of subcultures can results in mutations of cells and may cause loss of the ability of the affected cells to undergo embryogenesis. Incorporation of charcoal cultures can alleviate this problem.
- Activated charcoal prevented discoloration by adsorbing phenolics and rendered polyphenol oxidase and peroxidase inactive
- Adsorption of plant growth regulators in *in vitro* culture
- Activated charcoal is able to adsorb high concentrations of the growth regulators BA, IAA, IBA, NAA and Kinetin (Weatherhead *et al*.1978) in both liquid and solid media (Nissen *et al*., 1990).

The inclusion of AC in the growth medium has also had detrimental effects, such as the inhibition of root formation and the adsorption of various medium components (George *et al*., 1984; Zaghmout *et al*., 1988; Pullman *et al*., 2002)

AC promotes somatic embryogenesis, androgenesis, rooting, stems elongation, and bulb formation, but inhibits browning (Mohamed-Yasseen *et al*., 1994)

Sharma *et al*., 2005 found that rooting of shoots (87.6%) with maximum fasciculate roots occurred on kop medium containing iron and vitamins of MS medium with IBA and AC.

Addition of 300 mg/l AC with 0.3 mg/l NAA and 0.2 mg/l IBA lead to increase in rooting of the plant *Begonia fimbristipula* as found by Chen *et al*., 2012.

4. Methodology

The experiments performed in this study of plant tissue culture are carried out in the Plant Tissue Culture Laboratory in the Department of Biotechnology, Delhi Technological University. The procedures and methodologies followed to conduct this study are presented below.

4.1 Glassware

For this study borosilicate glassware are preferred since they are more stable and they are less soluble in alkali. Before use they must be autoclaved for 20 minutes at 121°C at pressure of 15 lbs/square inch.

4.2 Chemicals

Chemicals used for preparing MS media with certain growth hormones such as BAP, 2-4, D, NAA, IBA and IAA.

To sterilization and disinfecting plant materials, glassware and certain tools ethanol and mercuric chloride were used.

Bevistin and streptomycin were used as antibiotics.

Activated charcoal is used as supplement.

4.3 Plant material

Three different excisions of *Chlorophytum borivilianum* were used for micropropagation taken from the tissue culture laboratory.

Explant:-

Plant materials used for in vitro propagation of the plant are leave and shoot base. Shoot base was found to give the best result for micropropagation.

4.4 Media formulation

Murashige and Skoog (MS) medium is used for the micropropagation of *Chlorophytum borivilianum* with composition given below**.** Stock solutions of MS basal medium and plant growth regulator (PGRs).

The media used in micropropagation consists of micro and macronutrients, aminoacids, sugars (mainly sucrose) and vitamins.

Stock solutions of the major and minor salts were prepared. MS media were prepared by mixing the standard concentration of these salts**.**

4.4.1 Materials:

- Glass beakers
- Measuring cylinders
- disposable syringe filter
- BAP
- NAA
- Agar = 8grams
- Sucrose= 3%
- pH - 5.7 was maintained.

4.5 Culture establishment

4.5.1 Sterilization

The first step taken in tissue culture technique is to sterilize the explants. First the explants were washed in tap water for 3-4 times in order to remove contaminants. The explants were then soaked under detergent tween-20. Fungicide like bavistin was also used as disinfectant.

Then explants were soaked in 70 % alcohol for 5 minutes and surface sterilization was done with 0.1% mercuric chloride for 5 minutes. And finally these explants were washed with double distilled water for 3-4 times before inoculation.

The other materials used for micropropagation were also sterilized with autoclave like forceps, scalpels, glassware etc. Sterilized blades were used for cutting the plants.

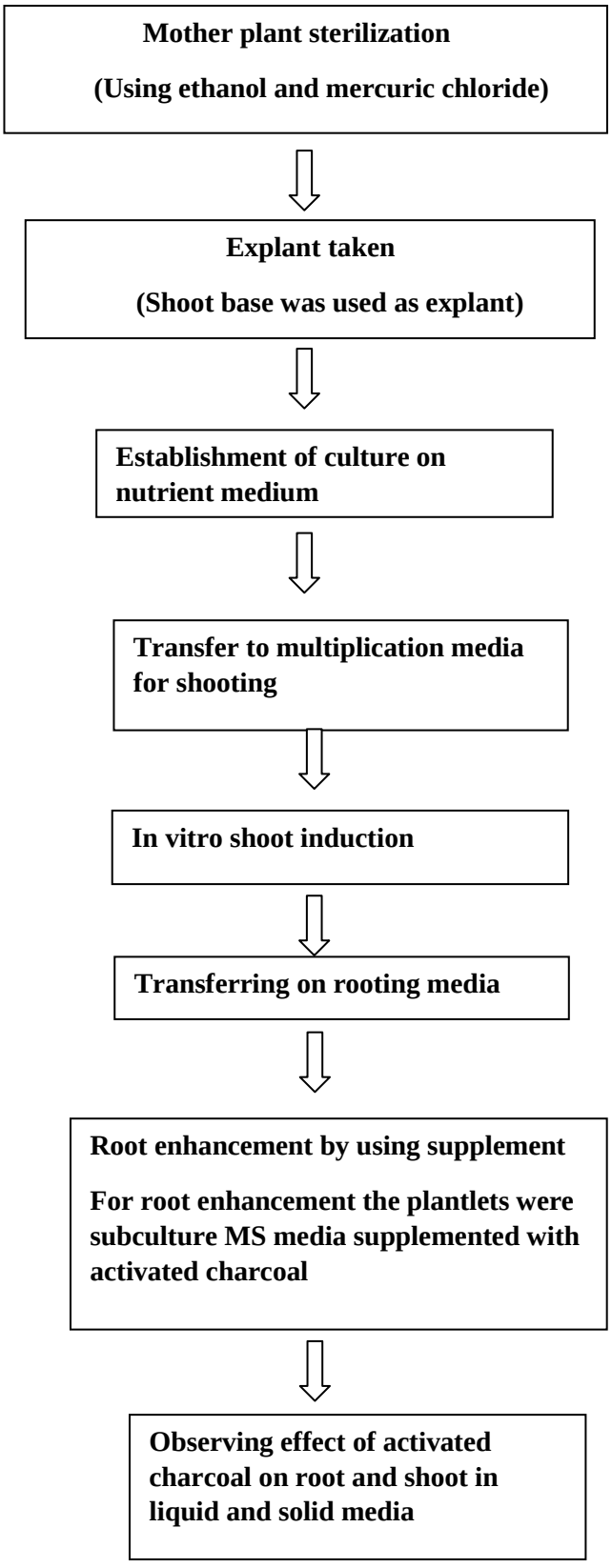
4.5.2 Inoculation

Culture establishment was done in laminar flow for maintaining the aseptic conditions. Explants were taken and inoculated in the MS media. Explants in *Chlorophytum borivilianum* are the shoot base. They should be taken carefully.

The cultures were incubated in an air conditioned room at a temperature of 25±2°C. The photoperiods for the plant should be maintained according to the ratio 16:8 light and dark periods respectively.

Finally the plant growth was measured.

Representation of root enhancement by culturing *Chlorophytum borivilianum* in solid and liquid media



4.6 Experiment design

3.6.1 Experiment 1:- To determine the effect of solid and liquid MS media containing BAP (with AC) on number of shoots and shoot length in 5 weeks

Single shoots of about 0.7-1 cm in length were cultured on full strength MS media containing 3% sucrose and 2% agar. The media is supplemented with 3 different concentrations of BAP.

Concentration of AC used was 250 mg/l in all media.

Table 1

Treatments	Solid (A1)	Liquid (A2)
Aa1	MS+ 1 mg/l BAP	MS+ 1 mg/l BAP
Aa2	MS+ 2 mg/l BAP	MS+ 2 mg/l BAP
Aa3	MS+ 3 mg/l BAP	MS+ 3 mg/l BAP

4.6.2 Experiment 2:- To determine the effect of solid and liquid MS media containing kinetin (with AC) on number of shoots and shoots length in 5 weeks.

Single shoots of about 0.7-1 cm in length were cultured on full strength MS media containing 3% sucrose and 2% agar. The media is supplemented with 3 different concentrations of kinetin.

Concentration of AC used was 250 mg/l in all media.

Table 2

Treatments	Solid (B1)	Liquid (B2)
Bb1	MS+ 1.5 mg/l Kinetin	MS+ 1.5 mg/l Kinetin
Bb2	MS+ 2 mg/l Kinetin	MS+ 2 mg/l Kinetin
Bb3	MS+ 2.5 mg/l Kinetin	MS+ 2.5 mg/l Kinetin

4.6.3 Experiment 3- To determine the effect of solid and liquid MS media containing IAA and NAA (with AC) on number of roots and root length in 6 weeks.

Single shoots of about 1.5-2 cm in length were cultured on full strength MS media containing 3% sucrose and 2% agar. The media is supplemented with 3 different concentrations of NAA and IAA.

Concentration of AC used was 250 mg/l in all media.

Table 3

Treatment	Solid (C1)	Liquid (C2)
Cc1	0.8 mg/l IAA + 1 mg/l IAA	0.8 mg/l NAA + 1 mg/l IAA
Cc2	1 mg/l IAA + 0.8 mg/l IAA	1 mg/l NAA +0.8 mg/l IAA
Cc3	1.2 mg/l NAA + 1.2 mg/l IAA	1.2 mg/l NAA + 1.2 mg/l IAA

4.6.4 Experiment 4:- To determine the effect of solid MS media containing BAP and kinetin (without AC) on number of shoots and shoot length in 5 weeks

Single shoots of about 0.7-1 cm in length were cultured on full strength MS media containing 3% sucrose and 2% agar. The media is supplemented with 3 different concentrations of BAP.

Table 1

Treatments	Solid (D1)
Dd1	MS+ 1 mg/l BAP
Dd2	MS+ 2 mg/l BAP
Dd3	MS+ 3 mg/l BAP
Dd4	MS+ 1.5 mg/l Kinetin
Dd5	MS+ 2 mg/l Kinetin
Dd6	MS+ 2.5 mg/l Kinetin

4.6.5 Experiment 5 - To determine the effect of solid MS media containing IAA and NAA (without AC) on number of roots and root length in 6 weeks.

Single shoots of about 1.5-2 cm in length were cultured on full strength MS media containing 3% sucrose and 2% agar. The media is supplemented with 3 different concentrations of NAA and IAA.

Table 5

Treatment	Solid (C1)
Ee1	0.8 mg/l IAA + 1 mg/l NAA
Ee2	1 mg/l IAA + 1 mg/l NAA
Ee3	1mg/l IAA + 1.2 mg/l NAA
Ee4	1.2 mg/l NAA + 1.2 mg/l NAA

4.6.7 TWO WAY ANOVA- STATISTICAL ANALYSIS

Factorial completely randomized design was used for analysis. SPSS software version 19 was used to subject the data to ANOVA.

5. Results

5.1 To determine the effect of solid and liquid MS media containing BAP (with AC) on number of shoots and shoot length in 5 weeks

Table 6

Treatment	Shoot length mean	Shoot number mean
Aa11	6.17	3.66
Aa12	4.12	5.00
Aa13	7.73	6.00
Aa21	4.47	12.66
Aa22	3.53	12.66
Aa23	4.53	10.66

5.1.1 Statistical analysis of interaction of shoot length with BAP (PGRS).

Between-Subjects Factors

		N
TREATMENT	A1	9
	A2	9
PGRS	1.00	6
	2.00	6
	3.00	6

Tests of Between-Subjects Effects

Dependent Variable:AC

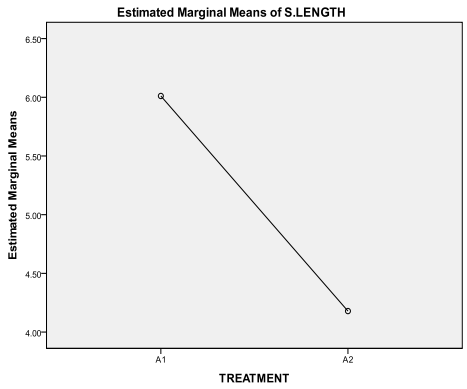
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	36.677[a]	5	7.335	5.015	.010
Intercept	467.262	1	467.262	319.425	.000
TREATMENT	15.107	1	15.107	10.327	.007
PGRS	16.422	2	8.211	5.613	.019
TREATMENT * PGRS	5.148	2	2.574	1.760	.214
Error	17.554	12	1.463		
Total	521.493	18			
Corrected Total	54.231	17			

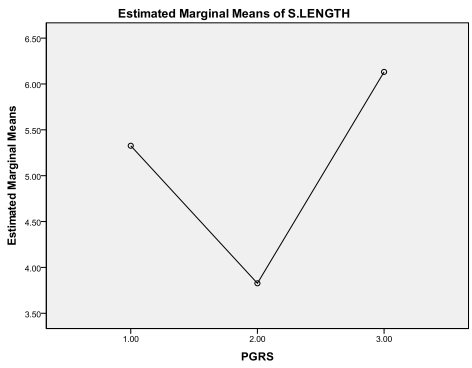
Table 7 R Squared = .676 (Adjusted R Squared = .541)

Statistics

			Statistic	Bootstrap[a] Bias	Std. Error	95% Confidence Interval Lower	Upper
N	Valid	SHOOT	18	0	0	18	18
		PGRS	18	0	0	18	18
	Missing	SHOOT	0	0	0	0	0
		PGRS	0	0	0	0	0
Mean		SHOOT	5.0950	.0208	.4033	4.3750	5.9133
		PGRS	2.0000	.0053	.1898	1.6111	2.3333
Std. Error of Mean		SHOOT	.42098				
		PGRS	.19803				

Table 7 Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples





5.1.2 Statistical analysis of interaction of shoot number with BAP (PGRS).

Between-Subjects Factors

		N
TREATMENT	B1	9
	B2	9
PGRS	1.00	6
	2.00	6
	3.00	6

Tests of Between-Subjects Effects

Dependent Variable:SHOOTS

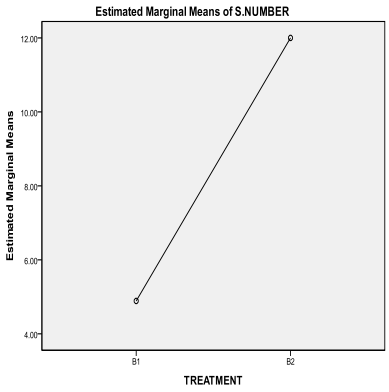
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	243.778[a]	5	48.756	5.015	.010
Intercept	1283.556	1	1283.556	132.023	.000
TREATMENT	227.556	1	227.556	23.406	.000
PGRS	1.444	2	.722	.074	.929
TREATMENT * PGRS	14.778	2	7.389	.760	.489
Error	116.667	12	9.722		
Total	1644.000	18			
Corrected Total	360.444	17			

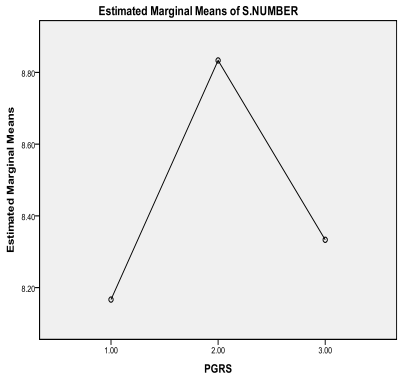
Table 7 R Squared = .676 (Adjusted R Squared = .541)

Statistics

				Bootstrap[a]			
						95% Confidence Interval	
			Statistic	Bias	Std. Error	Lower	Upper
N	Valid	SHOOTS	18	0	0	18	18
		PGRS	18	0	0	18	18
	Missing	SHOOTS	0	0	0	0	0
		PGRS	0	0	0	0	0
Mean		SHOOTS	8.4444	-.0129	1.0151	6.4459	10.4986
		PGRS	2.0000	.0031	.1999	1.6111	2.3889
Std. Error of Mean		SHOOTS	1.08532				
		PGRS	.19803				

a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples


Estimated Marginal Means of S.NUMBER
Estimated Marginal Means
12.00
10.00
8.00
6.00
4.00
B1
B2
TREATMENT


Estimated Marginal Means of S.NUMBER
Estimated Marginal Means
8.80
8.60
8.40
8.20
1.00
2.00
3.00
PGRS

5.2 To determine the effect of solid and liquid MS media containing kinetin (with AC) on number of shoots and shoots length in 5 weeks

Table 7

Treatment	Shoot length mean	Shoot number mean
Bb11	0.33	0.33
Bb12	0.66	0.66
Bb13	1.92	2.00
Bb21	2.66	6.33
Bb22	4.41	7.00
Bb23	1.12	5.00

5.2.1 Statistical analysis of interaction of shoot number with kinetin.

Between-Subjects Factors

		N
TREATMENT	B1	9
	B2	9
PGRS	1.00	6
	2.00	6
	3.00	6

Tests of Between-Subjects Effects

Dependent Variable:SHOOTS

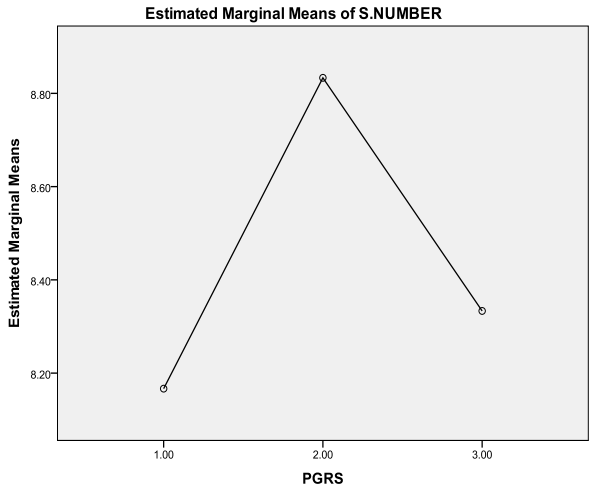
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	243.778[a]	5	48.756	5.015	.010
Intercept	1283.556	1	1283.556	132.023	.000
TREATMENT	227.556	1	227.556	23.406	.000
PGRS	1.444	2	.722	.074	.929
TREATMENT * PGRS	14.778	2	7.389	.760	.489
Error	116.667	12	9.722		
Total	1644.000	18			
Corrected Total	360.444	17			

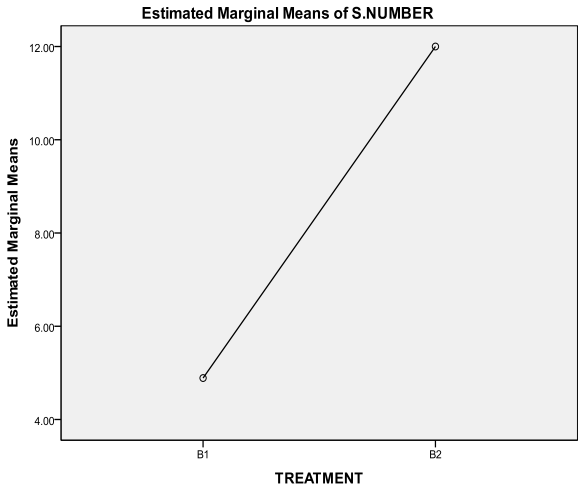
a. R Squared = .676 (Adjusted R Squared = .541)

Statistics

				Bootstrap[a]			
						95% Confidence Interval	
			Statistic	Bias	Std. Error	Lower	Upper
N	Valid	SHOOTS	18	0	0	18	18
		PGRS	18	0	0	18	18
	Missing	SHOOTS	0	0	0	0	0
		PGRS	0	0	0	0	0
Mean		SHOOTS	8.4444	-.0129	1.0151	6.4459	10.4986
		PGRS	2.0000	.0031	.1999	1.6111	2.3889
Std. Error of Mean		SHOOTS	1.08532				
		PGRS	.19803				

a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples





5.2.2 Statistical analysis of interaction of shoot length with kinetin.

Between-Subjects Factors

		N
TREATMENT	B1	9
	B2	9
KINETIN	1.50	6
	2.00	6
	2.50	6

Tests of Between-Subjects Effects

Dependent Variable:S.LENGTH

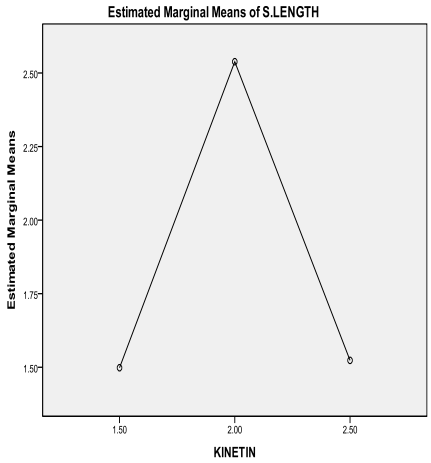
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	34.363[a]	5	6.873	4.487	.015
Intercept	61.827	1	61.827	40.370	.000
TREATMENT	13.869	1	13.869	9.056	.011
KINETIN	4.225	2	2.112	1.379	.289
TREATMENT * KINETIN	16.269	2	8.135	5.311	.022
Error	18.378	12	1.532		
Total	114.569	18			
Corrected Total	52.741	17			

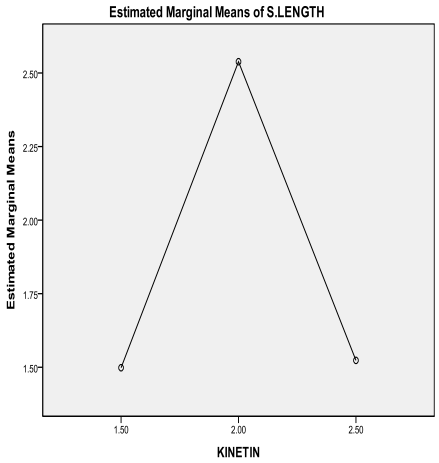
a. R Squared = .652 (Adjusted R Squared = .506)

Statistics

				Bootstrap[a]			
						95% Confidence Interval	
			Statistic	Bias	Std. Error	Lower	Upper
N	Valid	KINETIN	18	0	0	18	18
		S.LENGTH	18	0	0	18	18
		TREATMENT	18	0	0	18	18
	Missing	KINETIN	0	0	0	0	0
		S.LENGTH	0	0	0	0	0
		TREATMENT	0	0	0	0	0
Mean		KINETIN	2.0000	-.0041	.0973	1.8056	2.1944
		S.LENGTH	1.8533	-.0035	.3918	1.1990	2.6866
Std. Error of Mean		KINETIN	.09901				
		S.LENGTH	.41516				

a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples





5.3 To determine the effect of solid and liquid MS media containing IAA and NAA (with AC) on number roots and roots length in 6 weeks

Table 8

Treatments	Root number mean	Root length mean
Cc11	1.33	1.33
Cc12	1.00	1.66
Cc13	1.66	4.66
Cc21	16.66	8.00
Cc22	16.66	18.00
Cc23	12.44	18.33

5.3.1 Statistical analysis of interaction of root number with IAA and NAA

Between-Subjects Factors

		N
IAA	.80	6
	1.00	6
	1.20	6
NAA	.80	7
	1.00	5
	1.20	6

Tests of Between-Subjects Effects

Dependent Variable:R.NUMBER

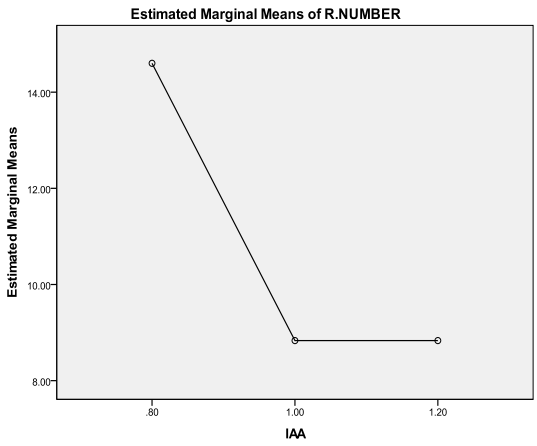
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	235.311[a]	3	78.437	1.141	.367
Intercept	1608.861	1	1608.861	23.402	.000
IAA	172.024	1	172.024	2.502	.136
NAA	235.200	1	235.200	3.421	.086
IAA * NAA	.000	0	.	.	.
Error	962.467	14	68.748		
Total	2620.000	18			
Corrected Total	1197.778	17			

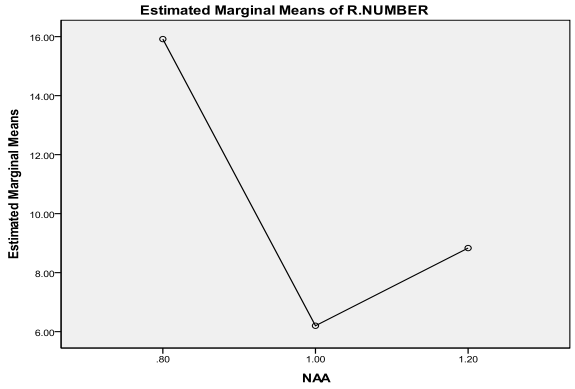
a. R Squared = .196 (Adjusted R Squared = .024)

Statistics

				Bootstrap[a]			
						95% Confidence Interval	
			Statistic	Bias	Std. Error	Lower	Upper
N	Valid	R.NUMBER	18	0	0	18	18
		IAA	18	0	0	18	18
		NAA	18	0	0	18	18
	Missing	R.NUMBER	0	0	0	0	0
		IAA	0	0	0	0	0
		NAA	0	0	0	0	0
Mean		R.NUMBER	8.8889	-.1017	1.9284	4.8347	12.7764
		IAA	1.0000	.0011	.0380	.9222	1.0667
		NAA	.9889	.0004	.0395	.9111	1.0667
Std. Error of Mean		R.NUMBER	1.97846				
		IAA	.03961				
		NAA	.04113				

a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples





5.3.2 Statistical analysis of interaction of root length with IAA, NAA and treatment

Between-Subjects Factors

		N
IAA	.80	6
	1.00	6
	1.20	6
NAA	.80	7
	1.00	5
	1.20	6

Tests of Between-Subjects Effects

Dependent Variable:R.LENGTH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	136.119[a]	3	45.373	.630	.607
Intercept	1094.745	1	1094.745	15.206	.002
IAA	50.820	1	50.820	.706	.415
NAA	110.170	1	110.170	1.530	.236
IAA * NAA	.000	0	.	.	.
Error	1007.932	14	71.995		
Total	2200.672	18			
Corrected Total	1144.052	17			

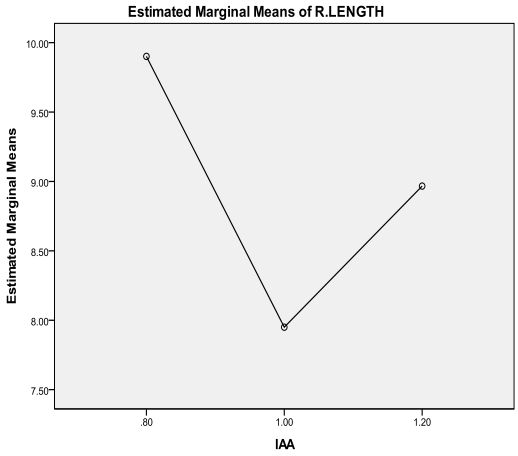
a. R Squared = .119 (Adjusted R Squared = -.070)

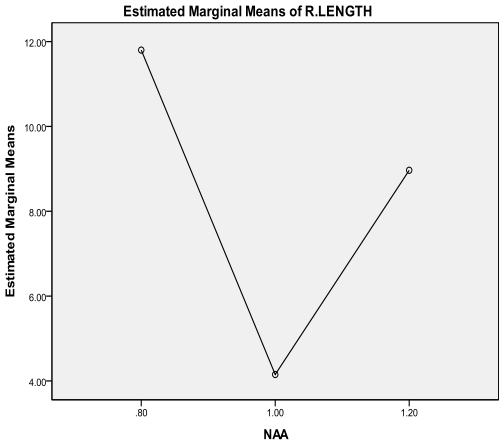
Tests of Between-Subjects Effects

Dependent Variable:R.LENGTH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1089.278[a]	6	181.546	18.406	.000
Intercept	1473.356	1	1473.356	149.372	.000
IAA	30.083	1	30.083	3.050	.109
NAA	60.167	1	60.167	6.100	.031
TREATMENT	820.628	1	820.628	83.197	.000
IAA * NAA	.000	0	.	.	.
IAA * TREATMENT	.000	0	.	.	.
NAA * TREATMENT	.000	0	.	.	.
IAA * NAA * TREATMENT	.000	0	.	.	.
Error	108.500	11	9.864		
Total	2620.000	18			
Corrected Total	1197.778	17			

a. R Squared = .909 (Adjusted R Squared = .860)





5.4 Response on interactions of cytokinins concentrations on shoot growth without activated charcoal in 5 weeks

Table 9

Treatment	Number of shoots per explant 3 weeks	Mean Shoot length in 3 weeks (in cm)	Number of shoots per explant 6 weeks	Mean Shoot length in 6 weeks (in cm)
Dd1	6	Highest length- 3.1	7	Highest length- 4.2
Dd2	8	Highest length -4.2	9	Highest length -4.9
Dd3	9	Highest length -7.7	11	Highest length - 9.9
Dd4	4	Highest length -2.7	5	Highest length - 8.1
Dd5	6	Highest length -3.5	8	Highest length- 11.0
Dd6	7	Highest length -4.0	7	Highest length -5.6

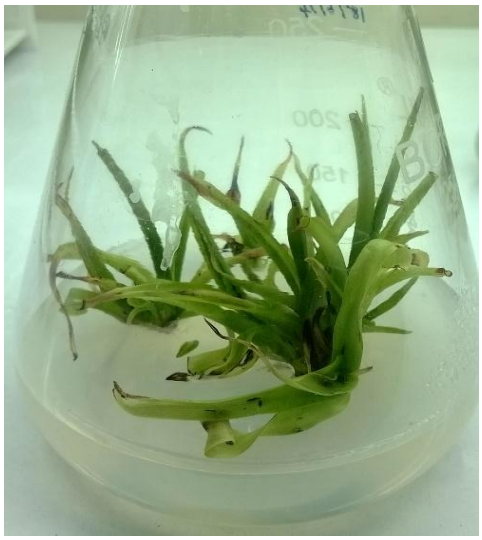
5.5 Response of auxins on root growth on MS media without activated charcoal in 6 weeks.

Table 10

Treatment	Mean Number of roots per explant after 3 weeks	Length of roots per explant after 3 weeks (in cm)	Number of roots per explant 6 weeks	Mean Length of roots per explant after 6 weeks (in cm)
Ee1	8	Highest length - 0.7	23	Highest length - 3.5
Ee2	12	Highest length - 0.3	33	Highest length - 5.5
Ee3	5	Highest length - 0.3	26	Highest length - 3.6
Ee3	9	Highest length - 0.5	29	Highest length - 4.9

Figures

Fig1. Shooting in MS media containing BAP in 3 weeks



Showing result with 3 mg/l BAP

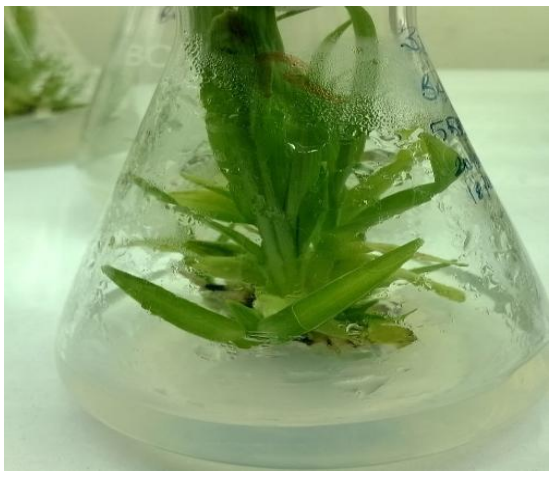
Fig2. Shooting in MS media containing BAP in 5 weeks:-

Showing result with 3 mg/l BAP

Fig3. Shooting in MS media containing kinetin in 3 weeks

Showing result with 2 mg/l kinetin

Fig4. Shooting in MS media containing kinetin in 5 weeks



Showing result with 2 mg/l kinetin

Fig5. Rooting in MS containing IAA and NAA in 4 weeks

Showing result with 1 mg/l IAA and 1 mg/l NAA.

Fig6. Rooting in MS media containing IAA and NAA in 6 weeks

Showing result with 1 mg/l IAA and 1 mg/l NAA.

Fig7. Shooting MS media containing BAP with activated charcoal in 3 weeks

Showing result with 2 mg/l BAP

Fig8. Shooting MS media containing BAP with activated charcoal in 5 weeks

Showing result with 2 mg/l BAP

Fig9. Rooting MS media containing IAA and NAA with Activated charcoal in 4 weeks

Showing result with 1 mg/l IAA and 0.8 mg/l NAA

Fig10. Rooting MS media containing IAA and NAA with Activated charcoal in 6 weeks

Showing result with 1 mg/l IAA and 0.8 mg/l NAA

Fig11. Liquid MS media containing IAA and NAA with activated charcoal in 3 weeks

Showing result with 1 mg/l IAA and 0.8 mg/l NAA

Fig12. Liquid MS media containing IAA and NAA with activated charcoal in 6 weeks

Showing result with 1 mg/l IAA and 0.8 mg/l NAA

Discussion and future prospective

The conventional vegetative propagation of *Chlorophytum borivilianum* takes about 8-10 months for the multiplication. This is due to the longer dormancy period of the roots and low seed viability. Introduction of in vitro multiplication of enhance the rate of multiplication of the plant and also reduce the cost of production. It also gives a better yield in short interval of time. Through micropropagation the multiplication rates of the species can be increase in 3 weeks up to 2.5 to 3 folds and 4 folds in 4 weeks.

Addition of 300 mg/l AC with 0.3 mg/l NAA and 0.2 mg/l IBA led to increase in rooting of the plant *Begonia fimbristipula* as found by Chen *et al*., 2012.

5.1 Interaction of BAP and kinetin for shoot induction in solid and liquid media with AC.

Interaction of BAP in solid and liquid media with AC was analyzed. It was found that that BAP has a significant difference for solid and liquid media. Shoot length in solid significantly differ from that of liquid media. Better shoot length was obtained in liquid media. Highest shoot length was found to be 4.53 cm. However shoot number was not effected with the concentration of BAP. Only it was significantly differ in liquid media and highest were 12.66.

Kinetin was also analyzed in both liquid and solid media. It was analyzed that kinetin concentration didn't affect the shoot number. But it differs significantly in both the media. Shoot length was affected by both the concentration of kinetin and media. Best shoot length obtained was 4.11 and best number of shoot obtained was 7.

5.2 Interaction of combination of NAA and IAA for root induction in solid and liquid media with AC.

The combination of both IAA and NAA was used for checking the effect on rooting. The addition of auxins enhances the root formation from the shoot base. The best result for rooting was with 1 mg/l NAA and 1 mg/l IAA. Kemat *et al.,* 2003 and Quraishi *et al.,* 2004 used IAA and NAA separately to induce rooting. The combination of IAA and NAA induce rooting in comparatively less time.

Interaction of IAA and NAA were analyzed with solid and liquid media. Liquid media differs significantly in response to the root length with solid media. Highest root length was obtained in 1 mg/l IAA and 0.8 mg/l NAA with activated charcoal in liquid media.

5.3 Response of BAP for shoot induction in solid media without AC.

For the rapid multiplication of *Chlorophytum borivilianum* different cytokinins effect on media were analyzed. The plantlets in basal MS media without any growth regulators were found to have retarded growth and long multiplication time. The addition of cytokinins has enhanced the growth rate in a small interval of time.

Numbers of days taken for initial sprout are different with respect to different growth regulators. In case of BAP, growth is fast as compared to the kinetin. The number of days taken for initial sprouting in BAP is 14 days and in case of kinetin it is 21 days.

The number of days of sprouting decrease with the increase in concentration of BAP while it increase in case of kinetin.

Dd3 i.e. 3 mg/l BAP gave the best result in number of shoots and Dd5 i.e. 2 mg/l kinetin gave best result in shoot length.

The best result for shooting came out from 3% BAP similar to as found by Dave *et al.,* 2003. BAP has also reduced the time for multiplication of the plant.

The highest shoot length was observed with kinetin as 11cm with the concentration of 2 mg/l. Kinetin increase the shoot length as shown by Ashraf *et al.,* 2014.

5.5 Response kinetin for shoot induction in solid media without AC

Days for root initiation were different from different concentration of root growth regulators. Different compositions of IAA and NAA are examined to determine the best result for rooting media. The best result came from the composition Ee2 i.e. 1 mg/l IAA and 1 mg/l NAA. Rooting in the media was initiated after 3 weeks in the media.

Future prospective:-

Activated charcoal has so far given appreciable result for induction of roots in liquid culture. Other supplements can also be used in order to enhance roots. Liquid medium has many disadvantages which should be taken into consideration.

5.5 Rooting in solid MS media containing combination of IAA and NAA without AC

Liquid MS media with IAA and NAA was also supplemented with 250 mg/l AC. All the media compositions gave better result for rooting the best result found in 0.8 mg/l IAA and 1 mg/l NAA with AC. The number of roots enhances both in number and length. Time interval for root induction was reduced in case of liquid media as compared to solid media.

Same compositions of growth hormones were added in both solid and liquid media having activated charcoal. Liquid media with AC gives the best result for rooting as compared to all other media compositions so far studied.

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