# *IN VITRO* REGENERATION OF *Aloe vera* L. IN A NEW PLANT TISSUE CULTURE MEDIUM

**CHHANDA SARKER**

**DEPARTMENT OF BIOTECHNOLOGY**

**SHER-E-BANGLA AGRICULTURAL UNIVERSITY**

**DHAKA-1207, BANGLADESH**

**JUNE, 2022**

# *IN VITRO* REGENERATION OF *Aloe vera* L. IN A NEW PLANT TISSUE CULTURE MEDIUM

**BY**

**CHHANDA SARKER**

**REGISTRATION NO. 15-06680**

Mobile No. : 01704830608

Email: chhandasarker235@gmail.com

A thesis Paper

Submitted to the faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of

**MASTER OF SCIENCE**

**IN**

**BIOTECHNOLOGY**

**SEMESTER: JANUARY-JUNE, 2022**

**Approved by:**

……

**Dr. Md. Ekramul Hoque**
**Professor**
Department of Biotechnology,
Sher-e-Bangla Agricultural University, Dhaka
**Supervisor**

……

**Dr. Homayra Huq**
**Professor**
Department of Biotechnology
Sher-e-Bangla Agricultural University, Dhaka
**Co-supervisor**

**…….....**

**Dr. Md. Rafiqul Islam**
**Associate professor**
Department of Biotechnology, SAU
**Chairman**
**Examination Committee**

**DEPARTMENT OF BIOTECHNOLOGY**
Sher-e-Bangla Agricultural University (SAU)
Sher-e-Bangla Nagar, Dhaka-1207

# CERTIFICATE

This is to certify that thesis paper entitled "**REGENERATION OF *Aloe vera* L. IN A NEW PLANT TISSUE CULTURE MEDIUM**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN BIOTECHNOLOGY**, embodies the result of a piece of bona fide research work carried out by **CHHANDA SARKER** Registration no**. 15-06680** under my supervision and guidance. No part of the dissertation has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

**Dated: June, 2022**
**Place: Dhaka, Bangladesh**

**Dr. Md. Ekramul Hoque**
**Professor**
Department of Biotechnology
SAU, Dhaka
**Supervisor**


DEDICATED
TO
MY BELOVED PARENTS

# ACKNOWLEDGEMENT

*All the praises due to the Almighty, the cherisher and sustainer of the world. His blessings have enabled the author to complete her thesis paper leading to MS in Biotechnology degree.*

*The author expresses her heartiest gratitude sincere appreciation, indebtedness and deep sense of respect to her adorable teacher, venerable Supervisor* ***Professor Dr. Md. Ekramul Hoque****, Department of Biotechnology, Sher-e-Bangla Agricultural University for his planning, painstaking and scholastic guidance, support, extraordinary kind concern, everlasting encouragement, inestimable cooperation and intellectual encircling the till final preparation of the thesis.*

*She expresses her profuse gratitude, cordial appreciation and gratefulness to her thoughtful, co-supervisor* ***Dr. Homayra Huq****, Professor, Department of Biotechnology, Sher-e-Bangla Agricultural University, for her valuable suggestions, guidance constant encouragement and inestimable during the entire period of study.*

*With due regards, she thanks to the honorable Chairman* ***Dr. Md. Rafiqul Islam****, Department of Biotechnology, Sher-e-Bangla Agricultural University, for the facilities provided, in carrying out this work. She also acknowledges with deep regards the help and cooperation received from her respected teachers and staff of the Department of Biotechnology, Sher-e-Bangla Agricultural University while carrying out this work.*

*She is really indebted to the esteemed staff members for their co-operation including tissue culture laboratory of Department of Biotechnology, Sher-e-Bangla Agricultural University for their friendly relationship, friendly gesture and for their help directly or indirectly regarding the work.*

*The author is also deeply indebted to the Ministry of Science and Technology, Government of the People's Republic of the Bangladesh for financial support to complete this study by giving NST- fellowship.*

*She expresses her heartiest gratitude sincere appreciation, indebtedness and deep sense of respect to her parents for their sincere and affectionate support and love, extraordinary kind concern, everlasting encouragement and inestimable cooperation during the entire period of study.*

***The Author***

# ABBREVIATIONS AND ACRONYMS

| | | |
|---|---|---|
| Agril. | : | Agriculture |
| Biol. | : | Biological |
| cm | : | Centimeter |
| CRD | : | Completely Randomized Design |
| DMRT | : | Duncan's Multiple Range Test |
| Conc. | : | Concentration |
| DAI | : | Days After Inoculation |
| *et al.* | : | And others (at elli) |
| FAO | : | Food and Agricultural Organization |
| IASC | : | International Aloe Science Council |
| g/L | : | Gram per litre |
| BAP | : | 6- Benzyl Amino Purine |
| BA | : | Benzyladenine |
| KIN | : | Kinetine |
| IAA | : | Indole acetic acid |
| IBA | : | Indole butyric acid |
| NAA | : | *a*- Napthalene acetic acid |
| 2, 4-D | : | 2,4- Dichlorophenoxy acetic acid |
| Int. | : | International |
| 2-ip | : | 2-isopentenyladenine |
| J. | : | Journal |
| Mol. | : | Molecular |
| mg/L | : | Milligram per litre |
| µM | : | Micromole |
| MS | : | Murashige and Skoog |
| PGRs | : | Plant Growth Regulators |
| Res. | : | Research |
| Sci. | : | Science |
| TDZ | : | Thidiazuron |
| PVP | : | Polyvinylpyrrolidone |
| PLB | : | Protocorm-like bodies |
| CV | : | Co-efficient of Variation |
| $^{o}$C | : | Degree Celsius |
| etc. | : | Etcetera |
| WAI | : | Weeks After Inoculation |

# TABLE OF CONTENTS

CERTIFICATE ..... iii
ACKNOWLEDGEMENT ..... v
ABBREVIATIONS AND ACRONYMS ..... vi
ABSTRACT ..... xii
**CHAPTER I**
**INTRODUCTION ..... 1**
**CHAPTER II**
**REVIEW OF LITERATURE ..... 6**
2.1. Explant: ..... 6
2.2. Sterilization/Decontamination: ..... 9
2.3. Effect of growth regulators ..... 11
**CHAPTER III**
**MATERIALS AND METHODS ..... 19**
3.1. Time and location of the experiment ..... 19
3.2. Experimental materials ..... 19
3.2.1. Source of material ..... 19
3.2.2. Plant materials ..... 19
3.2.3. Instruments ..... 20
3.2.4. Glass ware ..... 20
3.2.5. Culture medium ..... 21
3.2.6. Stock solution for MS (1962) Medium ..... 22
3.2.7. Preparation of stock solution I for SAU Tissue Culture Medium ..... 23
3.2.8. Preparation of stock solution II ..... 24
3.2.9. Preparation of stock solution III ..... 25
3.2.10. Preparation of stock solution IV ..... 25
3.2.11. Preparation of stock solution V ..... 25
3.2.12. Solutions for pH adjustment ..... 26
3.2.13. Disinfection of culture room and laminar airflow ..... 26
3.2.14. Experiment ..... 27
3.2.15. Sterilization of the explants ..... 30
3.2.16. Inoculation of culture ..... 31
3.2.17. Incubation ..... 32
3.2.18. Acclimatization ..... 33

3.2.19. Data compilation ..... 33
3.2.20. Statistical analysis ..... 34
**CHAPTER IV**
**RESULT AND DISCUSSION ..... 35**
4.1. Sub experiment 1. *In vitro* culture of *Aloe vera* in different tissue culture media ..... 35
4.1.1. Shoot initiation and percent of shoot regeneration ..... 35
4.1.2. Number of shoots per explant ..... 37
4.1.3. Number of leaves per explant ..... 40
4.1.4. Length of shoot (cm) per explant ..... 42
4.1.5. Days to root initiation ..... 45
4.1.6. Length of root (cm) ..... 47
4.2. Sub experiment-2. *In vitro* culture of *Aloe vera* in different tissue culture media supplemented with phytohormone ..... 50
4.2.1. Shoot initiation and percent of shoot regeneration ..... 50
4.2.2. Number of shoots per explant ..... 52
4.2.3. Number of leaves per explant ..... 54
4.2.4. Length of shoot (cm) per explant ..... 56
4.2.5. Days to root initiation of each explant ..... 59
4.2.6. Length of root (cm) ..... 60
4.3. Acclimatization of plantlets ..... 63
**CHAPTER V**
**SUMMARY AND CONCLUSION ..... 67**
**RECOMMENDATIONS ..... 70**
**REFERENCES ..... 71**
**APPENDICES ..... 78**
Appendix I. ..... 78
Appendix II. ..... 79
Sub experiment-1 ..... 79
a. Analysis of variance on days to shoot initiation ..... 79
b. Analysis of variance on number of shoots at 21 days after initiation ..... 79
c. Analysis of variance on number of shoots at 28 days after initiation ..... 79
d. Analysis of variance on number of shoots at 35 days after initiation ..... 79
e. Analysis of variance on number of shoots at 42 days after initiation ..... 80
f. Analysis of variance on length of shoot at 21 days after initiation ..... 80
g. Analysis of variance on length of shoot at 28 days after initiation ..... 80

h. Analysis of variance on length of shoot at 35 days after initiation .....80
i. Analysis of variance on length of shoot at 42 days after initiation .....81
j. Analysis of variance on number of leaves at 21 days after initiation .....81
k. Analysis of variance on number of leaves at 28 Days after initiation.....81
l. Analysis of variance on number of leaves at 35 Days after initiation.....81
m. Analysis of variance on number of leaves at 42 Days after initiation .....82
n. Analysis of variance on days to root initiation.....82
o. Analysis of variance on length of root at 28 days after initiation .....82
p. Analysis of variance on length of root at 35 days after initiation .....82
Appendix III. .....83
Sub experiment-2 .....83
a. Analysis of variance on days to shoot initiation .....83
b. Analysis of variance on number of shoots at 21 days after initiation .....83
c. Analysis of variance on number of shoots at 28 days after initiation .....83
d. Analysis of variance on number of shoots at 35 days after initiation .....83
e. Analysis of variance on number of shoots at 42 days after initiation .....83
f. Analysis of variance on length of shoot at 21 days after initiation .....84
g. Analysis of variance on length of shoot at 28 days after initiation .....84
h. Analysis of variance on length of shoot at 35 days after initiation .....84
i. Analysis of variance on length of shoot at 42 days after initiation .....85
j. Analysis of variance on number of leaves at 21 days after initiation .....85
k. Analysis of variance on number of leaves at 28 Days after initiation.....85
l. Analysis of variance on number of leaves at 35 Days after initiation.....85

# LIST OF TABLES


| Table no. | Table title | Page no. |
|---|---|---|
| **Sub Experiment-1** | | |
| 1. | Potentiality of different tissue culture media (treatments) on shoot initiation and percent of regeneration in *Aloe vera* | 36 |
| 2. | Effect of different tissue culture media (treatments) on number of shoots per explant at different days after initiation in *Aloe vera* | 38 |
| 3. | Effect of different tissue culture media (treatments) on number of leaves per explant at different days after initiation in *Aloe vera* | 41 |
| 4. | Effect of different tissue culture media (treatments) on days to root initiation and percent of regeneration in *Aloe vera* | 45 |
| **Sub Experiment-2** | | |
| 5. | Potentiality of different tissue culture media (treatments) on shoot initiation and percent of regeneration in *Aloe vera* supplemented with phytohormone | 50 |
| 6. | Effect of different tissue culture media (treatments) on number of shoots per explant at different days after initiation in *Aloe vera* supplemented with phytohormone | 53 |
| 7. | Effect of different tissue culture media (treatments) on number of leaves per explant at different days after initiation in *Aloe vera* supplemented with phytohormone | 55 |
| 8. | Effect of different tissue culture media (treatments) on days to root initiation and percent of regeneration in *Aloe vera* supplemented with phytohormone | 60 |
| **Acclimatization** | | |
| 9. | Survival rate of *In vitro* regenerated plants of *Aloe vera* | 63 |


# LIST OF PLATES


| Plate no. | Plate title | Page no. |
|---|---|---|
| 1. | Preparation of different stock solution | 26 |
| 2. | Incubated culture vial in the growth chamber | 32 |
| **Sub Experiment-1** | | |
| 3. | Shoot initiation of *Aloe vera* at 14 Days in different treatments | 37 |
| 4. | Number of shoots of *Aloe vera* per explant at 42 days in different treatment | 39 |
| 5. | Number of leaves of *Aloe vera* at 28 days in different treatments | 42 |

| Plate no. | Plate title | Page no. |
|---|---|---|
| 6. | Length of shoot (cm) of *Aloe vera* at 42 days in different treatents | 44 |
| 7. | Highest length of root (cm) at 42 days in the SAU Tissue Culture Medium | 48 |
| **Sub Experiment-2** | | |
| 8. | Shoot initiation of *Aloe vera* at 14 Days in different treatments supplemented with phytohormone | 51 |
| 9. | Number of leaves of *Aloe vera* at 42 Days in different treatments supplemented with phytohormone | 56 |
| 10. | Length of shoot (cm) of *Aloe vera* at 42 days in different treatments supplemented with phytohormone | 55 |
| 11. | Highest length of root (cm) at 42 days in the SAU Tissue Culture Medium +1.00 mg/L KIN+2.00 mg/L NAA in *Aloe vera* | 62 |
| **Acclimatization** | | |
| 12. | Acclimatization of *Aloe vera* in the growth chamber (14 days) | 64 |
| 13. | Acclimatization of *Aloe vera* in the shade house (28 days) | 65 |
| 14. | Acclimatization of *Aloe vera* in field condition (45 days) | 66 |


# LIST OF FIGURES


| Figure no. | Figure title | Page no. |
|---|---|---|
| **Sub Experiment-1** | | |
| 1. | Effect of different tissue culture media on length of shoot (cm) at different days after initiation in *Aloe vera* | 43 |
| 2. | Effect of different tissue culture media on length of root (cm) at different days after root initiation in *Aloe vera* | 47 |
| **Sub Experiment-2** | | |
| 3. | Effect of different tissue culture media on length of shoot (cm) at different days after initiation in *Aloe vera* with hormone | 58 |
| 4. | Effect of different tissue culture media on length of root (cm) at different days after root initiation in *Aloe vera* with hormone | 61 |

# *IN VITRO* REGENERATION OF *Aloe vera* L. IN A NEW PLANT TISSUE CULTURE MEDIUM

BY

**CHHANDA SARKER**

## ABSTRACT

The experiment was conducted at the Biotechnology Laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from July 2021 to June 2022, to evaluate the regeneration of *Aloe vera* in a new tissue culture medium (SAU Tissue Culture Medium or Hoque medium) which was marked as Treatment-3. MS (1962) medium was used as Treatment-1 and MS Powder (Duchefa, The Netharland) was used as Treatment-2. It was observed that, the highest percent (90%) of shoot regeneration was observed in the SAU Tissue Culture Medium. The same treatment showed maximum number of shoot in all the study period. The highest number of leaves were found in MS powder (Duchefa, The Netherland) medium and it was lowest in MS (1962) medium. Longest length of root (7.00) cm was observed in SAU Tissue Culture Medium and it was shortage (5.10) cm in MS (1962) Medium. Those three treatments were supplemented with same dose of phytohormone (1.00mg/L KIN + 2.00 mg/L NAA) to find out the ability of media in respect of hormonal condition. It showed that there was no significant difference in respect of days to shoot initiation and percent of shoot regeneration among the SAU Tissue Culture Medium and MS powder (Duchefa, The Netharland) when supllimented with phytohormone. Number of shoot per explant and number of leaves also showed non-significant difference between the Treatment-3 (SAU Tissue Culture Medium) and Treatment-2{MS powder (Duchefa, The Netherland)}. Percent of root induction and length of root also showed similar result among the Treatment-2 and Treatment-3. The MS (1962) medium showed lowest result for all the parameter under studied. The study highlighted the potentiality of SAU Tissue Culture Medium on *In vitro* regeneration of *Aloe vera.* It has indicated that the newly developed SAU Tissue Culture Medium (Hoque Medium) has excellent result on plantlet regeneration in *Aloe vera*. Hence, the formulation of SAU Tissue Culture Medium is a successful innovation in the field of tissue culture technology which can be applied for any other plant species.

# CHAPTER I

# INTRODUCTION

# CHAPTER I

# INTRODUCTION

*Aloe vera* L. is a plant species that is a member of the Aloe genus of the Liliaceae family that also goes by several names, such as "Lily of the Desert," "Medicinal Plant," "Burn Plant," "First Aid Plant," "Miracle Plant," and "Ghritkumari" in Ayurveda (Tanabe and Horiuchi, 2006). The name "*Aloe vera*" is derived from two different languages, Arabic and Latin. "Alloeh" in Arabic translates to "shining bitter substance," while "vera" in Latin means "true." The plant's rich cultural history stretches back thousands of years, with various civilizations valuing it for its medicinal properties. Greek scientists regarded *Aloe vera* as a universal panacea around 2000 years ago, indicating its widespread usage and reputation for healing. The Egyptians also held *Aloe vera* in high esteem, referring to it as "the plant of immortality." They believed the plant had mystical and rejuvenating properties, and it was frequently used in their embalming practices. These historical anecdotes emphasize the long-standing recognition of the medicinal properties of *Aloe vera* and its importance in different cultures throughout history.

It has been recognized as an essential plant in various fields, such as traditional and modern medicine, food, cosmetics, and pharmaceutical industries, due to its numerous therapeutic properties. As a result, it has been widely cultivated in many countries, particularly in arid and semi-arid regions, where it is adapted to the environment and thrives. In addition to its medicinal properties, *Aloe vera* has become increasingly popular in recent years for its use in skincare and cosmetic products due to its anti-inflammatory, antioxidant, and moisturizing effects. Therefore, it is not only an all-purpose herbal or medicinal plant, but it is also a valuable resource for various industries. Numerous studies have been conducted on this plant to understand its chemical composition, therapeutic properties, and potential applications. The cited sources, Cock (2015), Hasanuzzaman *et al.* (2008), and Reynolds (2004), demonstrate the plant's significant importance and widespread cultivation.

According to several sources, such as Lanka (2018), Hamman (2008), and Surjushe *et al.* (2008), the leaves of the *Aloe vera* plant contain various essential nutrients, including fat compounds, carbohydrates, proteins, lipids, 18 essential amino acids, vitamins such as A, C, E, vitamin B12, folic acid, and minerals. *Aloe vera* leaves are reported to contain more than 160 metabolites, as cited by Supe (2007). With the most important compounds being barbaloin and homonataloin, as noted by Groom and Reynolds (1987). The plant also contains eight essential amino acids out of the 22 required by the human body, and daily consumption of aloin-free *Aloe vera* gel is believed to be beneficial for improving natural immunity, as cited by Jeyanthi *et al.* (2013).

The plant also contains secondary metabolites like alkaloids, aloins, lectins, lignin, saponins, tannins, and phenolic and glukomannan, as mentioned by Boudreau and Beland (2006) and Darini *et al.* (2013). *Aloe vera* is commonly used as a functional food supplement and food preservative due to the presence of antioxidant molecules, high carbohydrates, and vitamins as its constituents, as stated by Gupta *et al.* (2020). Moreover, the plant is well-known for its widespread use in cosmetology and medicine, as emphasized by sources such as Lanka (2018), Cock (2015), Akev *et al.* (2015), Eshun and He (2004), and Hamman (2008).

*Aloe vera*, is a xerophytic plant that is characterized by its perennial nature. It belongs to the monocotyledonous group and has thick, fleshy, and triangular-shaped leaves with serrated edges. The leaves are usually green to grey-green in color and can grow up to 60-100 cm long. The yellow tubular flowers of the plant are pendulous and grow in an inflorescence that is about 90 cm tall. Each flower has a yellow tubular corolla that measures 2-3 cm and is hermaphrodite. The yellow perianth of the flower is divided into six lobes, each about 2.5 cm long, with scattered bracts.

*Aloe vera* spreads by producing offsets and root sprouts, and its flowers produce fruits that contain numerous seeds. The plant's flowers also have six protruding stamens and three ovaries with a long style. The plant prefers light, sunny weather and requires well-drained soil. However, as noted by Cock (2015), *Aloe vera* is highly adaptable and can even grow in nutritionally poor soil.

The conventional method of *Aloe vera* cultivation has several limitations. First, it is dependent on environmental factors such as soil, weather, and water availability, which can be unpredictable and uncontrollable. Additionally, conventional cultivation often relies on the use of chemical fertilizers and pesticides, which can have adverse effects on both the environment and human health.

Tissue culture, on the other hand, offers a more controlled and sustainable method of *Aloe vera* cultivation. Through tissue culture, a single plant can be used to produce a large number of genetically identical plants in a short period of time. This method also allows for the manipulation of growing conditions, such as light, temperature, and nutrient levels, to optimize plant growth and development.

Furthermore, tissue culture can produce *Aloe vera* plants that are free from diseases and pests, which reduces the need for chemical pesticides. This method also eliminates the variability in plant quality that can occur with conventional cultivation, ensuring that each plant has the desired medicinal properties.

The use of tissue culture techniques offers a rapid method for vegetative propagation of *Aloe vera*, demonstrating significant potential. There is limited information available on the impact of changes to the MS medium for regeneration of *Aloe vera* and any other crops.

When preparing culture media for tissue culture work, a combination of at least 17 macro and micro plant nutrients is required. The Murashige and Skoog (MS) media composition is widely used for rapid micro propagation and meristem culture techniques. This media's recommended nutrient dose has been successfully used for the past 50-60 years. Ammonium nitrate is a macro nutrient that is often used in the preparation of MS media, with a recommended dose of 16.50 gm per liter. It is a good source of nitrogen for the media, as it contains approximately 35% nitrogen. However, ammonium nitrate is an explosive chemical and has significant disadvantages in human civilization. It is used as an oxidizing agent in explosives and is involved in destructive activities such as bomb-making. Although ammonium nitrate is an important ingredient in tissue culture media, it is now completely banned in some countries due to its explosive nature.

Terrorism is a significant issue in many countries, including our own. To combat this problem, the government has taken strong measures to control the production of destructive materials such as bombs, including monitoring and regulating the supply of raw materials. As a result, suppliers and importers are prohibited from selling ammonium nitrate ($NH_4NO_3$) in the country, which had a negative impact on tissue culture work. To address this challenge, various research institutions and private tissue culture companies have turned to alternate approaches, such as using ready-made MS powder manufactured in abroad. Some well-known companies that produce these powders include Duchefa in the Netherlands, Sigma in the USA and Germany, and SRL in India. However, these ready-made solutions are quite expensive, with 220gm of Duchefa's MS powder costing 8000 taka and yielding only up to 50 liters of MS solution.

In contrast, manually preparing stock solutions is much cheaper and more user-friendly. Dr. Md. Ekramul Hoque, a respected professor in the Department of Biotechnology at Sher-e-Bangla Agricultural University (SAU) in Dhaka, Bangladesh, developed an alternative chemical to ammonium nitrate ($NH_4NO_3$), which is cost-effective, environmentally friendly, and available in Bangladesh. With relentless experiments and trials, the Department of Biotechnology was developed a new plant tissue culture medium where $NH_4NO_3$ is absence. It was named as SAU Tissue Culture Medium. This medium was tested for regeneration of potato and a remarkable success was achieved for *In vitro* regeneration and plantlet production in potato crop.

In order to confirm the regenerative potential of the new media, it is important to assess its effectiveness with other crops. Therefore, a research proposal was initiated to evaluate the regeneration potentiality of the SAU Tissue Culture Medium in the plant *Aloe vera*.

Considering the above facts, the present experiment has been undertaken with the following specific objectives:

- To study the comparative performance of SAU Tissue Culture Medium with other standard media
- To study the effect of SAU Tissue Culture Medium supplemented with phytohormone

- To study the regeneration ability of *Aloe vera* in SAU Tissue Culture Medium
- To develop regeneration protocol in *Aloe vera*

# CHAPTER-II

# REVIEW OF LITERATURE

# CHAPTER II

# REVIEW OF LITERATURE

Plant tissue culture is a fundamental component of plant biotechnology that encompasses various methods, such as micro propagation, somaclonal induction, somatic hybridization, cryopreservation, and regeneration of transgenic plants (Altman and Ziv, 1997). It involves cultivating any part of a plant on a sterile nutrient medium with regulated light and temperature conditions to encourage growth. The idea of plant tissue culture originated from Schwann's cell theory in 1839. Tissue culture techniques have been extensively utilized in micropropagation of horticultural and ornamental plants for many years, with the first successful plant tissue culture achieved in horticultural plants (Altman and Ziv, 1997). These methods have been useful in disease control and vegetative propagation (Hussey, 1979). Currently, tissue culture is a prevalent practice worldwide for studying different aspects of *Aloe vera* (Altman and Ziv, 1997), but unfortunately, it is not widely used in Bangladesh. The present study involved a review of relevant literature from various domestic and international research institutions. As part of this review, we have included citations for some of the most significant studies in the field.

## 2.1. Explant:

An explant is a part of the plant by which a whole plant can be produced through plant tissue culture technique. Several researchers have suggested using shoot tips and apical meristems for micropropagation of *Aloe vera*, as evidenced by the studies of Debiasi *et al.* (2007), Liao *et al.* (2004), Aggarwal and Barna (2004), and Campestrini *et al.* (2006). Other explants, with the exception of young stem segments, ceased to grow entirely after releasing brown substances from the explant. This phenomenon may be due to the fact that juvenile tissues have a greater capacity for regeneration (Murashige and Skoog, 1962) or produce fewer phenols (Roy and Sarkar, 1991).

In a study by Baksha *et al.* (2005), *Aloe barbadensis* Mill. was micropropagated through *In vitro* culture of shoot tip explants. The authors obtained ten multiple

shoots per explant by culturing shoot tip explants on MS media supplemented with BAP 2.0 mg/L and NAA 0.5 mg/L. Furthermore, the authors reported that approximately 95% of the micro-shoots rooted successfully when cultured on half-strength MS media supplemented with NAA 0.5 mg/L, and that the rooted plantlets showed a 70% survival rate when transferred to soil.

Daneshvar *et al.* (2013) investigated the impact of various media on shoot proliferation from the shoot tip of *Aloe vera* L. According to the authors, MS media containing 1.5 mg/L KIN and 0.3 mg/L NAA yielded the highest percentage of proliferated shoots. The authors also found that MS media containing 2.0 or 2.5 mg/L BAP and 0.15 mg/L NAA resulted in significantly higher rates of shoot proliferation compared to other treatments.

Hongxing *et al.* (2004) investigated the correlation between aloin accumulation in *Aloe vera* var. chinensis and callus produced from different plant parts as explants, including roots, stems, and leaves. The researchers determined the aloin content in the callus through HPLC and TLC. They found that the callus induced from leaves had the highest differentiation degree and contained the most aloin when cultured on MS medium supplemented with 1.0 mg/L NAA + 0.5 mg/L BAP. Conversely, callus from stems had a low aloin content. Callus from root did not contain any aloin. Furthermore, when cultured on MS medium with 1.0 mg/L 2,4-D + 0.5 mg/L BAP, callus differentiation was low and aloin was not present, regardless of the plant part used.

Kumawat (2013) conducted a study on the *In vitro* regeneration of *Aloe barbadensis* Mill. (commonly known as Ghritkumari) using micro shoots, leaves, and roots as explants. The study used two genotypes, JA-1 and JA-2, which were inoculated on a medium consisting of MS supplemented with a standard callus induction protocol of 0.5 mg/L KIN and 2.0 mg/L NAA. The cultures were grown at 25 ± 20C under a 14:10 photoperiod with a light intensity of 3000 lux. Semi-friable, pale yellow callus was obtained from the base of micro shoot explants that were inoculated on a medium with 0.25 mg/L KIN and 1.0 mg/L 2,4-D, along with different levels of antioxidants such as ascorbic acid, activated charcoal, and polyvinylpyrrolidone for de novo shoot regeneration.

In a research conducted by Lee and colleagues (2011), the induction and proliferation of adventitious root from *Aloe vera* leaves tissues was investigated as a means of *In vitro* production of aloe-emodin. The study found that a combination of 0.5 mg/L NAA and 0.2 mg/L BAP in Murashige & Skoog (MS) medium was effective in inducing adventitious roots, but root proliferation was impeded by the accumulation of phenolic compounds in the media. This problem was resolved by pre-washing the adventitious roots with more than 4 g/L of polyvinylphyrollidine (PVP) analogs, which increased the survival rate by up to 60%. Further analysis of aloe-emodin contents in various adventitious root grown on different basal media revealed that B5 medium produced significantly higher levels of aloe-emodin compared to MS medium.

Lobine *et al.* (2015) conducted a study on developing a successful tissue culture protocol for the conservation of endangered Mascarene Aloes. The study focused on the micropropagation and restoration of four threatened endemic species of Masacrene Aloes: *Aloe lomatophylloides, Aloe macra*, *Aloe purpurea*, and *Aloe tormentorii*. The researchers utilized explants consisting of 2cm long hypocotyls with radicles and cultured them on Murashige and Skoog's (MS) basal medium supplemented with various plant growth regulators, including Thidiazuron, Benzyl amino purine, and Naphthalene acetic acid (TDZ, BAP, NAA).

MS basal medium was used by Rathore *et al.* (2011) to develop a tissue culture method for high frequency plantlet regeneration from callus cultures of sweet aloe genotype. The callus cultures were supplemented with 6.0 mg/L of 2,4-D and 100 mg/L activated charcoal and additives. Meanwhile, all explants used by Lobine *et al.* (2015) produced a significantly ($p<0.05$) higher number of PLBs on MS containing 0.01 mg/L of NAA, except for A. tormentor explant which produced more PLBs on MS with both NAA and TDZ. Acclimatization of the rooted plantlets resulted in a survival rate of over 95%.

To maintain regenerative callus cultures, a carbohydrate source of 4% was added to MS medium supplemented with 1.5 mg/L 2,4-D and 0.25 mg/L Kinetin. All regenerated plantlets were successfully hardened in the greenhouse and stored under an agro net house. In a different study, Wang *et al.* (2002) utilized adventitious buds as explants for in vitro propagation of *Aloe vera* tetraploids.

The authors found that the addition of 1 mg/L BAP to MS medium was optimal for adventitious bud differentiation and successive culture transfer. Meanwhile, MS medium supplemented with 2 mg/L IBA, 0.3 mg/L NAA, and 0.3% AC was optimal for successful propagation and differentiation of roots.

### 2.2. Sterilization/Decontamination:

To successfully regenerate plants, sterilization of the plant material is crucial and should be done prior to the inoculation process. The use of a sterile laminar airflow cabinet is necessary during all operations. Various sterilization agents such as $HgCl_2$, NaOCl, and ethanol can be used. For instance, sterilization treatment for *Solanum tuberosum* involves dipping in 0.5% $HgCl_2$ solution for 3-5 minutes and then washing with autoclaved distilled water. For dissected segments of sprouts used in an experiment by Yasmin *et al.* (2003), surface sterilization was achieved by treating with 10% commercial bleach containing polyoxyethylene sorbitan monolaurate (Tween-20) for 10 minutes.

According to Rao (2008), both NaOCl and $HgCl_2$ are oxidizing agents that damage microorganisms by oxidizing their enzymes. However, Sirivastava *et al.* (2010) suggest that NaOCl may be a mild sterilizing agent, which could explain its lower effectiveness compared to $HgCl_2$. While $HgCl_2$ is reportedly a better sterilizing agent, it is more toxic, requires special handling, and is difficult to dispose of (Maina *et al.* 2010). Moreover, Muna *et al.* (1999) warn that mercuric chloride can be toxic to plant tissues if used in higher concentrations, in addition to its effectiveness as a sterilizing agent.

Biswas and colleagues (2013) conducted a study on micro-propagation of *Aloe indica* L. using shoot tip culture as the explant. The shoot tips were disinfected with 2% NaOCl and rinsed with sterile water. Results showed that the medium containing 2 mg/L Benzyladenine with 0.5 mg/L Naphthaleneacetic acid had the best proliferation, with an average of 7.8 shoots per explant after 8 weeks. The lowest average number of shoots per explant was 0.9 with a medium containing 0.5 mg/L Benzyladenine. The highest average number of roots per explant was 5.2, which was produced with a 0.5 mg/L Napthaleneacetic acid concentration. Conversely, the lowest average number of roots per explant was zero with 0.1 mg/L Indole-3-acetic acid concentrations.

De Oliveira and Crocomo (2009) conducted research on the mass production of *Aloe vera* through micropropagation. They evaluated the efficacy of two disinfectants on the survival of the explant in various treatments. The explants were cultured on a semisolid Murashige and Skoog (MS) medium containing 6-benzylaminopurine (6-BAP; 2 mg/L) and multiplied four times at 30-day intervals. The elongation and rooting stages were conducted in the same MS medium without 6-BAP. Ultimately, they obtained a total of 40,495 *Aloe vera* microplants with a yield of 300 microplants per apical bud at a ratio of 1:5.3 during each 30-day multiplication period. They successfully acclimatized 38,480 *Aloe vera* microplants by transferring them to 36 and 64 cell polyethylene trays containing appropriate substrate in two different ex vitro greenhouse conditions. (De Oliveira and Crocomo, 2009)

In a study by Hashemabadi and Kaviani (2008), they investigated the rapid micro-propagation of *Aloe vera* L. through shoot multiplication. Using shoot tip as an explant for in vitro culture, they disinfected the explant with 2% NaOCl and washed it thoroughly with sterile water. The medium supplemented with 0.5 mg/L benzyladenine + 0.5 mg/L naphthaleneacetic acid showed the best proliferation of shoot per explant (9.67) and rooting after 8 weeks. After successful rooting, the plantlets were gradually acclimatized in plastic pots containing a mixture of cocopeat and perlite (1:1) covered with transparent plastic. Nearly all (95%) of the transplanted plantlets survived.

Monge *et al.* (2008) carried out research on somatic embryogenesis and plant regeneration in Aloe (*Aloe barbadensis* Mill.). The study investigated different disinfection treatments for the explants using various combinations of sonication and 4% v/v NaOCl for 2, 3, 4, 5, 10 and 15 minutes. The researchers also looked into the explant source and growth regulators. The highest survival rate (85%) and lowest contamination (15%) were achieved using 5 minutes of sonication. The greatest number of shoots was obtained from embryo genic calluses derived from zygotic embryos, and a medium containing 0.05 mg/L 2, 4-D and 2 mg/L BAP was found to be most effective. It has been established that the explant source is a crucial factor for micro propagation in *Aloe vera*. Furthermore, since not all explants exhibit the same regenerability, it is likely that different selective

pressures would act on different explants, leading to varying frequencies and spectra of regeneration among plants from different explants.

In a study conducted by Sharifkhani *et al.* (2011), an alternative sterilization method for *Aloe barbadensis* Mill. Explants were investigated with the aim of replacing mercuric chloride, a hazardous chemical. The researchers utilized Clorox, a commercial sodium hypochlorite solution, with different concentrations of Tween 80 as a sterilizing agent. Results of a nonparametric Kreskas-Wallis test showed that a solution of 5% sodium hypochlorite with 20 minutes of shaking produced the highest number (91.7%) of viable and sterilized explants with regeneration potential on a Murashige and Skoog medium supplemented with IBA, TDZ, and Zeatin. This study offers a new, safe approach to explant sterilization in *Aloe barbadensis* Mill. micro propagation.

### 2.3. Effect of growth regulators

Shukla and colleagues (2012) noted that adjusting the types and amounts of plant growth regulators (PGRs) is a common experimental strategy for refining in vitro micro propagation techniques.

Aggarwal and Barna (2004) developed a micro propagation protocol for an elite variety of *Aloe vera* through enhanced axillary branching. They found that the best multiplication was achieved using MS medium supplemented with 1.0 mg/L BA and 0.2 mg/L IBA. Addition of 10 mg/L citric acid to the liquid medium improved the shoot multiplication. All the micro-shoots produced rooted plantlets within 15 days of culture on hormone-free agar medium. However, the use of liquid medium during the rooting stage decreased the frequency of rooting. The transferred plants were successfully grown in soil and displayed similar morphology to the mother plants.

Bhandari *et al.* (2010) conducted a study on the *In vitro* propagation of *Aloe vera* and investigated the effect of various plant growth regulators (PGRs) including BA, IBA, KIN, and adenine sulphate. The results indicated that *Aloe vera* growth was promoted in different concentrations of PGRs, especially BA and KIN, which showed indications of shoot proliferation after 2 weeks of incubation. Among these PGRs, BA at a concentration of 1.0 mg/L exhibited better shoot proliferation

(3.3 ± 1.1) compared to KIN. Furthermore, all the cultures treated with BA-containing medium showed 100% shoot proliferation.

Chaudhuri and Mukundan (2001) conducted an experiment to investigate the effects of different growth regulators on shoot tip cultures of *Aloe vera*. They used full strength MS medium containing 3% sucrose and supplemented with adenine sulfate (Ads), benzyladenine (BA), IAA and IBA alone or in combinations. The treatments resulted in differentiation of leaves and shoot in most cases. After 60 days of culture, the 2-3 cm shoots were sub-cultured for 4 weeks on half-strength MS medium supplemented with 1.0 mg/L IAA. The formation of multiple shoots was observed due to the presence of cytokinin and auxin. However, only the presence of either auxin or cytokinin in the culture medium resulted in root or callus formation.

The most suitable medium for achieving the maximum number of shoots was a full-strength MS medium supplemented with 10 mg/L BA, 160 mg/L AS, and 0.1 mg/L IBA. When the leafy shoot was cultured on half-strength MS medium containing 1 mg/L IAA, up to 20 shoots per explant were produced. The fresh gel exudate from both control plants and tissue culture-derived acclimatized plants contained approximately 1% dry matter. The average level of soluble solids was 0.6-0.7%, and the fresh weight and fiber content were 0.075%. The gels from both types of plants were similar and mainly composed of reducing sugars (mannose and glucose in a 3:1 ratio)

Choudhary *et al.* (2011) reported successful induction of callus from shoot tip of *Aloe vera* on MS medium containing 0.5 mg/L KIN and 0.5 mg/L 2,4-D. Shoot formation was achieved on medium supplemented with 1.0 mg/L BAP and 0.5 mg/L NAA. The study also revealed that some plants with high polyphenolic content can hinder primary cultures by causing the media to turn brown due to the oxidation of polyphenolic compounds from explants. To avoid this issue, the explants were transferred to fresh medium every two weeks.

Chukwujekwu (2001) carried out a study on the micropropagation and acclimatization of *Aloe polyphylla* and *Platycerium bifurcatum*. To initiate shoot cultures of *Aloe polyphylla,* young shoot explants were taken from *In vitro* grown plants and cultured on MS medium supplemented with myo-inositol and sucrose.

Various cytokinins, alone or in combination with auxins (IBA and NAA), were evaluated for their ability to induce shoot proliferation. All the cytokinins tested (kinetin, zeatin, iP, and BA) showed good shoot proliferation response. The gelling agent used in the medium was agar. Shoot cultures of *Aloe polyphylla* were initiated using MS medium supplemented with myo-inositol and sucrose as the basal medium. Various cytokinins, alone or in combination with auxins, were tested for their ability to promote shoot proliferation. All cytokinins tested showed good shoot proliferation response, with optimal concentrations for each as follows: zeatin (0.5 mg/L), kinetin (1.5 mg/L), iP (1.0 mg/L), and BA (1.5 mg/L). Optimal combinations with auxins were also identified. However, BA was found to cause hyperhydricity. Temperature and sucrose concentration were found to have an impact on shoot proliferation, with optimal values being 25°C and 30 g/L, respectively. Rooting of plantlets was successful in plant growth regulator-free MS medium, while the best potting mixture for survival was soil:sand:vermiculite (1:1:1 v/v).

Haque and Ghosh (2013) investigated the microcloning potential of *Aloe vera* using the nodal portion of the rhizomatous stem. They cultured the explants on MS medium supplemented with various cytokinins and *Aloe vera* leaf gel (AvG) as an organic supplement. The number of proliferated shoots per explant increased with the regeneration cycles, and the highest number of shoots (38.5 ± 0.44) per explant was induced on the 3rd regeneration cycle on MS medium supplemented with 2.5 mg/L 6-benzylaminopurine and 10.0% (v/v) AvG. After transferring the individual shoots to a one-third strength MS medium containing 20.0% (v/v) AvG, all the shoots formed whole plantlets with a maximum number (9.6 ± 0.29) of roots per shoot. The regenerated plantlets showed a high survival rate (95.0%) in the poly-greenhouse, and their true-to-type conformity was confirmed through molecular cytogenetic assessment of two-year-old field-grown regenerated plants.

Hashemabadi and Kaviani (2010) conducted a study on *In vitro* proliferation of *Aloe vera*, an important medicinal plant. Shoot tips of *Aloe vera* L. were cultured on Murashige and Skoog (MS) medium with varying concentrations of benzyladenine (BA), Indol-3-butyric acid (IBA), and α-naphthalene acetic acid (NAA). The best proliferation of shoot per explant (9.67) and rooting was

observed on medium supplemented with 0.5 mg/L BA + 0.5 mg/L NAA. The largest number of roots was obtained on medium supplemented with 0 mg/L IBA + 1 mg/L NAA (9.71). The longest (8.75 cm) and thickest (4.3 cm) root was achieved on medium supplemented with 1 mg/L IBA + 1 mg/L NAA. The control plants produced the minimum number of microshoots. After hardening, regenerated plants were transferred to a mixture of cocopeat and perlite (1:1) and showed 100% survival in all stages of the experiment.

A study was conducted by Nayanakantha and colleagues (2010) to establish an efficient micro propagation protocol for *Aloe vera* L. using lateral shoot explants. Shoot induction and elongation were significantly improved in a culture medium supplemented with 4mg/L BAP, 0.2mg/L NAA, and 1g/L PVP, with 16 shoots per explant produced. The addition of 10mg/L citric acid and 0.5g/L activated charcoal to the medium resulted in the development of more adventitious buds (21.5 shoots per explant). The micro-shoots were further elongated and rooted by sub-culturing on MS basal medium containing 0.5g/L activated charcoal. The survival rate of the rooted plantlets was 100% after acclimatization.

According to Patel and Sail (2012), the highest amount of callus was obtained when using a culture medium that contained 0.5 mg/L NAA. After sub culturing the callus in the same medium, an increase in the quantity of callus was observed within a short period of 6 days.

In a study conducted by Tanabe and Horiuchi (2006), they investigated the possibility of growing *Aloe barbadensis* Mill. plants in an *Ex vitro* autotrophic culture. To achieve this, they used an economical basal medium consisting of Excel 15-5-15 plus minor's fertilizer formulation and Plant Preservative Medium (PPM) biocide. Micropropagated plantlets were regenerated in a medium containing 2 g/L sucrose and 2.2 μM 6-Benzylamino purine (BA) and then used to initiate ex vitro cultures in open vessels. The loss of ex vitro media due to evaporation was minimized by sealing the culture vessel around the plantlets using parafilm or aluminum foil and by adjusting the level of gellan gum. The use of parafilm and higher levels of gellan gum significantly reduced ex vitro media loss, but plants grown in 6 g/L or 8 g/L gellan gum had lower plant weight gains compared to those grown in 4 g/L gellan gum after 25 weeks of transplanting.

According to Ujjwala's research in 2006, shoot tip segments showed a higher regeneration frequency of shoots (80%) when cultured in MS medium supplemented with 4 mg/L BA and 1 mg/L NAA. The best rooting of the regenerated shoots was observed in the medium containing 1 mg/L IBA and 0.5% activated charcoal. After successful hardening, the regenerated plantlets, with well-developed shoots and roots, were transferred to soil.

Wu and Xie (2002) conducted a study to investigate the effect of hormones, sugar and activated charcoal (AC) on shoot propagation and root formation in *Aloe vera* using tender or lateral buds explants. They found that the most effective medium for shoot propagation was MS media supplemented with 5 mg/L BA, 0.1 mg/L NAA, 30 gm/L sugar and 0.5% activated charcoal, resulting in a propagation coefficient of 51-54 after 20-25 days of culture. For root formation, the optimal medium was MS media supplemented with 0.8 mg/L NAA, 30 gm/L sugar, and 0.3% activated charcoal, which resulted in the growth of 4-5 roots and 5-6 cm shoot height after 15 days of culture.

Yadav (2008) conducted a study on *Aloe barbadensis* Mill. and found that MS medium supplemented with 0.5 mg/L KIN and 1.5 mg/L NAA was effective in inducing profuse callus in adventitious buds. In addition, Yadav observed that the maximum shoot induction in microshoot explants of *Aloe vera* occurred when cultures were incubated at a photoperiod of 14:10 hours followed by 12:12 hours.

Callus induction is an important step in plant tissue culture as it is a mass of undifferentiated cells that can be used for the regeneration of plantlets. Adventitious buds are formed from undifferentiated cells that arise from explants that do not normally produce shoots. KIN (kinetin) is a plant hormone that promotes cell division and differentiation, while NAA (1-naphthaleneacetic acid) is a synthetic auxin that promotes cell elongation and root formation.

In addition, Yadav noted that the callus remained potent during subculture. Subculture is the process of transferring cells or tissue from one medium to another, and it is essential for maintaining the quality and quantity of callus. By keeping the callus potent during subculture, researchers can ensure that the callus will continue to produce shoots and roots, which can then be used to regenerate whole plants.

In their study, Zakia *et al.* (2013) developed a successful protocol for the micropropagation of *Aloe vera* using shoot tip explants. The explants were sterilized using a combination of mercuric chloride and sodium hypochlorite, followed by thorough washing with autoclaved distilled water. Solid MS medium was used with various concentrations of 6-benzyl aminopurine (BAP) and α-naphthalene acitic acid (NAA). The most favorable shoot growth (11.18 shoots) and length (12.15 cm) were obtained using 0.5 mg/l BAP and the same concentration of NAA after 7 weeks of inoculation. The rooting of the shoots was best achieved using a medium containing 1.5 mg/L of indole butyric acid (IBA), with a success rate of 84.67%. The rooted explants were acclimatized and gradually transferred to a greenhouse.

In Gurjar's study in 2009, it was found that the maximum de novo shoot regeneration in callus cultures of *Aloe vera* occurred when they were cultured on a medium supplemented with 0.25 mg/L KIN + 1 mg /L 2,4-D and kept in a photoperiod of 16:8 hours followed by 14:10 hours. On the other hand, de novo shoot regeneration from callus culture was completely prevented at 8:16 hours photoperiod.

Jakhar *et al.* (2012) also observed callus induction in shoot apex explant of *Aloe vera*. They found that the most effective medium for callus induction was MS medium supplemented with 0.5 mg/L KIN + 2.0 mg/L NAA, and this medium was incubated at 25 ± 20C with a photoperiod of 14:10 hours.

Kawai *et al.* (2006) found that callus formation in *Aloe arborescens* tissue was induced on MS medium supplemented with 3% sucrose, 0.1- 0.5 μM/L KIN, and 10-15 μM/L NAA with an incidence rate of 20-32%. Callus formation was further promoted by replacing 3% sucrose with 10% Alpha modification of Eagles medium and 3% glucose. On the other hand, Saggoo and Kaur (2010) reported shoot proliferation in *Aloe vera* callus induced from shoot disc on MS medium supplemented with 1.0 mg/L 2.4-D + 0.2 mg/L kinetin and cultured on MS + 0.2 mg/L BA + 0.2 mg/L IBA. They also found that rooting was most successful on MS + 0.3 mg/L NAA. Regenerated plantlets were acclimatized, hardened, and transferred to garden soil. According to the authors, plant regeneration from callus can introduce variability in *Aloe germplasm* for further improvement.

Singh and Sood (2009) conducted a study on the influence of explant preparation and sizing in *Aloe vera* L. for an efficient method for *In vitro* multiple shoot induction. They used stem nodal explants and shoot tips and cultured them on modified Murashige and Skoog's medium supplemented with different concentrations of growth regulators. The optimal medium composition was found to be MS medium supplemented with IAA, IBA, and BA. The study also found that explant sizing, pruning, and retention of mother tissue significantly influenced the induction of multiple shoot and root. The stem nodal explants with ensheathing leaves base performed better than those without such covering. A high number of shoot and root grew from these explants. The study successfully acclimatized and transferred the rooted plantlets to the green house conditions and finally to field conditions.

According to Bhojwani and Razdan (1992), the plants produced from micropropagation are fragile and not well-suited to the natural environment. Ahmed *et al.* (2007) conducted a study on a rapid micropropagation method for *Aloe vera* L. using shoot tip explants. The best medium for shoot proliferation was found to be MS medium with a combination of BA 2.0 mg/L, KIN 0.5 mg/L and NAA 0.2 mg/L, with 98.96% of shoots proliferated and the highest number of shoots per explant achieved in just 5 weeks. For adventitious rooting, MS medium with NAA 0.2 mg/L and NAA 0.5 mg/L was the most effective, with 80.25% rooting and the highest number of roots per culture (6.71). After transplanting rooted shoots into a mixture of garden soil, compost, and sand (2:1:1), 80% survivability was achieved after 5 weeks. Regenerated plants were successfully acclimatized and transferred to soil with 82% survival rate.

Dwivedi *et al.* (2014) developed a method for micropropagation of *Aloe vera* using axillary shoots, which resulted in the simultaneous growth of new shoots and roots in MS medium supplemented with BAP 1.5 mg/L. Shoot multiplication could be continued for a year by transferring the divided shoots to the same medium. The regenerated plantlets were successfully transferred to soil with 83% survival.

Gholamreza *et al.* (2013) investigated the effect of different explant types on shoot induction and rooting in *Aloe vera* using MS, B5, and SH media

supplemented with various combinations of NAA, BAP, and Kn. They found that the highest shoot proliferation was achieved using MS medium containing 4 mg/L BA. The optimal rooting response was observed on B5 medium supplemented with 2 mg/L NAA, resulting in 100% of regenerated shoots developing an average of 7.8 roots per shoot within 3 weeks. The plantlets were successfully acclimatized and transferred to the greenhouse with 95% success.

# CHAPTER-III

# MATERIALS AND METHODS

# CHAPTER III

# MATERIALS AND METHODS

## 3.1. Time and location of the experiment

The research was conducted between July 2021 and June 2022 in the Biotechnology Laboratory of the Department of Biotechnology at Sher-e-Bangla Agricultural University, which is located in the Sher-e-Bangla Nagar area of Dhaka-1207. The laboratory was chosen as the site for this study due to its well-equipped facilities and experienced staff. The researcher spent approximately twelve months in the laboratory, working diligently to carry out the necessary experiments and collect data.

## 3.2. Experimental materials

### 3.2.1. Source of material

The *Aloe vera* specimens used in this research were sourced from the Agargaon Nursery, which is located in the Sher-e-Bangla Nagar area of Dhaka-1207. The collection of appropriate planting materials is a critical first step in any plant tissue culture work, as the quality of the starting materials can have a significant impact on the success of the experiment. By obtaining the *Aloe vera* specimens from a reputable source like the Agargaon Nursery, the researchers can be confident that the specimens are healthy, well-suited for tissue culture, and have not been exposed to any potential contaminants or diseases that could interfere with the experiment.

### 3.2.2. Plant materials

Fresh and healthy suckers from *Aloe vera* were collected in a beaker filled with water to ensure their cleanliness. The suckers were then washed with running tap water to remove soil from the roots. Shoots with young leaves were selected from high-performing plants with good biomass yield, and excess leaves were removed. Finally, the shoots were trimmed to 2-3 cm in size for further experimentation.

### 3.2.3. Instruments

To ensure sterility, various metal instruments such as forceps, scalpels, needless, spatulas, and aluminum foils were subjected to autoclaving. The sterilization process involved exposure to a temperature of 121°C for 30 minutes at a pressure of 1.06 kg/cm$^2$ (15 PSI).

### 3.2.4. Glass ware

All experiments were conducted using Borosil glassware including Erlesn meyer flasks, culture bottles, flat bottom flasks, pipettes, petridishes, beakers and measuring cylinders (ranging from 25 ml to 1000 ml) for media preparation. Prior to use, the glassware was washed with a liquid detergent (Trix) and thoroughly rinsed with tap water to remove any remaining detergent. They were then rinsed with distilled water and sterilized in an oven at a temperature of 160-180ºc for a duration of 3-4 hours.

**List of glassware along with other instruments and equipment used**:

Used glasswares, instruments and equipments**:**

- Measuring cylinders
- Petridis
- Culture vials
- Autoclave sterilizer
- Microwave oven
- Beaker
- Laminar Air Flow Cabinet
- Freeze
- Hotplate with magnetic stirrer
- Electric balances
- Pipettes
- Plant growth chamber
- pH meter
- Distilled water plant
- Automatic drying oven

- Forceps, needles, spatula, brush, scissors, cotton, surgical blade.

**List of necessary chemicals used:**

- MS media powder ( Duchefa, The Netherland )
- All inorganic, organic and vitamins for MS (1962) medium
- All ingredient of SAU Tissue Culture Medium (Hoque Medium)
- Sterilizing chemicals (70% Ethanol, $HgCl_2$ , tween-20)
- Sucrose
- Agar powder
- NaOH (1N)
- HCl
- Sterilized and deionized distilled water ( $H_2O$ )
- Absolute Ethanol (100 percent )
- Methyl alchohol spirit

### 3.2.5. Culture medium

The process of preparing media is a critical step in tissue culture. Murashige and Skoog medium (MS medium) is a commonly used plant growth medium for regenerating plant cell cultures. All plant cells require water, nutrients, and growth regulators to grow. To satisfy these needs, various stock solutions are employed, consisting of inorganic nutrients such as macro and micro molecules, as well as organic nutrients like sucrose, vitamins, and supplements. Five distinct types of stock solutions were created utilizing these nutrients.

The inventory of the stock solutions was presented below:

- Stock solution I
- Stock solution II
- Stock solution III
- Stock solution IV
- Stock solution V

### 3.2.6. Stock solution for MS (1962) Medium

#### 3.2.6.1. Preparation of stock solution I

The necessary quantity of chemicals, measured by weight, was dissolved in distilled water using a 1L beaker and a magnetic stirrer. Due to the slow rate of precipitation for $CaCl_2.2H_2O$, it was liquefied separately before being added to the solution. The final volume is adjusted to 1L, and the resulting mixture is placed in the primary glass container and refrigerated at $4^0$c. It was prepared in 10x concentration. The amount of chemicals required for a stock solution I solution is listed in the table below.

| SL | Chemical name | Amount (g/L) |
|---|---|---|
| 1. | $KNO_3$ ( Potassium Nitrate) | 19.00 |
| 2. | $NH_4NO_3$ (Ammonium Nitrate) | 16.50 |
| 3. | $MgSO_4$ .$7H_2O$ ( Magnesium Sulphate) | 3.70 |
| 4. | $KH_2PO_4$ ( Potassium biphosphate) | 1.70 |
| 5. | $CaCl_2.2H_20$ ( Calcium chloride dehydrate) | 4.40 |

#### 3.2.6.2. Preparation of stock solution II

To produce a 100x concentrated solution for stock solution II with a total volume of 1000 ml, the chemicals required along with their respective amounts are provided below.

| SL | Chemical name | Amount (mg/L) |
|---|---|---|
| 1. | KI ( Potassium Iodate) | 83.00 |
| 2. | $H_3BO_3$ (Boric Acid ) | 620.00 |
| 3. | $MnSO_4.4H_2O$ ( Manganese Sulphate ) | 2230.00 |
| 4. | $ZnSO_4.7H_2O$ ( Zinc Sulphate ) | 860.00 |
| 5. | $Na_2MoO_4.2H_2O$ ( Sodium Molybdate) | 25.00 |
| 6. | $CuSO_4.5H_2O$ ( Copper Sulphate ) | 10.00 |
| 7. | $CoCl_2.6H_2O$ ( Carbonyle dichloride) | 10.00 |

### 3.2.6.3. Preparation of stock solution III

The required chemicals for 1000 ml solution of 100x higher concentration are given below

| SL | Chemical name | Amount (g/L) |
|---|---|---|
| 1. | $FeSO_4$ .$7H_2O$ ( Ferrous Sulphate) | 2.78 |
| 2. | $Na_2EDTA.2H_2O$ ( Sodium- EDTA) | 3.73 |

### 3.2.6.4. Preparation of stock solution IV

The required chemicals for 1000 ml solution of 100x higher concentration are given below

| SL | Chemical name | Amount (mg/L) |
|---|---|---|
| 1. | Myo-inositol | 10000.00 |
| 2. | Nicotinic Acid | 50.00 |
| 3. | Pyridoxine HCl | 50.00 |
| 4. | Thiamine HCl | 20.00 |
| 5. | Glycine | 200.00 |

### 3.2.7. Preparation of stock solution I for SAU Tissue Culture Medium

The nutrient composition of SAU Tissue Culture Medium is totally different from MS (1962) medium. Ammonium nitrate was not used in SAU Tissue Culture Medium. A new chemical was identified from the Department of Biotechnology under the leadership of Professor Dr. Md. Ekramul Hoque, which was used as an alternate of Ammonium nitrate ($NH_4NO_3$). Due to the privacy and patent related issue the name of the chemical was secrete for public domain. Hence it was denoted as a ***- chemical**. This ***- chemical** was used in stock solution I of SAU Tissue Culture Medium in absence of Ammonium nitrate ($NH_4NO_3$). The concentration of other compounds of major salts also changed in SAU Tissue Culture Medium. The composition of Stock solution II & III was more or less similar with MS (1962) medium.

Myo-inositol is a sugar hexitol. It has several function like sugar transport, carbohydrate metabolism, membrane structure and cell wall formation. It needs

in higher concentration. The amount of Myo-inositol is 10.00gm/l. The other component of stock solution IV of MS (1962) medium needs very small amount. For example Thiamine-HCl needs only 10.00 mg/L. For the preparation of stock solution IV in MS (1962) medium, Researchers have to mix 10,000 mg/L Myo-inositol in 10 mg/L Thiamine-HCl which is quite imbalance proportion for medium preparation.

Hence, considering the function and the concentration of Myo-inositol it was separated from stock solution-IV and grouped as Stock Solution-V in the SAU Tissue Culture Medium. The composition and concentration of SAU Tissue Culture Medium were given below.

| SL | Chemical name | Amount (g/L) |
|---|---|---|
| 1. | $KNO_3$( Potassium Nitrate) | Different concentration from MS (1962) medium |
| 2. | *-Chemical (Star Chemical) | Specific dose (hidden) |
| 3. | $MgSO_4.7H_2O$(Magnesium Sulphate) | Different concentration from MS (1962) medium |
| 4. | $KH_2PO_4$ (Potassium biphosphate) | Different concentration from MS (1962) medium |
| 5. | $CaCl_2.2H_20$ (Calcium Chloride) | Different concentration from MS (1962) medium |

### 3.2.8. Preparation of stock solution II

To produce a 100x concentrated solution for stock solution II with a total volume of 1000 ml, the chemicals required along with their respective amounts are provided below.

| SL | Chemical name | Amount(mg/L) |
|---|---|---|
| 1. | KI ( Potassium Iodate ) | 83.00 |
| 2. | $H_3BO_3$ (Boric Acid ) | 620.00 |
| 3. | $MnSO_4.4H_2O$( Manganese Sulphate ) | 2230.00 |
| 4. | $ZnSO_4.7H_2O$ ( Zinc Sulphate ) | 860.00 |
| 5. | $Na_2MoO_4.2H_2O$( Sodium Molybdate ) | 25.00 |
| 6. | $CuSO_4.5H_2O$ ( Copper Sulphate ) | 10.00 |
| 7. | $CoCl_2.6H_2O$ ( Carbonyle dichloride ) | 10.00 |

### 3.2.9. Preparation of stock solution III

The required chemicals for 1000 ml solution of 100x higher concentration are given below

| SL | Chemical name | Amount(g/L) |
|---|---|---|
| 1. | $FeSO_4$ .$7H_2O$ (Ferrous Sulphate) | 2.78 |
| 2. | $Na_2EDTA.2H_2O$ ( Sodium- EDTA ) | 3.73 |

### 3.2.10. Preparation of stock solution IV

The required chemicals for 1000 ml solution of 100x higher concentration are given below

| SL | Chemical name | Amount(mg/L) |
|---|---|---|
| 1. | Nicotinic Acid | 50.00 |
| 2. | Pyridoxine HCl | 50.00 |
| 3. | Thiamine HCl | 20.00 |
| 4. | Glycine | 200.00 |

### 3.2.11. Preparation of stock solution V

| SL | Chemical name | Amount (g/L) |
|---|---|---|
| 1. | Myo -inositol | 10.00 |

**Plate 1. Preparation of different stock solution**

### 3.2.12. Solutions for pH adjustment

In order to regulate the pH of the culture medium, solutions of NaOH and HCl were employed. To make a 1N NaOH solution, 40gm of the compound was mixed with distilled water, while for a 1N HCl solution, 36.5 gm of HCl was added to 1 liter of distilled water. These solutions were utilized to decrease the pH, and the target pH value for the media was set at 5.8.

### 3.2.13. Disinfection of culture room and laminar airflow

Formaldehyde spray was used to disinfect the tissue culture room and it was closed for a day. The floor and rake were cleaned with a detergent that had antifungal properties, and wiped down with 70% ethanol. Prior to beginning work with the explants, the laminar airflow cabinet was also cleaned with 70% ethanol. The UV ray was switched on for a period of 30 minutes before commencing any work.

### 3.2.14. Experiment

Two sub-experiments were conducted to fulfill the objectives.

**Sub experiment-1**

***In vitro* regeneration of *Aloe vera* in different tissue culture media**

Three different tissue culture media were used to establish regeneration protocol of *Aloe vera*. Among them, the well-established MS (1962) medium was used as a standard check medium. It was formulated on the basis of stock solution method. The MS (1962) Medium was treated as treatment-1 ($T_1$). The second medium was Readymade MS powder which was manufactured by Duchefa, The Netharland. It was mentioned as treatment-2 ($T_2$). The third medium was newly developed from the Department of Biotechnology, SAU, Dhaka under the leadership of Professor Dr. Md. Ekramul Hoque and it was named as SAU Tissue Culture Medium (Hoque medium). This medium was named as treatment-3 ($T_3$). S0 the total number of treatment was three. In brief the treatments were as follows-

Treatment-1 ($T_1$) = Murashige and skoog (1962) medium

Treatment-2 ($T_2$) = Readymade MS powder (Duchefa, The Netherland)

Treatment-3 ($T_3$) = SAU Tissue Culture Medium (Hoque medium)

Any kind of Phytohormone was not applied in those three treatment. The objectives of the present sub experiment to investigate the comparative performance of these three treatment for *In vitro* regeneration of *Aloe vera.*

#### 3.2.14.1 Media preparation procedure of each treatment

**Treatment-1 ($T_1$) = Murashige and skoog (1962) medium**

The procedure for preparing 1 liter of Murashige & Skoog (1962) medium was as follows:

1. Eight hundred (800) ml of sterilized distilled water was poured into a 1L glass beaker.
2. Stock solution-I of treatment-1 ($T_1$) was taken 100 ml and 10 ml of each of the other stock solutions were carefully added to the beaker. The mixture was then gently stirred with a magnetic stirrer.
3. Thirty (30) gm of sucrose was added to the mixture.

4. The volume of the solution was increased to 1 liter with distilled water.
5. The pH of the media was adjusted to 5.8 using either NaOH or HCl, while monitoring the pH using a pH meter.
6. Five (5) gm of agar powder was weighed and added to the solution.
7. The mixture was heated in an oven to melt the agar
8. The media was carefully transferred to clean glassware and sterilized once again before use.

**Treatment-2 ($T_2$) = Readymade MS powder (Duchefa, The Netherland)**

The process of preparing 1L Readymade MS powder (Duchefa, The Netherland) is outlined below:

1. A 1L glass beaker was used to hold 800ml of sterilized distilled water.
2. Five (5) gm of MS powder was separately weighed on an electric balance and added to the beaker to form the solution.
3. Thirty (30) gm of sucrose was added to the beaker.
4. The beaker was placed on a magnetic stirrer to mix the contents.
5. Additional distilled water was added to the solution to bring the final volume up to 1L.
6. The pH of the medium was adjusted to 5.8 using either HCl or NaOH.
7. Finally, 5gm of agar powder was weighed and added to the solution. The mixture was then heated in an oven to melt the agar powder.
8. Then the media were aliquot to the culture vial and autoclaved for further use.

**Treatment-3 ($T_3$) = SAU Tissue Culture Medium (Houque Medium)**

The preparation of SAU Tissue Culture Medium was outlined as follows:

1. Eight hundred (800) ml of distilled water was placed in a 1L beaker.
2. One hundred (100) ml of stock 1 for treatment-3 ($T_3$) was added to the beaker.
3. Other stock solutions were added to the beaker in 10ml portions.
4. Thirty (30) g of sucrose was weighed and added to the solution.
5. The volume was adjusted to 1000ml.

6. The pH of the solution was measured and adjusted to 5.8 by the addition of an acid or base.
7. Five (5) g of agar was added to the solution.
8. The mixture was heated in an oven to melt the agar.
9. Finally, the prepared media was poured into glassware and sterilized once again in an autoclave before use.

**Sub Experiment-2**

***In vitro* culture of *Aloe vera* in different tissue culture media supplemented with Phytohormone**

The previous three media were used in this sub experiment. The major difference between sub experiment-1 and sub experiment-2 was additional supplement of both Auxin and Cytokinine group of hormone in sub experiment-2. Hence, the treatment was design by the supplement of same concentration of NAA and Kinitine.

The treatments were-

Treatment-1 ($T_1$) = Murashige and skoog (1962) medium +1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA)

Treatment-2 ($T_2$) = Readymade MS powder (Duchefa, The Netherland) +1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA)

Treatment-3 ($T_3$) = SAU Tissue Culture Medium+1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA)

The objectives of this sub experiment was to investigate the potentiality of those media when supplemented with both combined phytohormone.

**Detail treatment procedure of Sub Experiment-2**

Media preparation procedure of each treatments

Treatment-1 ($T_1$) = Murashige and skoog (1962) medium + 1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA)

For the preparation of treatment-$T_1$, the same steps were followed as the sub experiment-1, with the inclusion of two hormones: 1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA)

Treatment-2 ($T_2$) = Readymade MS powder (Duchefa, The Natherland) +1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA)

The preparation process for treatment-$T_2$ followed the same steps as the sub experiment-1 of treatment-$T_1$, but with the addition of two hormones: 1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA)

Treatment-3 ($T_3$) = SAU Tissue Culture Medium+1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA)

The steps for preparing of treatment-$T_3$ were identical to those of sub experiment-1of treatment-$T_3$, with the exception of adding two hormones: 1.00mg/L Kinetin (KIN) + 2.00mg/L $\alpha$ −naphthaleneacetic acid (NAA

### 3.2.15. Sterilization of the explants

The shoot tips that were trimmed were thoroughly washed under running tap water and autoclaved distilled water for multiple times. The explants were then placed in a 250 ml sterilized beaker in a laminar airflow cabinet and were constantly agitated during the sterilization process. Following this, they were subjected to a treatment of 70% ethanol for 1-2 minutes and washed several times with autoclaved distilled water. To eliminate any chemical residues, the explants were immersed in a beaker containing 0.1% HgCl2 and 3-4 drops of Tween-20 for approximately 4-5 minutes while being agitated in both clockwise and anticlockwise directions. Finally, the explants were washed 3-4 times with autoclaved distilled water to ensure they were free of chemicals and could be inoculated into the culture media.

### 3.2.16. Inoculation of culture

The sterilized explants were inoculated with great care after undergoing proper sterilization procedures within a laminar airflow cabinet. Before starting the work, the surface of the laminar flow bench was cleaned with 70% ethyl alcohol and the interior was sprayed with the same. All glassware, instruments, and media were sterilized in an autoclave. During the process, instruments were placed in a beaker of 70% ethanol and flamed repeatedly with a spirit burner. The worker's hands and forearms were washed thoroughly with soap and water and sprayed with 70% alcohol several times throughout the work. The mouth of the culture vial was flamed before and after positioning the explant on the medium. The explants were transferred to a large sterile glass petri dish or plate with sterile forceps under strict aseptic conditions. Further trimming and removal of extra outer leaves were done with a sterile scalpel blade to make the explants a suitable size. After cutting the explants into a suitable size of 1.5-2 cm, they were transferred to culture bottles containing medium with a plant growth regulator. After vertically inoculating the explants singly in the culture bottles, the mouth of the bottle was quickly flamed and tightly capped. After proper labeling with media code, date of inoculation, etc., the bottles were transferred to the growth room.

### 3.2.17. Incubation

The culture bottles were placed on the culture racks and placed in a controlled environment for growth. The cultures were grown at a temperature of 23±2 °C with a light intensity ranging from 4000–5000 lux (23 W white bulbs) using white fluorescent lamps. The photoperiod followed a pattern of 14 hours of light and 10 hours of darkness with a relative humidity of 70%.

**Plate 2. Incubated culture vial in the growth chamber**

### 3.2.18. Acclimatization

The process of acclimatization, also known as "hardening", involves the adaptation of plants that were propagated *In vitro* to an environment outside of the laboratory setting.

**Step 1:** Plantlets that had been growing on rooting media for 35 days were carefully removed from their culture vials using forceps, taking care not to damage the newly formed roots. They were then dipped in water to remove any traces of solidified agar media, in preparation for acclimatization. Plastic pots measuring 6 x 6 cm, filled with a mixture of garden soil and compost in a 1:1 ratio, were prepared for hardening. The plantlets were immediately transplanted into the pots with great care, right after removing the solidified agar media from their roots.

**Step-2:** Once transplanted, the plantlets were watered thoroughly and kept under controlled conditions with a temperature range of 23±2 °C and a light intensity of 4000-5000 lux. The photoperiod was set to 16 hours light and 8 hours dark with a relative humidity of 60-70%, and they were irrigated regularly for a week.

**Step-3:** After one week, the plants were moved to a shade house with indirect sunlight and lower humidity. Transparent plastic sheets were used to cover the tops of the pots, and the plants were grown at room temperature with a relative humidity of 50-60% for 14 days. They were irrigated every two days.

**Step-4:** Three weeks later, the plants were removed from their original pots and transferred to new pots with larger sizes. They were watered periodically, and the top layer of the soil was mulched as needed.

### 3.2.19. Data compilation

In the *In vitro* situation, the research data of the following unit of those parameters were shown by using a scale to measure length, and for other properties, visual observation is also done. The data collection was done on 7, 14, 21, 28, 35 and 42 days after inoculation of explant on culture media. The mean value of the data was calculated in case of each unit of different parameters.

Data were collected for the following parameters-

- Data on days to shoot initiation
- Shoot length (cm)
- No. of shoot per explant
- Shoot diameter
- No. of node per plantlet
- No. of leaves per plantlet
- No. of root and
- Root length (cm) per plantlet

### 3.2.20. Statistical analysis

The obtained data for various parameters were analyzed using Statistix 10 software, and an ANOVA table was generated through an 'F' test to identify any significant differences among the treatments. Mean values for each parameter were also calculated. To estimate the significance of the differences among treatment means, an LSD test was performed at a 5 percent level of probability. The LSD was calculated to compare the differences between three treatment means.

# CHAPTER-IV

# RESULTS AND DISCUSSION

# CHAPTER IV

# RESULT AND DISCUSSION

The aim of this thesis was to investigate the potential of a new plant tissue culture medium (SAU Tissue Culture Medium), for the *In vitro* regeneration of *Aloe vera*. In addition, the performance of (SAU Tissue Culture Medium) was compared with other standard media. The results obtained from the experiments conducted in this study have been presented in this chapters. It will provide a detailed analysis and discussion of the results, focusing on the effect of different tissue culture media on the regeneration and growth of *Aloe vera*. Furthermore, the acclimatization process of regenerated plantlets in natural conditions will also be discussed in this chapter. The findings of this study will contribute to the development of a more efficient and cost-effective protocol for the regeneration of *Aloe vera*, which can have potential implications in the commercial production of *Aloe vera* plants. However, the major findings were presented in different sub – heading.

## 4.1. Sub experiment 1. *In vitro* culture of *Aloe vera* in different tissue culture media

### 4.1.1. Shoot initiation and percent of shoot regeneration

The potentiality of different tissue culture media on shoot initiation and the percentage of regeneration in *Aloe vera* were presented at Table-1. The treatments used in the experiment are labeled as $T_1$, $T_2$, and $T_3$, representing different media formulations. The first treatment, ($T_1$) refers to the Murashige & Skoog (1962) medium, which is a commonly used standard medium in tissue culture. The second treatment, ($T_2$) involves the use of a readymade MS powder (Duchefa, The Netherlands), while the third treatment, ($T_3$) is the SAU Tissue Culture Medium.

The Table presented two important parameters: the number of days required for shoot initiation and the percentage of regeneration. The minimum days (10.67) was required in treatment-3, (SAU Tissue Culture Medium) which was statically non-significant with the treatment $T_2$ but significant with $T_1$. In terms of shoot initiation, the treatment-1 **(**$T_1$**)** exhibited a mean of 13.00 days. The Treatment-2 ($T_2$) showed a lower mean of 11.33 days which indicating a significant difference compared to $T_1$. The Treatment-3 ($T_3$) demonstrated the shortest mean duration of 10.67 days.

**Table 1. Potentiality of different tissue culture media (treatments) on shoot initiation and percent of regeneration in *Aloe vera***

| Treatments | Days to shoot initiation | Percent of regeneration (%) |
|---|---|---|
| **$T_1$**= Murashige & Skoog (1962) medium | 13.00 a | 75 |
| **$T_2$**= Readymade MS Powder (Duchefa, The Netherland) | 11.33 ab | 85 |
| **$T_3$**=SAU Tissue Culture Medium | 10.67 b | 90 |
| LSD(0.05) | 1.8 | |
| CV(%) | 8.16 | |

The second parameter, the percentage of regeneration, represents the proportion of explants that successfully initiated shoot growth. The Treatment-1 ($T_1$) had a regeneration rate of 75%, while $T_2$ exhibited a higher rate of 85%. The Treatment-3 ($T_3$) showed the highest regeneration rate at 90% (Plate-3). The LSD (0.05) value listed in the Table-1 represents the least significant difference between treatment means for shoot initiation. This value, 1.8, quantifies the minimum difference required to conclude that two treatment means are significantly different. The coefficient of variation (CV) value, 8.16%, represents the percentage of variation relative to the mean and serves as an indicator of experimental precision. A lower CV value suggests higher precision and consistency in the data. The results indicate that all three tissue culture media have the potential to induce shoot initiation and regeneration in *Aloe vera*. However, the treatment-3, $T_3$ (SAU Tissue Culture Medium) demonstrated the shortest time

to shoot initiation and the highest percentage of regeneration, suggesting its superior performance compared to the other media tested. Alauddin (2014) stated that the minimum days to shoot induction was 23 days in 0.5 mg/L of BAP concentration and maximum 41 days in control. He also reported that, the highest percentage (60%) of shoot induction was induced 0.5 mg/L BAP and lowest percentage (35%) was induced in hormone free media.

**$T_1$**=Murashige & Skoog (1962) medium

**$T_2$**=Readymade MS Powder (Duchefa, The Netherland)

**$T_3$**=SAU Tissue Culture Medium

**Plate 3. Shoot initiation of *Aloe vera* at 14 Days in different treatments**

### 4.1.2. Number of shoots per explant

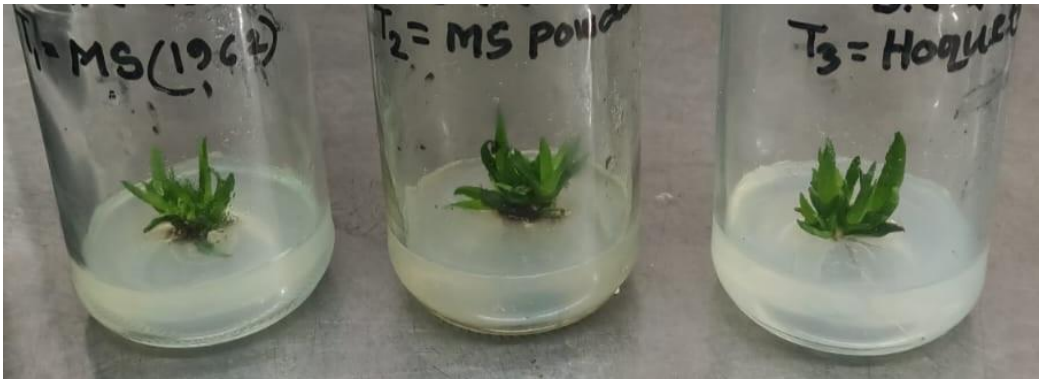
The Table-2 presented the effect of different tissue culture media (treatments) on the number of shoots per explant at various days after initiation (DAI) in *Aloe vera*. The Table provides data for four different time points: 21 DAI, 28 DAI, 35 DAI, and 42 DAI. The treatments used in the experiment are labeled as $T_1$, $T_2$, and $T_3$, representing different media formulations. The first treatment, ($T_1$) refers to the Murashige & Skoog (1962) medium, which is a commonly used standard medium in tissue culture. The second treatment, ($T_2$) involves the use of a readymade MS powder (Duchefa, The Netherlands), while the third treatment, ($T_3$) is the SAU Tissue Culture Medium.

The results in the Table indicated the average number of shoots per explant for each treatment at different DAI. The values presented in the table represent the means of multiple observations and are accompanied by standard deviations (LSD) and coefficients of variation (CV). The standard deviations provide a measure of the variation or spread of the data around the mean, while the coefficients of variation indicated the relative variability expressed as a percentage.

Analyzing the data, it was observed that the number of shoots per explant generally increases over time for all treatments. At 21 DAI, the highest number of shoots (2.70) per explant is recorded for the Treatment-3, followed by $T_2$ (2.37) and $T_1$ (1.93). As the culture progresses to 28 DAI, 35 DAI, and 42 DAI, all treatments showed an increasing trend in the number of shoots per explant (plate-4). However, the Treatment-3 consistently exhibits the highest values at all-time points, indicating its efficacy in promoting shoot growth. The treatment-3 ($T_3$) also demonstrated comparable performance to $T_2$, while $T_1$ shows relatively lower shoot formation.

**Table 2. Effect of different tissue culture media (treatments) on number of shoots per explant at different days after initiation in *Aloe vera***

| Treatments | Number of shoots per explant at different days after initiation (DAI) | | | |
|---|---|---|---|---|
| | **21 DAI** | **28 DAI** | **35 DAI** | **42 DAI** |
| **$T_1$**= Murashige & Skoog (1962) medium | 1.93 d | 3.02 d | 4.58 d | 5.91 c |
| **$T_2$**= Readymade MS Powder (Duchefa, The Netherland) | 2.37 cd | 3.63 bc | 5.64bc | 6.80 b |
| **$T_3$**=SAU Tissue Culture Medium | 2.70 bc | 3.7 b | 5.83 b | 6.91 b |
| LSD (0.05) | 0.86 | 0.75 | 0.88 | 0.50 |
| CV (%) | 14.76 | 8.72 | 6.56 | 3.09 |




**$T_1$**=Murashige & Skoog (1962) medium | **$T_2$**=Readymade MS Powder (Duchefa, The Netherland) | **$T_3$**=SAU Tissue Culture Medium

**Plate 4. Number of shoots of *Aloe vera* per explant at 42 days in different treatment**

The statistical analysis provided further insights into the significance of the differences among the treatments. The LSD values indicated the minimum significant difference between means, with a significance level of 0.05. The CV values reflect the variability in the data, with lower values indicating less variability.

The results from Table 2 suggested that the readymade MS powder ($T_2$) and the SAU Tissue Culture Medium ($T_3$) show promising outcomes in terms of promoting shoot formation in *Aloe vera*. These treatments consistently yield a higher number of shoots per explant compared to the Murashige & Skoog medium ($T_1$) at different DAI. However, it is essential to consider the LSD values and the coefficients of variation to understand the significance and reliability of the observed differences. These findings provide valuable insights into selecting suitable tissue culture media for optimizing the growth and development of *Aloe vera* plants. Biswas *et al.* (2013) achieved the best proliferation of average number of shoots (7.8) per explant of *Aloe vera* was for the medium containing of 2 mg/L with 0.5 mg/L NAA after 8weeks. Baksha *et al*. (2005) noticed (3.2) shoots per explant in media supplemented with 2.0 mg/L BAP.

### 4.1.3. Number of leaves per explant

The Table-3 presented the effect of different tissue culture media (treatments) on the number of leaves per explant at various days after initiation (DAI) in *Aloe vera*. Data were recorded on the following time scheduled: 21 DAI, 28 DAI, 35 DAI, and 42 DAI. The first treatment $T_1$, refers to the Murashige & Skoog (1962) medium, which is a commonly used standard medium in tissue culture. The second treatment, $T_2$, involves the use of a readymade MS powder (Duchefa, The Netherlands), while the third treatment, $T_3$, and was the SAU Tissue Culture Medium.

The highest number of leaves (8.02) at 42DAI was recorded in the treatment-2 ($T_2$), and it was lowest in MS (1962) medium. Analyzing the data, it is observed that the number of leaves per explant generally increases over time for all treatments. At 21 DAI, the highest number of leaves per explant is recorded for the treatment-2, $T_2$ (5.40), followed by $T_3$ (5.06) and $T_1$ (3.82). As the culture progresses to 28 DAI, 35 DAI, and 42 DAI, all treatments showed an increasing trend in the number of leaves per explant (Plate-5). However, the treatment-2 ($T_2$) consistently exhibits the highest values at all-time points, indicating its effectiveness in promoting leaf development. The ttreatment-3 ($T_3$) also demonstrated comparable performance to $T_2$, while $T_1$ shows relatively lower leaf formation.

The statistical analysis provided further insights into the significance of the differences among the treatments. The LSD values indicated the minimum significant difference between means, with a significance level of 0.05. The CV values reflect the variability in the data, with lower values indicating less variability.

**Table 3. Effect of different tissue culture media (treatments) on number of leaves per explant at different days after initiation in *Aloe vera***

| Treatments | Number of leaves per explant at different days after initiation (DAI) | | | |
|---|---|---|---|---|
| | **21 DAI** | **28 DAI** | **35 DAI** | **42 DAI** |
| **$T_1$**= Murashige & Skoog (1962) medium | 3.82 b | 4.95 b | 6.46 b | 7.46 b |
| **$T_2$**=Readymade MS Powder (Duchefa, The Netherland) | 5.4 a | 6.2 a | 7.51 a | 8.02 a |
| **$T_3$**=SAU Tissue Culture Medium | 5.06 a | 5.74 a | 7.2 a | 7.87 a |
| LSD(0.05) | 0.70 | 0.71 | 0.34 | 0.27 |
| CV(%) | 7.36 | 6.31 | 2.47 | 1.79 |

In conclusion, the results from Table 3 suggest that the readymade MS powder ($T_2$) and the SAU Tissue Culture Medium ($T_3$) show promising outcomes in terms of promoting leaf formation in *Aloe vera*. These treatments consistently yield a higher number of leaves per explant compared to the Murashige & Skoog medium ($T_1$) at different DAI. However, it is essential to consider the LSD values and the coefficients of variation to understand the significance and reliability of the observed differences. These findings provide valuable insights into selecting suitable tissue culture media for optimizing leaf growth and development in *Aloe vera* plants. Alauddin (2014) noticed that the highest (16.8) leaves per explant of *Aloe vera* were recorded with 1.5 mg/L BAP + 1.0 mg/L NAA .

| $T_1$=Murashige & Skoog (1962) medium | $T_2$=Readymade MS Powder (Duchefa, The Netherland) | $T_3$=SAU Tissue Culture Medium |
|---|---|---|

**Plate 5. Number of leaves of *Aloe vera* at 28 Days in different treatments**

### 4.1.4. Length of shoot (cm) per explant

The Figure-1 presented a bar diagram illustrating the effect of different tissue culture media on the length of shoot in centimeters (cm) at various days after initiation (DAI) in *Aloe vera*. The diagram provided a visual representation of the data for four time points: 21 DAI, 28 DAI, 35 DAI, and 42 DAI. The three treatments used in the experiment are labeled as $T_1$, $T_2$, and $T_3$, representing different tissue culture media formulations.

At 21 DAI, the length of the shoot for treatment-1, (Murashige & Skoog medium) is 1.36 cm, while $T_2$ (Readymade MS Powder) shows a slightly longer shoot of 1.57 cm, and $T_3$ (SAU Tissue Culture Medium) exhibits the highest shoot length of 1.83 cm. As the culture progresses to 28 DAI, there is an overall increase in shoot length for all treatments. The Treatment-1 reached 2.03 cm, $T_2$ shows a slightly longer shoot at 2.33 cm, and $T_3$ demonstrates the highest shoot length of 2.7 cm.

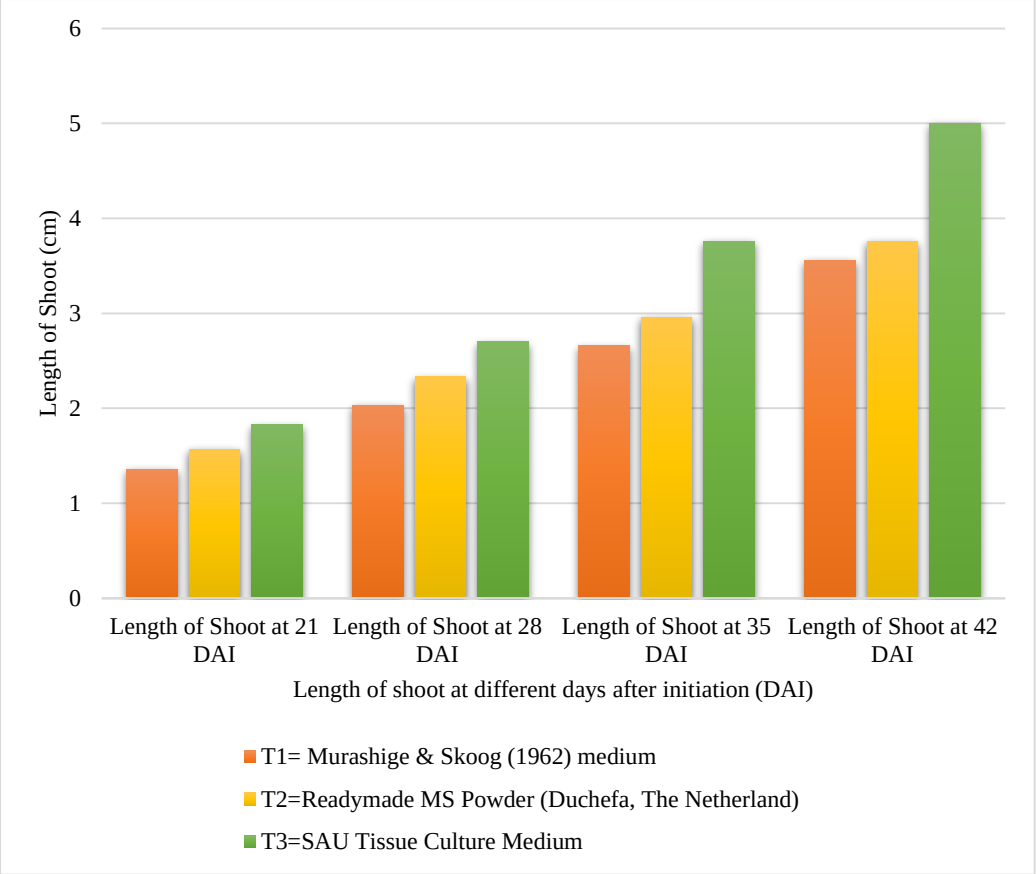



**Figure 1. Effect of different tissue culture media on length of shoot (cm) at different days after initiation in *Aloe vera***

At 35 DAI, all treatments continue to show a positive trend in shoot growth. The treatment-1, has recorded a shoot length of 2.66 cm, $T_2$ increased to 2.96 cm, and $T_3$ exhibits the longest shoot length of 3.76 cm. Finally, at 42 DAI, the shoot length further increases for all treatments. The treatment-1, has reached 3.56 cm, $T_2$ showed a shoot length of 3.76 cm, and $T_3$ demonstrated the highest shoot length of 5.00 cm (Plate-6).

The bar diagram visually represents the differences in shoot length among the different tissue culture media treatments at each time point. It allows for easy comparison of the shoot lengths and provides a clear understanding of the trends in shoot growth over time. The diagram highlights that $T_3$ (SAU Tissue Culture Medium) consistently leads to the longest shoot lengths, followed by $T_2$ (Readymade MS Powder), while $T_1$ (Murashige & Skoog medium) shows relatively shorter shoot lengths.

These findings suggest that the SAU Tissue Culture Medium ($T_3$) promotes the most significant shoot growth in *Aloe vera*, followed by the Readymade MS Powder ($T_2$), while the Murashige & Skoog medium ($T_1$) exhibits comparatively lower shoot lengths. This information is valuable for selecting the most effective tissue culture medium to enhance shoot development in *Aloe vera* plants during in vitro culture. Hasembadi and kaviani (2010) obtained the highest length of shoot 8.67 cm on medium supplemented with 0.5 mg/L BA + 0.5mg/L NAA. MD. Alauddin (2014) has noticed the highest length of shoot 9.62 cm from the 1-5 mg/L BAP + 1.0 mg/L NAA, whereas the minimum 3.30 cm in control.


**Plate 6. Length of shoot (cm) of *Aloe vera* at 42 days in different treatments**

### 4.1.5. Days to root initiation

The Table-4 presented the results of the experiment investigating the effect of different tissue culture media on days to root initiation and the percent of regeneration in *Aloe vera*. The table consists of three treatments: $T_1$ (Murashige & Skoog (1962) medium), $T_2$ (Readymade MS Powder (Duchefa, The Netherlands)), and $T_3$ (SAU Tissue Culture Medium).

The second column, "Days to root initiation," represents the number of days required for root initiation to occur in each treatment. The values reported are 21.87 days for $T_1$, 19.20 days for $T_2$, and 20.11 days for $T_3$.

The third column, "Percent of regeneration (%)," indicates the percentage of successful regeneration observed in each treatment. The values provided are 41% for $T_1$, 57% for $T_2$, and 61% for $T_3$.The fourth row represents the LSD (Least Significant Difference) at a significance level of 0.05. It is a statistical value used to determine if there are significant differences between the means of different treatments. In this case, the LSD is 0.42.

The last row shows the CV (Coefficient of Variation) as a percentage. It is a measure of the variability in the data. The CV value reported is 1.06%, indicating relatively low variation in the observed measurements.

**Table 4. Effect of different tissue culture media (treatments) on days to root initiation and percent of regeneration in *Aloe vera***

| Treatments | Days to root initiation | Percent of regeneration (%) |
|---|---|---|
| **$T_1$**= Murashige & Skoog (1962) medium | 21.87 a | 41 |
| **$T_2$**=Readymade MS Powder (Duchefa, The Netherland) | 19.20 c | 57 |
| **$T_3$**=SAU Tissue Culture Medium | 20.11 b | 61 |
| LSD(0.05) | 0.42 | |
| CV(%) | 1.06 | |

Based on the results, the treatment-2, (Readymade MS Powder) exhibited the shortest average time to root initiation (19.20 days) compared to $T_1$ (21.87 days) and $T_3$ (20.11 days). The treatment-3, had the highest percent of regeneration (61%) followed by $T_2$ (57%) and $T_1$ (41%). The LSD value suggests that there are significant differences between the treatments' means, indicating that the choice of tissue culture media can significantly impact root initiation and regeneration in *Aloe vera*. The low CV value suggested that the data is relatively consistent and reliable. Baksha *et al*. (2005) noticed that the *Aloe vera* required 10 days for root inuction in the medium with 0.5 mg/L of NAA.

### 4.1.6. Length of root (cm)

The Figure-2 depicts a bar diagram that illustrates the impact of different tissue culture media on the length of root in centimeters (cm) at different days after root initiation (DAI) in *Aloe vera*. The diagram presented data for three time points: 21 DAI, 28 DAI, and 35 DAI. The three treatments used in the experiment are labeled as $T_1$, $T_2$, and $T_3$, representing different tissue culture media formulations.

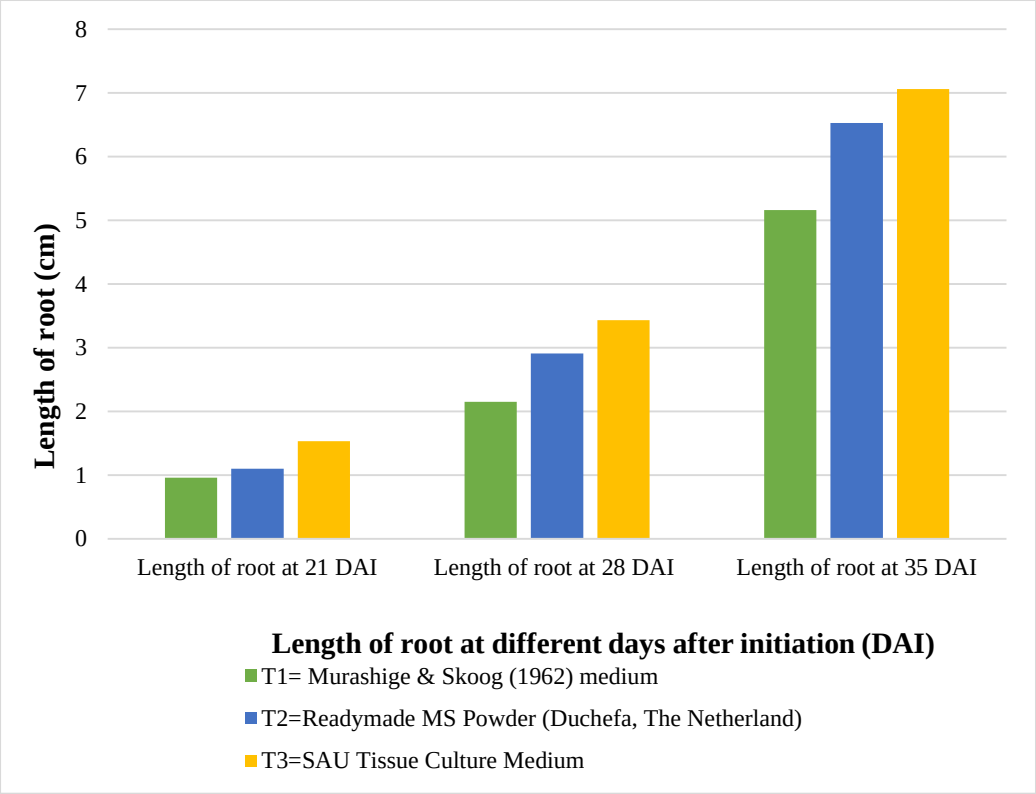
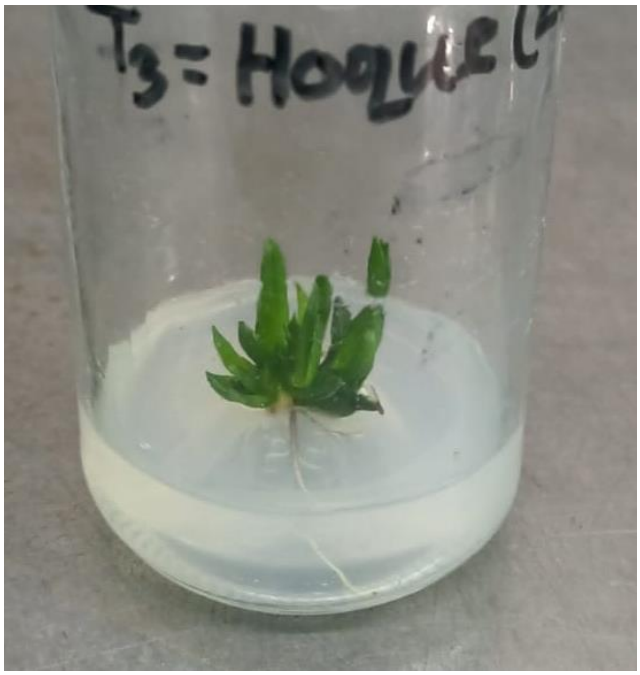



**Figure 2. Effect of different tissue culture media on length of root (cm) at different days after root initiation in *Aloe vera***

At 21 DAI, the length of the root for the treatment -1, (Murashige & Skoog medium) is 0.96 cm, $T_2$ (Readymade MS Powder) exhibits a slightly longer root of 1.1 cm, and $T_3$ (SAU Tissue Culture Medium) demonstrates the longest root length of 1.53 cm. As the culture progressed to 28 DAI, there is a substantial increase in root length for all treatments. The Treatment-1, showed a root length of 2.15 cm, $T_2$ exhibits a longer root at 2.91 cm, and $T_3$ displays the highest root length of 3.43 cm.

At 35 DAI, all treatments continue to display significant root growth. The treatment-1, $T_1$ recorded a root length of 5.16 cm, $T_2$ increases to 6.53 cm, and $T_3$ exhibits the longest root length of 7.06 cm (Plate-7).

The bar diagram visually presented the variations in root length among the different tissue culture media at each time point. It enabled easy comparison of the root lengths and facilitates a clear understanding of the trends in root growth over time. The diagram highlighted that $T_3$ (SAU Tissue Culture Medium) consistently leads to the longest root lengths, followed by $T_2$ (Readymade MS Powder), while $T_1$ (Murashige & Skoog medium) exhibits relatively shorter root lengths.



**$T_3$**=SAU Tissue Culture Medium

**Plate 7. Highest length of root (cm) at 42 days in the SAU Tissue Culture Medium**

These findings indicated that the SAU Tissue Culture Medium ($T_3$) promotes the most significant root growth in *Aloe vera*, followed by the Readymade MS Powder ($T_2$), while the Murashige & Skoog medium ($T_1$) showed comparatively shorter root lengths. This information is valuable for selecting the most effective tissue culture medium to enhance root development in *Aloe vera* plants during in vitro culture. NMC Nayanakantha *et al*. (2011) has reported that the longest (8.75)

cm root was achieved on. Hashembadi and kaviani (2010) obtained the highest length (8.75) cm root was achieved on media supplemented with 1 mg/L IBA + 1mg/L NAA. Daneshvar *et al.* (2013) noticed (6.32) cm root length in 1.00 mg/L. Jafari and Hamidoghli *et al*. (2009) has also explained that the concentration of 2 mg/L IBA has given the maximum root length of *Aloe vera.*

## 4.2. Sub experiment-2. *In vitro* culture of *Aloe vera* in different tissue culture media supplemented with phytohormone

In this particular sub experiment, all treatments of sub experiment-1 were supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA. The experimental results and subsequent discussions are presented below.

### 4.2.1. Shoot initiation and percent of shoot regeneration

The Table-5 provided a comprehensive analysis of the potentiality of different tissue culture media supplemented with specific hormones on shoot initiation and the percentage of regeneration in *Aloe vera*. The treatments evaluated were treatment-1, which utilized Murashige & Skoog (1962) Medium supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA; the treatment-2, which employed Readymade MS Powder (Duchefa, The Netherlands) supplemented with the same hormone concentrations; and the treatment-3, which involved SAU Tissue Culture Medium (Hoque medium) supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA.

**Table 5. Potentiality of different tissue culture media (treatments) on shoot initiation and percent of regeneration in *Aloe vera* supplemented with phytohormone**

| Treatments | Days to shoot initiation | Percent of regeneration (%) |
|---|---|---|
| **$T_1$**= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA | 10.00 a | 85 |
| **$T_2$**= Readymade MS Powder (Duchefa, The Netherland) +1.00 mg/L KIN+2.00 mg/L NAA | 8.73 b | 90 |
| **$T_3$**= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA | 8.36 b | 90 |
| LSD(0.05) | 1.2 | |
| CV(%) | 6.84 | |

The table presented two important parameters: the number of days required for shoot initiation and the percentage of regeneration. In terms of shoot initiation, the treatment-1, exhibited a mean of 10.00 days. The treatment-2, showed a lower mean of 8.73 days, which indicated a significant difference compared to $T_1$ (Plate-8). The treatment-3, demonstrated the shortest mean duration of 8.36 days. It was statistically non-significant with the treatment-2 but significant with the treatment-1.

**$T_1$**= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA

**$T_2$**= Readymade MS Powder (Duchefa, The Netherland) +1.00 mg/L KIN+2.00 mg/L NAA

**$T_3$**= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA

**Plate 8. Shoot initiation of *Aloe vera* at 14 Days in different treatments supplemented with phytohormone**

The second parameter, the percentage of regeneration, represented the proportion of explants that successfully initiated shoot development. The treatment-1, had a regeneration rate of 85%, while both $T_2$ and $T_3$ exhibited a higher rate of 90%. These values indicate that a higher percentage of explants regenerated shoots when treated with $T_2$ and $T_3$, compared to $T_1$.

The LSD (0.05) value listed in the table represented the least significant difference between treatment means for shoot initiation. This value, 1.2, quantified the minimum difference required to conclude that two treatment means are significantly different.

The coefficient of variation (CV) value, 6.84%, represented the percentage of variation relative to the mean and serves as an indicator of experimental precision. A lower CV value suggested higher precision and consistency in the data.

Overall, the results indicated that all three tissue culture media supplemented with the specified hormone concentrations have the potential to induce shoot initiation and regeneration in *Aloe vera*. However, the treatment-3, (SAU Tissue Culture Medium) and $T_2$ (Readymade MS Powder) demonstrated shorter times to shoot initiation and higher percentages of regeneration compared to $T_1$ (Murashige & Skoog Medium), suggesting their superior performance in promoting shoot initiation and regeneration in *Aloe vera* under the experimental conditions. Nayanakantha *et al*. (2011) reported that, BAP alone was less favorable for shoot induction of *Aloe vera*. Sabina Yasmin *et al*. (2022) was found that, the explants cultured with MS medium supplemented with 4.0 mg/L BAP + 0.5 mg/L NAA showed the minimum number of days (5.00) for shoot induction and highest percent (90%) for regeneration of *Aloe vera*.

### 4.2.2. Number of shoots per explant

The Table-6 presented the results of the experiment investigating the effect of different tissue culture media supplemented with hormones on the number of shoots per explant at various days after initiation (DAI) in *Aloe vera*. The treatments evaluated were $T_1$, which involved Murashige & Skoog (1962) Media supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA; $T_2$, which utilized Readymade MS Powder (Duchefa, The Netherlands) supplemented with the same hormone concentrations; and $T_3$, which employed SAU Tissue Culture Medium supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA.

At 21 DAI, the treatment-1, exhibited a mean number of shoots per explant of 3.02. The treatment-2, had a slightly higher value of 3.14 which was non-significant to each other. The treatment-3, showed the highest mean number of shoots per explant at 3.21. Moving to 28 DAI, the treatment-1, had a mean of 3.11 shoots per explant. The treatment $T_2$ showed a higher mean of 4.74 shoots per explant. The treatment-3, had a mean of 4.93 shoots per explant which was insignificant among them. The trend continued at 35 and 42 DAI, with increasing

mean numbers of shoots observed for all treatments. It proved that, there was no significant difference between the treatment-2 and treatment-3.

The LSD (0.05) values provided in the table represented the least significant differences between treatment means. These values quantified the variability within the experiment, indicating the minimum difference required to conclude that two treatment means are significantly different. For the different days after initiation, the LSD values were 0.30, 0.67, 0.43, and 0.35, respectively.

The coefficient of variation (CV) values indicated the percentage of variation relative to the mean and serve as an indicator of experimental precision. Lower CV values suggest higher precision and consistency in the data. In this experiment, the CV values for the different days after initiation ranged from 4.95% to 7.89%, indicating a moderate level of variation in the results.

**Table 6. Effect of different tissue culture media (treatments) on number of shoots per explant at different days after initiation in *Aloe vera* supplemented with phytohormone**

| Treatments | Number of shoots per explant at different days after initiation (DAI) | | | |
|---|---|---|---|---|
| | **21 DAI** | **28 DAI** | **35 DAI** | **42 DAI** |
| **$T_1$**= Murashige & Skoog (1962) Media+1.00 mg/L KIN+2.00 mg/L NAA | 3.02a | 3.11b | 5.23b | 6.0b |
| **$T_2$**= Readymade MS Powder (Duchefa, The Netherland) +1.00 mg/L KIN+2.00 mg/L NAA | 3. 14a | 4.74a | 6.65a | 7.58a |
| **$T_3$**= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA | 3.21a | 4.93a | 6.83a | 7.96a |
| LSD (0.05) | 0.30 | 0.67 | 0.43 | 0.35 |
| CV (%) | 4.95 | 7.89 | 3.45 | 2.44 |

Overall, the results suggested that all three tissue culture media, supplemented with the specified hormone concentrations, influenced the number of shoots per

explant in *Aloe vera*. However, further statistical analysis is necessary to determine the significance of the observed differences between treatments and establish the most effective medium for shoot production in *Aloe vera* tissue culture. Bhandari *et al*. (2010) reported (3.3) shoots per explant in combination with BAP 1.0 mg/L + IBA .2mg/L after 4 weeks of subculture. Zuraida Ab Rahman *et al*. (2015) was recorded (5.7) shoots per explant cultured on MS media Supplemented with 1.0 mg/L BAP + 5.0mg/L NAA.

### 4.2.3. Number of leaves per explant

The Table-7 presented a detailed analysis of the effect of different tissue culture media supplemented with specific hormone concentrations on the number of leaves per explant at different days after initiation (DAI) in *Aloe vera*.

The table provided data for four different time points: 21 DAI, 28 DAI, 35 DAI, and 42 DAI. These time points represent the number of days after initiation of culture. The number of leaves per explant is recorded for each treatment and time point. At 21 DAI, the treatment-1, exhibited a mean of 4.16 leaves per explant. The treatment-2, showed a significantly higher mean of 6.09 leaves per explant which indicating a notable difference compared to $T_1$. Similarly, the treatment-3 also exhibited a higher mean of 6.22 leaves per explant which showed significant difference compared to $T_1$. This suggested that both $T_2$ and $T_3$ have a higher leaf count compared to $T_1$ at 21 DAI.

At 28 DAI, the treatment-1, displayed a mean of 5.07 leaves per explant while $T_2$ and $T_3$ demonstrated higher means of 7.23 and 7.26 leaves per explant, respectively. These results indicate that both $T_2$ and $T_3$ have a significantly greater leaf count compared to $T_1$ at this time point. At 35 DAI, the treatment-1, exhibited a mean of 6.49 leaves per explant while $T_2$ and $T_3$ showed higher means of 7.9 and 8.1 leaves per explant, respectively. Again, both $T_2$ and $T_3$ demonstrated a significantly higher leaf count compared to $T_1$.

Finally, at 42 DAI, treatment-1, had a mean of 7.5 leaves per explant whereas $T_2$ and $T_3$ exhibited higher means of 10.12 and 10.44 leaves per explant, respectively. Both $T_2$ and $T_3$ showed a significantly greater leaf count compared to $T_1$ at this final time point.

**Table 7. Effect of different tissue culture media (treatments) on number of leaves per explant at different days after initiation in *Aloe vera* supplemented with phytohormone**

| Treatments | Number of leaves per explant at different days after initiation (DAI) | | | |
|---|---|---|---|---|
| | **21 DAI** | **28 DAI** | **35 DAI** | **42 DAI** |
| **$T_1$**= Murashige & Skoog (1962) Media+1.00 mg/L KIN+2.00 mg/L NAA | 4.16 b | 5.07 b | 6.49 b | 7.5 b |
| **$T_2$**= Readymade MS Powder (Duchefa the Netherland) +1.00 mg/L KIN+2.00 mg/L NAA | 6.09 a | 7. 23 a | 7.9 a | 10.12 a |
| **$T_3$**= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA | 6.22 a | 7.26 a | 8.1 a | 10.44 a |
| LSD (0.05) | 0.66 | 0.68 | 0.50 | 0.61 |
| CV (%) | 6.09 | 5.26 | 3.38 | 3.27 |

**$T_1$**= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA

**$T_2$**= Readymade MS Powder (Duchefa, The Netherland) +1.00 mg/L KIN+2.00 mg/L NAA

**$T_3$**= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA

**Plate 9. Number of leaves of *Aloe vera* at 42 Days in different treatments supplemented with phytohormone**

The LSD (0.05) value listed in the table represented the least significant difference between treatment means for the number of leaves per explant. This value, ranging from 0.50 to 0.68, quantifies the minimum difference required to conclude that two treatment means are significantly different.

The coefficient of variation (CV) values, ranging from 3.27% to 6.09%, represent the percentage of variation relative to the mean and indicate the precision and consistency of the experimental data. Lower CV values suggest higher precision and consistency in the measurements.

The results of Table 8 demonstrated the impact of different tissue culture media supplemented with specific hormone concentrations on the number of leaves per explant in *Aloe vera* at various time points. The treatments, $T_2$ and $T_3$ consistently exhibited a significantly higher leaf count compared to $T_1$, indicating their superior efficacy in promoting leaf development. These findings provide valuable insights into the selection of optimal tissue culture media for maximizing leaf production during *Aloe vera In vitro* culture experiments.

### 4.2.4. Length of shoot (cm) per explant

The Figure-3 represented a bar diagram depicting the effect of different tissue culture media supplemented with specific hormone concentrations on the length of shoots per explant at different days after initiation (DAI) in *Aloe vera*. The treatments evaluated in this sub-experiment are $T_1$, which consists of Murashige & Skoog (1962) Media supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA; $T_2$, which involves Readymade MS Powder (Duchefa, The Netherlands) supplemented with the same hormone concentrations; and $T_3$, which utilizes SAU Tissue Culture Medium supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA.

The horizontal axis of the bar diagram represents the different days after initiation (21 DAI, 28 DAI, 35 DAI, and 42 DAI), indicating the time points at which the length of shoots was measured. The vertical axis represents the length of shoots per explant, measured in centimeters.


| | | |
|---|---|---|
| **$T_1$**=Murashige & Skoog (1962) medium+ 1.00 mg/L KIN + 2.00 mg/L NAA | **$T_2$**=Readymade MS Powder (Duchefa, The Netherland) + 1.00 mg/L KIN + 2.00 mg/L NAA | **$T_3$**=SAU Tissue Culture Medium+ 1.00 mg/L KIN + 2.00 mg/L NAA |

**Plate 10. Length of shoot (cm) of *Aloe vera* at 42 days in different treatments supplemented with phytohormone**

At 21 DAI, the lengths of shoots per explant for $T_1$, $T_2$, and $T_3$ are 1.56 cm, 1.96 cm, and 2.53 cm, respectively. The treatment-3 exhibits the longest shoots among the three treatments at this time point. At 28 DAI, the lengths of shoots per explant for $T_1$, $T_2$, and $T_3$ are 2.4 cm, 2.46 cm, and 3.36 cm, respectively. Once again, the treatment-3, demonstrated the longest shoots compared to $T_1$ and $T_2$. At 35 DAI, the lengths of shoots per explant for $T_1$, $T_2$, and $T_3$ are 2.86 cm, 3.8 cm, and 4.56 cm, respectively. $T_3$ continues to exhibit the longest shoots among the treatments at this time point.

Finally, at 42 DAI, the lengths of shoots per explant for $T_1$, $T_2$, and $T_3$ are 3.86 cm, 5.23 cm, and 5.56 cm, respectively. $T_3$ shows the longest shoots once again, followed closely by $T_2$ (Plate-10)

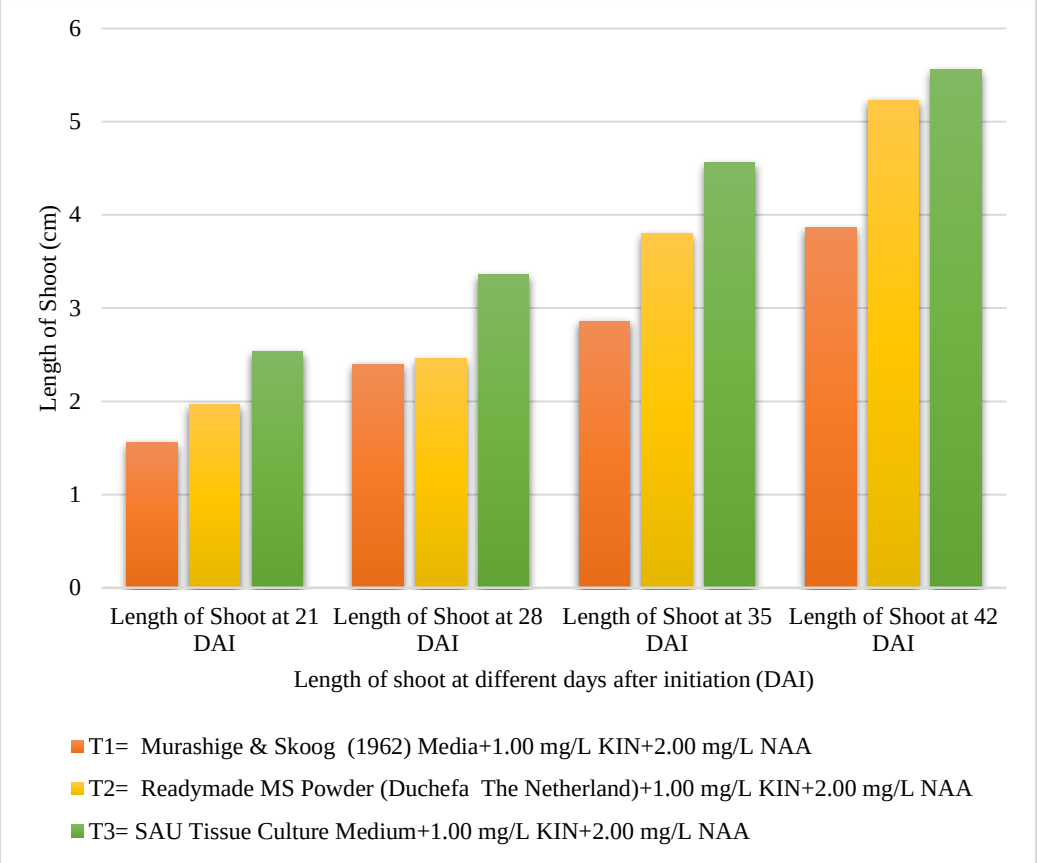



**Figure 3. Effect of different tissue culture media on length of shoot (cm) at different days after initiation in *Aloe vera* with hormone**

The bar diagram visually illustrated the differences in shoot lengths between the three treatments at different time points. It provided a clear comparison of the effectiveness of each tissue culture medium supplemented with specific hormone concentrations in promoting shoot growth in *Aloe vera*. Overall, the bar diagram in Figure-3 highlighted positive impact of $T_3$ (SAU Tissue Culture Medium supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA) on shoot length at multiple time points, suggesting its potential as an effective medium for enhancing shoot growth in *Aloe vera* tissue culture experiments. Manokari *et al* (2022) has reported that MS media containing 1.0 mg/L mT (meta-topolin) showed average length 3.3 cm and liquid MS medium containing 0.5mg/l mT (metatopolin) + 0.25 mg/L NAA showed maximum length 5.6 cm after 3$^{rd}$ sub culture. Baksha *et al* (2005) noticed that the highest length of shoot (4.0) cm on MS media supplemented with 2 mg/L BAP+0.5 mg/L NAA.

### 4.2.5. Days to root initiation of each explant

The Table-8 presented the results of the experiment investigating the effect of different tissue culture media supplemented with hormones on days to root initiation and the percent of regeneration in *Aloe vera*. The table provides data for three treatments: $T_1$ (Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA), $T_2$ (Readymade MS Powder (Duchefa, The Netherland) +1.00 mg/L KIN+2.00 mg/L NAA), and $T_3$ (SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA).

The first column, "Treatments," identified the different tissue culture media used in the experiment along with the specific concentrations of hormones added to each medium. The second column, "Days to root initiation," represented the number of days required for root initiation to occur in each treatment. The values reported are 18.17 days for the treatment-1, ($T_1$), 16.33 days for $T_2$, and 15.16 days for $T_3$. The third column, "Percent of regeneration (%)," indicated the percentage of successful regeneration observed in each treatment. The values provided are 85% for $T_1$, 90% for both $T_2$ and $T_3$. It indicated that, the treatment-2 and treatment-3 has no significant difference in respect of root induction percentage. The fourth row represented the LSD (Least Significant Difference) at a significance level of 0.05. It was a statistical value used to determine if there are significant differences between the means of different treatments. In this case, the LSD is 0.57.

The last row showed the CV (Coefficient of Variation) as a percentage. It was a measure of the variability in the data. The CV values reported are 1.73%, indicating relatively low variation in the observed measurements.

**Table 8. Effect of different tissue culture media (treatments) on days to root initiation and percent of regeneration in *Aloe vera* supplemented with phytohormone**

| Treatments | Days to root initiation | Percent of regeneration (%) |
|---|---|---|
| **$T_1$**= Murashige & Skoog (1962) Medium+1.00 mg/L KIN+2.00 mg/L NAA | 18.17 a | 85 |
| **$T_2$**= Readymade MS Powder (Duchefa, The Netherland) +1.00 mg/L KIN+2.00 mg/L NAA | `16.33b | 90 |
| **$T_3$**= SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA | 15.16 c | 90 |
| LSD(0.05) | 0.57 | |
| CV(%) | 1.73 | |

The results suggested that the treatment-3, (SAU Tissue Culture Medium+1.00 mg/L KIN+2.00 mg/L NAA) has the shortest time to root initiation compared to $T_1$ and $T_2$. Additionally, the treatments $T_2$ and $T_3$ both exhibit higher percentages of regeneration (90%) compared to $T_1$ (85%). The low LSD and CV values indicate that the results are reliable and the observed differences are statistically significant. Alauddin (2014) has reported that the maximum (25) days to root induction was required in media lack of growth regulator and minimum days (10.6) to root induction in case of 2.5 mg/L NAA. Sabina Yesmin *et al.* were noticed that minimum number of days (11+_0.79) and highest percentage (80+_1.97%) of root induction in *Aloe vera*.

### 4.2.6. Length of root (cm)

The Figure-4 presented a bar diagram illustrating the effect of different tissue culture media supplemented with hormones on the length of root in centimeters (cm) at different days after root initiation (DAI) in *Aloe vera.* The diagram provided a visual representation of the data for three time points: 21 DAI, 28 DAI, and 35 DAI. The three treatments used in the experiment are labeled as $T_1$, $T_2$,

and $T_3$, representing different tissue culture media formulations supplemented with the hormones KIN (kinetin) and NAA (naphthalene acetic acid).

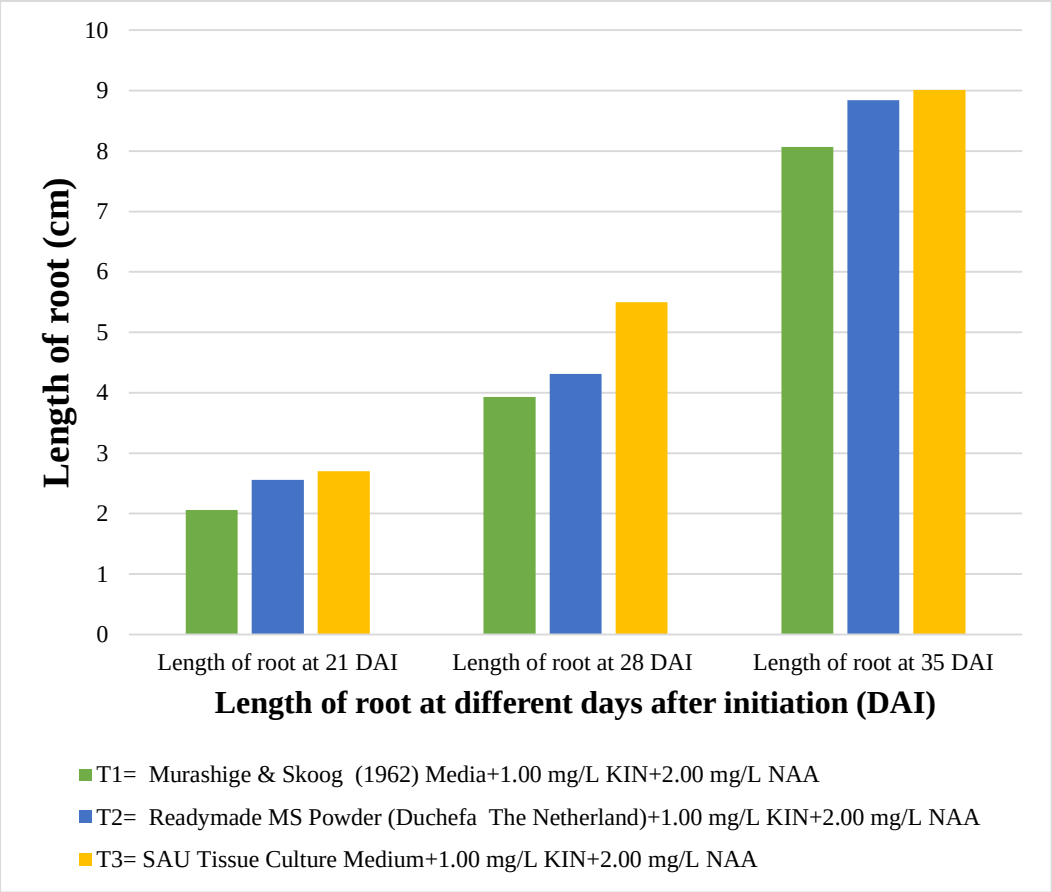



**Figure 4. Effect of different tissue culture media on length of root (cm) at different days after root initiation in *Aloe vera* with hormone**

At 21 DAI, the length of the root for the treatment-1, $T_1$ (Murashige & Skoog medium + 1.00 mg/L KIN + 2.00 mg/L NAA) is 2.06 cm, while $T_2$ (Readymade MS Powder + 1.00 mg/L KIN + 2.00 mg/L NAA) shows a slightly longer root length of 2.56 cm, and $T_3$ (SAU Tissue Culture Medium + 1.00 mg/L KIN + 2.00 mg/L NAA) exhibits the longest root length of 2.7 cm.

As the culture progresses to 28 DAI, there was a significant increase in root length for all treatments. The treatment-1, ($T_1$) reached 3.93 cm, $T_2$ shows a longer root at 4.31 cm, and $T_3$ demonstrated the highest root length of 5.5 cm.

At 35 DAI, all treatments continued to show a positive trend in root growth. The treatment-1, ($T_1$) recorded a root length of 8.07 cm, the treatment-2, ($T_2$) has increased to 8.84 cm, and $T_3$ exhibits the longest root length of 9.01 cm (Plate-11).

The bar diagram visually represented the differences in root length among the different tissue culture media supplemented with hormones at each time point. It allows for easy comparison of the root lengths and provided a clear understanding of the trends in root growth over time. The diagram highlighted that the treatmen-3, $T_3$ (SAU Tissue Culture Medium + 1.00 mg/L KIN + 2.00 mg/L NAA) consistently leads to the longest root lengths, followed by $T_2$ (Readymade MS Powder + 1.00 mg/L KIN + 2.00 mg/L NAA), while $T_1$ (Murashige & Skoog medium + 1.00 mg/L KIN + 2.00 mg/L NAA) shows relatively shorter root lengths.


**Plate 11. Highest length of root (cm) at 42 days in the SAU Tissue Culture Medium +1.00 mg/L KIN+2.00 mg/L NAA in *Aloe vera***

These findings suggested that the SAU Tissue Culture Medium supplemented with hormones ($T_3$) promotes the most significant root growth in *Aloe vera*, followed by the Readymade MS Powder supplemented with hormones ($T_2$), while the Murashige & Skoog medium supplemented with hormones ($T_1$) exhibits comparatively shorter root lengths. This information was valuable for selecting the most effective tissue culture medium and hormone combination to enhance root development in *Aloe vera* plants during *In vitro* culture. Jugabrata Das *et al.* (2017) was found longest root length (6.8) cm on medium supplemented with 2.0 mg/L IBA+0.5 mg/L NAA. Daneshvar *et al.* (2013) noticed (6.32) cm root length in 1.0 mg/L IBA.

### 4.3. Acclimatization of plantlets

The acclimatization of *Aloe vera* plantlets were evaluated based on the survival rate in different conditions. The plantlets were initially grown in a growth chamber for 14 days and then transferred to a shade house with less humidity and indirect sunlight for 28 days and in field condition for 45 days. Plantlet regeneration from both two sub experiment were accumulated or merge on the basis of culture medium. The plantlet of MS (1962) medium with and without hormone were aggregated together and finally total 30 plantlets were studied for acclimatization in the net house and open field condition. Same as plantlet regenerated from MS powder and SAU Tissue Culture Medium were studied. The hardening results were given below-

**Table 9. Survival rate of *In vitro* regenerated plants of *Aloe vera***

| Treatment | In growth chamber (14 Days) | | | In shade house (28 Days) | | | In field Condition (45 Days) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plantlets transferred | seedlings established | Survival rate (%) | plantlets transferred | seedlings established | Survival rate (%) | plantlets transferred | seedlings established | Survival rate (%) |
| **$T_1$**= Murashige & Skoog (1962) Medium | 30 | 28 | 94 | 30 | 27 | 90 | 30 | 26 | 86 |
| **$T_2$**=Readymade MS Powder (Duchefa,The Netherland) | 30 | 30 | 100 | 30 | 28 | 94 | 30 | 28 | 94 |
| **$T_3$**=SAU Tissue Culture Medium | 30 | 30 | 100 | 30 | 28 | 94 | 30 | 28 | 94 |

During this period, the survival rates were recorded and it was observed that the survival rate of the plantlets in the shade house ranged from 90% to 94%, with the highest rate seen in the treatments $T_2$, and $T_3$, indicating that the plantlets were able to adapt to the new conditions

After four weeks of hardening in the shade house, the plantlets were transplanted into the field and the survival rate was recorded for 45 days. It was observed that the survival rate of the plantlets in the field ranged from 86% to 94%, with the highest rate seen in bot sub experiment for the treatment $T_2$, and $T_3$ indicated that the plantlets were able to adapt to the new environment and established themselves in the soil.

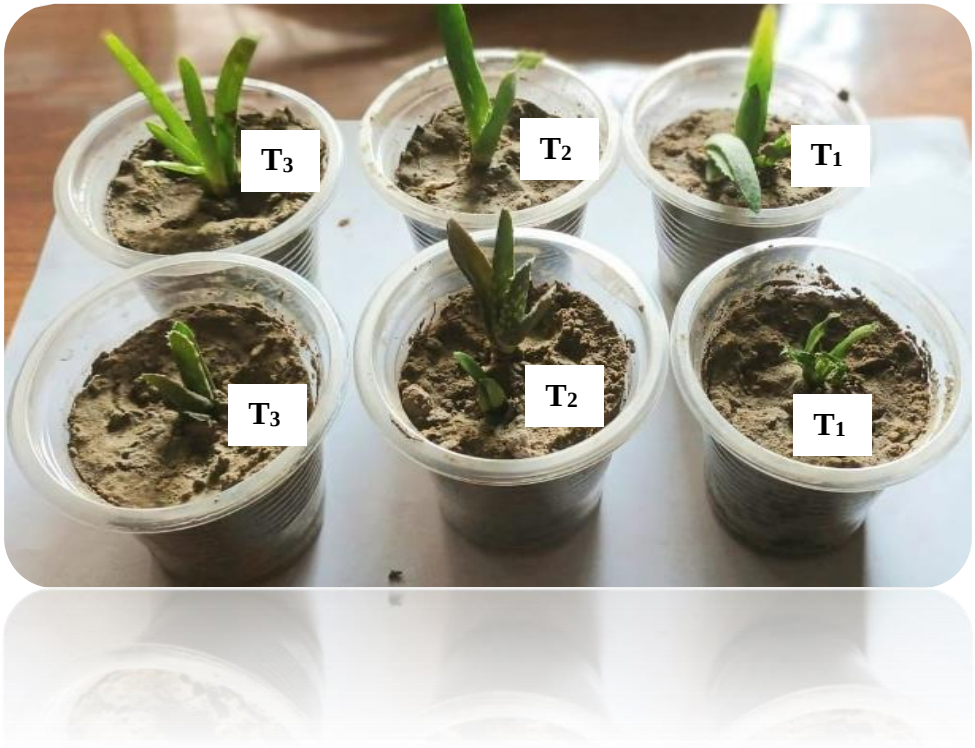



**Plate 12. Acclimatization of *Aloe vera* in the growth chamber (14 days)**

**$T_1$**=Murashige & Skoog (1962) medium
**$T_2$**=Readymade MS Powder (Duchefa, The Netherland)
**$T_3$**=SAU Tissue Culture Mediu

Comparing the survival rates of the plantlets in the growth chamber, shade house, and field, it was observed that all the plantlets survived during the growth chamber stage. In the shade house, the survival rates ranged from 90% to 94%, while in the field, the survival rates ranged from 86% to 94%. These results suggest that the plantlets were able to adapt to the new conditions during the hardening process in the shade house, as well as in the field.

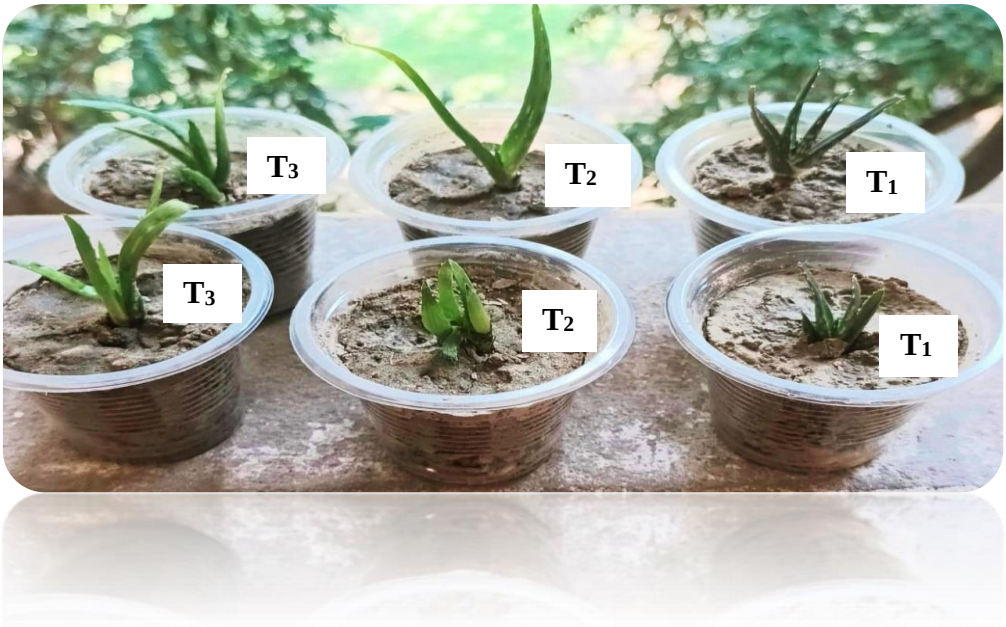



**Plate 13. Acclimatization of *Aloe vera* in the shade house (28 days)**

**T1**=Murashige & Skoog (1962) medium
**T2**=Readymade MS Powder (Duchefa, The Netherland)
**T3**=SAU Tissue Culture Medium

The findings of this study are consistent with previous studies on acclimatization of *Aloe vera* plantlets. Aggarwal and Barna (2004) observed a survival rate of 85% when plantlets were transferred to a polyhouse and then to a shade house with less humidity. Similarly, Dwivedi *et al.* (2014) reported a survival rate of 83% and Bhandari *et al.* (2010) reported survival rates of 90% and 80% under polyhouse and shade house conditions, respectively. Baksha *et al.* (2005) successfully transferred well-developed rooted plantlets to soil with a survival rate of 70%.


**Plate 14. Acclimatization of *Aloe vera* in field condition (45 days)**

**T1**=Murashige & Skoog (1962) medium
**T2**=Readymade MS Powder (Duchefa, The Netherland)
**T3**=SAU Tissue Culture Medium

The results suggest that *Aloe vera* plantlets have a high potential for acclimatization to new environmental conditions. The survival rates of the plantlets in the growth chamber, shade house, and field indicate that they can adapt to changes in humidity, temperature, and sunlight intensity. These findings are important for the commercial cultivation of *Aloe vera* plants, as they provide valuable information on the acclimatization potential of the plantlets and the best conditions for their establishment in the field.

# CHAPTER-V

# SUMMARY AND CONCLUSION

# CHAPTER V

# SUMMARY AND CONCLUSION

Two sub-experiments were carried out to explore the regeneration of *Aloe vera* in a new plant tissue culture media. The media used for the experiment were Murashige & Skoog (1962) Medium, Readymade MS Powder (Duchefa,The Netherland), SAU Tissue Culture Medium (Houque Medium) as $T_1$, $T_2$, $T_3$ respectively in sub experiment-1.The same media were used in sub experiment-2 but those media were supplemented with 1.00 mg/L KIN+ 2.00mg/L NAA. The major findings were given below-

**Sub experiment-1**

Regarding shoot initiation, the treatment-3, (SAU Tissue Culture Medium) exhibited the shortest time to shoot initiation (10.67 days) compared to $T_1$ (13.00 days) and $T_2$ (11.33 days). The percentage of regeneration was the highest for $T_3$ (90%), followed by $T_2$ (85%) and $T_1$ (75%). These findings suggest that, $T_3$ performed best than other media in terms of promoting shoot initiation and regeneration in *Aloe vera*. Analyzing the number of shoots per explant, the treatment-3, ($T_3$) consistently showed the highest values at all-time points (21 DAI, 28 DAI, 35 DAI, and 42 DAI). The treatment-2, ($T_2$) also demonstrated comparable performance, while $T_1$ exhibited relatively lower shoot formation.

Regarding the number of leaves per explant, the treatment-2, consistently had the highest values at all-time points, followed by $T_3$, while $T_1$ showed relatively lower leaf formation. For root initiation, $T_2$ (Readymade MS Powder) exhibited the shortest average time (19.20 days) compared to $T_1$ (21.87 days) and $T_3$ (20.11 days). The treatment-3, showed the highest percentage of regeneration (61%), followed by $T_2$ (57%) and $T_1$ (41%).

Regarding the length of roots, the treatment-3, consistently led to the longest root lengths at all-time points, followed by $T_2$, while $T_1$ exhibited relatively shorter root lengths.

Based on these results, it can be concluded that $T_3$ (SAU Tissue Culture Medium) showed superior performance compared to $T_1$ (Murashige & Skoog medium).The treatment-2 (MS Powder) and treatment-3 (SAU Tissue Culture Medium showed more or less similar performance for all the parameter under investigation. Sometimes there was no significant difference between two treatments. Hence it can be recommended that the SAU Tissue Culture Medium will be used as alternative to MS powder (Duchefa, The Netharland).

**Sub experiment-2**

In terms of shoot initiation, the treatment-1, (Murashige & Skoog Medium supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA) exhibited a mean of 10.00 days, while $T_2$ (Readymade MS Powder supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA) and $T_3$ (SAU Tissue Culture Medium supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA) showed shorter mean durations of 8.73 and 8.36 days, respectively. The percentage of regeneration was 85% for the treatment-1, and 90% for both $T_2$ and $T_3$.

Regarding the number of shoots per explant, the treatment-1, had a mean ranging from 3.02 to 6.0 shoots at different days after initiation (DAI), while $T_2$ and $T_3$ consistently showed higher means across all time points. The length of shoots per explant increased over time for all treatments. The treatment-3, consistently showed the longest shoots 5.6 at different 42 DAI, followed by $T_2$.The days to root initiation were 18.17, 16.33, and 15.16 days for the treatments $T_1$, $T_2$, and $T_3$, respectively. The percentage of regeneration was 85% for $T_1$ and 90% for both $T_2$ and $T_3$.The length of roots increased over time for all treatments. The treatment-3, consistently resulted in the longest roots 9.01 cm at 35 DAI followed by $T_2$.

Based on the results of sub-experiment 2, it can be concluded that all three tissue culture media supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA have the potential to induce shoot initiation and regeneration in *Aloe vera*. However, the treatment-3, (SAU Tissue Culture Medium) and $T_2$ (Readymade MS Powder) demonstrated shorter times to shoot initiation, higher percentages of regeneration, and superior performance in promoting shoot initiation, the

number of shoots per explant, the number of leaves per explant, the length of shoots, and the length of roots compared to $T_1$ (Murashige & Skoog Medium).

These findings suggest that treatment $T_3$ (SAU Tissue Culture Medium) supplemented with 1.00 mg/L KIN and 2.00 mg/L NAA and the $T_2$ (Readymade MS Powder ) are the most effective tissue culture medium for enhancing shoot initiation, shoot regeneration, leaf production, and root development in *Aloe vera* under the experimental conditions. Further statistical analysis would be necessary to confirm the significance of the observed differences and draw definitive conclusions regarding the optimal tissue culture medium for *Aloe vera* shoot initiation and regeneration when supplemented with specific hormone concentrations.

# RECOMMENDATIONS

Based on the findings and conclusion of the study, the following are a few notable recommendations:

***i.*** SAU Tissue Culture Medium (Hoque medium) and the same medium supplemented with 1.00 mg/L KIN+2.00 mg/L NAA showed the highest percentage of regeneration and shorter days to shoot initiation compared to other treatments. Therefore, these media could be used for commercial plantlet production and tissue culture-based cultivation method of *Aloe vera*.

***ii.*** Growth regulators significantly reduced the days to shoot initiation compared to media without growth regulators. Thus, it is recommended to use media supplemented with growth regulators for *Aloe vera* regeneration.

***iii.*** Further studies could be carried out to evaluate the effect of different plant growth regulators and their combinations on the regeneration of *Aloe vera*.

***iv.*** It is also suggested to investigate the potential of other explant types and culture conditions for *in vitro* regeneration of *Aloe vera* to identify more efficient and cost-effective protocols for commercial production.

***v.*** The newly developed SAU Tissue Culture medium (Houque Medium) can be used for *In vitro* regeneration of other crop species to validate the present findings.

# REFERENCES


Ahmed, S., Kabir, A.H., Ahmed, M.B., Razvy, M.A. and Ganesan, S. (2007). Development of rapid micropropagation method of *Aloe vera* L. *Sjemenarstvo*. **24**: 2.

Akev, N., Can, A., Sütlüpınar, N., Çandöken, E., Özsoy, N., Özden, T. Y. and Üzen, E. (2015). Twenty years of research on *Aloe vera*. *J. Fac. Pharm. Ist. Univ.* **45**(2): 191-215.

Alauddin. (2014). *In Vitro* Plant Regeneration of *Aloe vera* L. MS. Thesis. Department of Biotechnology. Sher-e-Bangla Agricultural University. Dhaka. Bangladesh.

Aggarwal, D. and Barna, K.S. (2004). Tissue culture propagation of elite plant of *Aloe vera* Linn. *J. Plant Biochem. Biotech.* **13**: 77-79.

Altman, A. and Ziv, M. (1997) Horticultural biotechnology: A historical perspective and future prospects. *Acta Hort*. **447**: 31-35.

Baksha, R., Jahan, M.A.A., Khatun, R. and Munshi, J.L. 2005. Micropropagation of *Aloe barbadensis* Mill. Through *In vitro* culture of shoot tip explant. *Plant Tiss. Cult. Biotech*. **15**(2): 121-126.

Bhandari, A.K., Negi, J.S., Bisht, V.K. and Bharti, M.K. (2010). *In vitro* propagation of *Aloe vera*-A plant with Medicinal Properties. *Nat. Sci.* **8**(8): 174-176.

Bhojwani, S.S. and Razdan, M.K. (1992). Plant tissue culture: Theory and practice. Elsevier. Amsterdam. London. New York.Tokyo.

Biswas, G.C., Miah, A.M., Hasan, S.M.M., Hossain, S.A.K.M., Shakil, S.K. and Howlader, M.S. (2013). Micropropagation of *Aloe indica* L. Through shoot tip culture. *J. Agric. Vet. Sci*. **5**(1): 30-35.

Boudreau, M. D., and Beland, F. A. (2006). An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller), *Aloe vera. J. Environ. Sci and Health C.* **24**(1): 103-154.

Campestrini, L.H., Kuhnen, S., Lemos, P.M.M., Bach, D.B., Dias, P.F. and Maraschin, M. (2006). Cloning protocol of *Aloe vera* as a study-case for tailormade biotechnology to small farmers. *J. Technol. Manag. Innov*. **1**: 76- 79.

Chaudhuri, S. and Mukundan, U. (2001). *Aloe vera* L. micropropagation and characterization of its gel. *Phytomo.* **51**(2): 155-157.

Choudhary, A.K., Ray, A.K., Jha, S. and Mishra, I.N. (2011). Callus formation, shoot initiation and *In vitro* culture of *Aloe vera*. *Biotechnol. Bioinf. Bioeng.* **1**(4): 551-553.

Chukwujekwu, J.C. (2001). Micropropagation acclimatization of *Aloe polyphylla* and *Platycerium bifurcatum*. M.Sc. Thesis. University of Natal. Pietermaritzburg.

Cock, I. E. (2015). The genus Aloe: Phytochemistry and therapeutic uses including treatments for gastrointestinal conditions and chronic inflammation. Novel natural products: therapeutic effects in pain, arthritis and gastro-intestinal diseases. 179-235

Daneshvar, M.H., Moallemi, N. and Abdolahzadeh, N. (2013). The effect of different media on shoot proliferation from the shoot tip of *Aloe vera* L. Jundishapur *J. Nat. Pharm. Prod*. **8**(2): 93-7

Darini MTh, Indradewa D, Shiddieq D, and Purwantoro A (2013). Response growth and *Aloe vera* contains aloin explant plantlets from different sources. *AgroUPY. J. Science*. **4(**5): 5-12.

De Oliveira, E.T. and Crocomo, O.J. (2009). Large-scale Micropropagation of of *Aloe vera*. *Hort. Sci*. **44**(6): 1675–1678

Debiasi C, Fraguas CB, Lima GPP. (2007). Study of polyamines in the morphogenesis *In vitro* of *Hemerocallis* sp. Ciencia Rural 37, 1014-1020. http://dx.doi.org/10.1590/S010384782007000400015

Dwivedi, N.K., Indiradevi, A., Asha, K.I., Asokan, N.R. and Suma, A. (2014). A protocol for micropropagation of *Aloe vera* L. (Indian Aloe) - a miracle plant. *Res. Biotech*. **5**(1): 1-5.

Eshun, K., and He, Q. (2004). *Aloe vera*: a valuable ingredient for the food, pharmaceutical and cosmetic industries-a review. *Crit. Rev. Food. Sci. Nutr*. **44**(2): 91-96.

Gholamreza, A., Hedayat, M. and Modarresi, M. (2013). *In vitro* micropropagation of *Aloe vera* – Impacts of plant growth regulators, media and types of explant. *J. Biol. Environ. Sci.* **7**(19): 19-24.

Groom, Q. J., & Reynolds, T. (1987). Barbaloin in *Aloe* species. *Plant. Med*. **53**(4): 345-348.

Gurjar, Y.R. (2009). Regeneration in callus cultures of Ghritkumari (*Aloe barbadensis* (Mill). Thesis. RAU. Bikaner.

Hamman, J. H. (2008). Composition and applications of *Aloe vera* leaf gel. *Mol.* **13**(8): 1599-1616.

Hasanuzzaman, M., Ahamed, K. U., Khalequzzaman, K. M., Shamsuzzaman, A. M. M., and Nahar, K. (2008). Plant characteristics, growth and leaf yield of *Aloe vera* as affected by organic manure in pot culture. *Aust. J. Crop Sci.* **2**(3): 158-163.

Hashembadi, D. and Kaviani, B. (2008). Rapid micro-propagation of *Aloe vera* L. via shoot multiplication. *African J. Biotech*. **7**(12): 1899-1902.

Hashembadi, D. and Kaviani, B. (2010). *In vitro* proliferation of an important medicinal plant Aloe- A method for rapid Production. Aust. *J. Crop Sci.* **4**(4): 216-222.

Haque, S.M. and Ghosh, B. (2013). High frequency microcloning of *Aloe vera* and their true-to-type conformity by molecular cytogenetic assessment of two years old field growing regenerated plants. **54**: 46.

Hongxing, W., Faqi, L., Taixia, W., Jianjun, L., Junying, L., Xiangfu, Y. and Jingyuan, L. (2004). Chong, Yao Cai, September. 27(9): 627-628.

Hussey, G. (1979). Tissue culture and its application to plant propagation [ornamental plants, citrus trees and crops, strawberries]. Plantsman.**1**:133-145.

Jakhar, M.L., Gurjar, Y.R., Choudhary, M.R. and Kakralya, B.L. (2012). Regeneration in callus cultures of Ghrithkumari (*Aloe barbadensis* Mill). *J. Pl. Sci. Res*. **28** (1): 131-136.

Kawai, K., Beppu, H., Koike, T., Fujita, K. and Marunouchi, T. (2006). Tissue culture of *Aloe arborescens* Miller var. natalensis Berger. Phytotherapy Res. **7** (7): 5- 10.

Kumawat, N. (2013). *In vitro* regeneration in Ghritkumari (*Aloe barbadensis* Mill.). M.Sc. Thesis. Swami Keshwanand Rajasthan Agricultural University. Bikaner.

Lanka, S. (2018). A review on *Aloe vera*-The wonder medicinal plant. *J. Drug Deliv. Ther*. **8**(5): 94-99.

Lee, Y.S., Yang, T.J., Park, S.U., Baek, J.H., Wu, S.Q. and Lim, K.B. (2011). Induction and proliferation of adventitious root from *Aloe vera* leaves tissues for *In vitro* production of aloe-emodin. *Plant Omics J*. **4**(4): 190-194.

Liao, Z., Chen, M., Tan, F., Sun, X. and Tang, K. (2004). Micropropagation of endangered Chinese aloe. *Plant Cell. Tiss. Org. Cult*. **76:** 83–86.

Lobine, D., Soulange, J.D., Sanmukhiya, M.R. and Lavergne, C. (2015). A tissue culture strategy towards the rescue of endangered Mascarene Aloes. *J. Agric. Biol. Sci.* **10(**1): 1990-6145

Maina, S.M., Quinata, E., Kiran, K., Sharma, K., Simon, T., Moses, G. and Santie, M.D.V. (2010). Surface sterilant effect on the regeneration efficiency from cotyledon explant of groundnut (*Arachis hypogea* L) varieties adapted to eastern and Southern Africa. *J. Biotech*. **9**(20): 2866- 2871.

Monge, G.G., Arias, A. and Melara, V.M. (2008). Somatic embryogenesis, Plant regeneration and acemannan detection in Aloe (*Aloe barbadensis* Mill.). *Agron. Costra*. **32** (2): 41-52.

Muna, A.S., Ahmad, A.K., Mahmoud, K. and Abdulrehman, K. (1999). *In vitro* propagation of a semi-dwarfing cherry roottock. *Plant Cell. Tiss. Org. Cult.* **59**: 203-208.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tabacco tissue cultures. *Plant Physiol*. **115**: 493-497.

Nayanakantha, N.M.C., Singh, B.R. and Kumar, A. (2010). Improved culture medium for micropropagation of *Aloe vera* L. *Trop. Agric. Res. Ext.* **13**(4): 87-93.

Rathore, M.S., Chikara, J. and Shekhawat, N.S. (2011). Plantlet regeneration from callus cultures of selected genotype of *Aloe vera* L. an ancient plant for modern herbal industries. *Appl. Biochem. Biotech*. **163**(7): 860-868

Rao, S.P.N. (2008). The role of endogenous auxin in root initiation. *Plant Growth Regul*. **13**(1): 77-84.

Reynolds, T. (Ed.). (2004). Aloes: The genus Aloe. CRC press.**23**:408.

Roy, S.C. and Sarkar, A. (1991). *In vitro* regeneration and micropropagation of *Aloe vera* L. *Sci. Hort*. **47**: 107-113.

Patel, D. and Sail, S.S. (2012). Studies seed tissue culture and organogensis of *Aloe vera*. *J. Cell Tiss. Res*. 12 (2): 3241-3244.

Saggoo, M.T.S. and Kaur, R. (2010). Studies in north Indian *Aloe vera*: Callus induction and regeneration of plantlets. *Arch. Appl. Sci. Res*. **2**(2): 241-245.

Sharifkhani, A., Saud, H.M. and Aziz, M.A. (2011). An alternative safer sterilization method for explant of *Aloe vera* barbadensis Mill. 2nd International conference on chemical engineering and applications. IPCBEE vol. 23 IACSIT Press. Singapore.

Shukla, M.R., Jones, A.M.P., Sullivan, J.A., Liu, C.Z., Gosling, S. and Saxena, P.K. (2012). *In vitro* conservation of American elm (*Ulmus americana*): Potential role of auxin metabolism in sustained plant proliferation. Can *J. Forest Res*. **42**: 686–697.

Singh, B. and Sood, N. (2009). Significance of explant preparation and sizing in *Aloe vera* L. A highly efficient method for *In vitro* multiple shoot induction. *Sci. Hort.* **122**: 146-151.

Srivastava, P., and Chaturvedi, R. (2010). Simultaneous determination and quantification of three pentacyclic triterpenoids-betulinic acid, oleanolic acid, and ursolic acid-in cell cultures of *Lantana camara* L. *In Vitro* Cellular & Developmental Biology-Plant. **46**: 549-557.

Supe UJ (2007). *In vitro* regeneration of *Aloe barbadensis*. *Biotech*. **6**(4): 601-603.

Surjushe, A., Vasani, R., and Saple, D. G. (2008). *Aloe vera*: a short review. *Indian J. Dermatol*. **53**(4): 163.

Tanabe, M. J., & Horiuchi, K. (2006). *Aloe barbadensis* Mill. *Ex vitro* autotrophic culture. *J. Hawajian Pacific Agric*.**13**: 55-59.

Ujjwala, S.J. (2006). Plant tissue culture laboratory. St. Thomas College Bhilai (CG). 490006.

Wang, L., Zheng, S.X. and Gu, Z.J. (2002). *In vitro* culture of tetraploids of *Aloe vera* (L.). *Acta. Hort.* **29** (2): 176-178.

Wu, Y.X. and Xie, Q.H. (2002). Some influential factors for shoot organogenesis and plant regeneration in *Aloe vera*. South West China *J. Agric. Sci*. **15**: 190-192.

Yadav, L. (2008). Standardization of micropropagation protocol in Ghritkumari (*Aloe barbadensis* Mill.). M.Sc. Thesis, Rajasthan Agricultural University, Bikaner.

Yasmin, S., Nasiruddin, K. M., Begum, R. and Talukder, S. K. (2003). Regeneration and establishment of potato plantlets through callus formation with BAP and NAA. Asian *J. Plant Sci*. **2**(12): 936-940.

Zakia, S., Zahid, N.Y., Yaseen, M., Abbasi, N. A., Hafiz, A.A. and Mahmood, N. (2013). Standardization of micropropgation techniques for *Aloe vera*: A pharmaceutically important plant. *Pak. J. Pharm. Sci.* **26**(6): 1083-1087.

# APPENDICES

## Appendix I.

**Composition of Duchefa Biochemic MS (Murashige and Skoog, 1962) medium including vitamins**

| Components | Concentrations (mg/L) | Concentrations |
|---|---|---|
| **Micro Elements** | **mg/L** | **µM** |
| $CoCl_2.6H_2O$ | 0.025 | 0.11 |
| $CuSO_4.5H_2O$ | 0.025 | 0.10 |
| Fe Na EDTA | 36.70 | 100.00 |
| $H_3BO_3$ | 6.20 | 100.27 |
| KI | 0.83 | 5.00 |
| $MnSO_4.H_2O$ | 16.90 | 100.00 |
| $Na_2MoO_4.2H_2O$ | 0.25 | 1.03 |
| $ZnSO_4.7H_2O$ | 8.60 | 29.91 |
| **Macro Elements** | **mg/L** | **mM** |
| $CaCl_2$ | 332.02 | 2.99 |
| $KH_2PO_4$ | 170.00 | 1.25 |
| $KNO_3$ | 1900.00 | 18.79 |
| $MgSO_4$ | 180.54 | 1.50 |
| $NH4NO_3$ | 1650.00 | 20.61 |
| **Vitamins** | **mg/L** | **µM** |
| Glycine | 2.00 | 26.64 |
| Myo-Inositol | 100.00 | 554.94 |
| Nicotinic acid | 0.50 | 4.06 |
| Pyridoxine HCl | 0.50 | 2.43 |
| Thiamine HCl | 0.10 | 0.30 |

Total concentration of Micro and Macro elements including vitamins: 4405.19 mg/L

Manufacturing Company: Duchefa Biochem

# Appendix II.

## Sub experiment-1

### a. Analysis of variance on days to shoot initiation

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 10.8889 | 5.44444 | 6.12 | 0.0355 |
| Error | 6 | 5.3333 | 0.88889 | | |
| Total | 8 | 16.2222 | | | |
| CV (%) | 8.16 | | | | |
| LSD | 1.8836 | | | | |

### b. Analysis of variance on number of shoots at 21 days after initiation

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 0.89616 | 0.44808 | 3.76 | 0.0874 |
| Error | 6 | 0.71480 | 0.11913 | | |
| Total | 8 | 1.61096 | | | |
| CV (%) | 14.76 | | | | |
| LSD | 0.6896 | | | | |

### c. Analysis of variance on number of shoots at 28 days after initiation

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 0.84802 | 0.42401 | 4.67 | 0.0599 |
| Error | 6 | 0.54520 | 0.09087 | | |
| Total | 8 | 1.39322 | | | |
| CV (%) | 8.72 | | | | |
| LSD | 0.6022 | | | | |

### d. Analysis of variance on number of shoots at 35 days after initiation

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 2.73389 | 1.36694 | 11.09 | 0.0097 |
| Error | 6 | 0.73967 | 0.12328 | | |
| Total | 8 | 1.39322 | | | |
| CV (%) | 6.56 | | | | |
| LSD | 0.7015 | | | | |

**e. Analysis of variance on number of shoots at 42 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 1.80942 | 0.90471 | 22.08 | 0.0017 |
| Error | 6 | 0.24587 | 0.04098 | | |
| Total | 17 | 12.0944 | | | |
| CV (%) | 3.09 | | | | |
| LSD | 0.4044 | | | | |

**f. Analysis of variance on length of shoot at 21 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 0.32889 | 0.16444 | 49.33 | 0.0002 |
| Error | 6 | 0.02000 | 0.00333 | | |
| Total | 8 | 0.34889 | | | |
| CV (%) | 3.63 | | | | |
| LSD | 0.1153 | | | | |

**g. Analysis of variance on length of shoot at 28 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 0.66889 | 0.33444 | 10.38 | 0.0113 |
| Error | 12 | 0.38667 | 0.03222 | | |
| Total | 17 | 3.48500 | | | |
| CV (%) | 7.62 | | | | |
| LSD | 0.3586 | | | | |

**h. Analysis of variance on length of shoot at 35 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 1.94000 | 0.97000 | 97.00 | 0.0000 |
| Error | 6 | 0.06000 | 0.01000 | | |
| Total | 8 | 2.00000 | | | |
| CV (%) | 3.19 | | | | |
| LSD | 0.1998 | | | | |

**i. Analysis of variance on length of shoot at 42 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 3.61556 | 1.80778 | 325.40 | 0.0000 |
| Error | 6 | 0.03333 | 0.00556 | | |
| Total | 8 | 3.64889 | | | |
| CV (%) | 1.81 | | | | |
| LSD | 0.1489 | | | | |

**j. Analysis of variance on number of leaves at 21 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 4.17529 | 2.08764 | 16.98 | 0.0034 |
| Error | 6 | 0.73787 | 0.12298 | | |
| Total | 8 | 4.91316 | | | |
| CV (%) | 7.36 | | | | |
| LSD | 0.7006 | | | | |

**k. Analysis of variance on number of leaves at 28 Days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 2.37109 | 1.18554 | 9.38 | 0.0142 |
| Error | 6 | 0.75827 | 0.12638 | | |
| Total | 8 | 3.12936 | | | |
| CV (%) | 6.31 | | | | |
| LSD | 0.7102 | | | | |

**l. Analysis of variance on number of leaves at 35 Days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 1.76727 | 0.88363 | 29.08 | 0.0008 |
| Error | 6 | 0.18233 | 0.03039 | | |
| Total | 8 | 1.94960 | | | |
| CV (%) | 2.47 | | | | |
| LSD | 0.3483 | | | | |

**m. Analysis of variance on number of leaves at 42 Days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 0.51069 | 0.25534 | 13.21 | 0.0063 |
| Error | 6 | 0.11600 | 0.01933 | | |
| Total | 8 | 0.62669 | | | |
| CV (%) | 1.79 | | | | |
| LSD | 0.2778 | | | | |

**n. Analysis of variance on days to root initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 4.69556 | 2.34778 | 51.89 | 0.0002 |
| Error | 6 | 0.27147 | 0.04524 | | |
| Total | 8 | 4.96702 | | | |
| CV (%) | 1.06 | | | | |
| LSD | 0.4250 | | | | |

**o. Analysis of variance on length of root at 28 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 0.52667 | 0.26333 | 47.40 | 0.0002 |
| Error | 6 | 0.03333 | 0.00556 | | |
| Total | 8 | 0.56000 | | | |
| CV (%) | 6.21 | | | | |
| LSD | 0.1489 | | | | |

**p. Analysis of variance on length of root at 35 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 2.46687 | 1.23343 | 11.98 | 0.0080 |
| Error | 6 | 0.61753 | 0.10292 | | |
| Total | 8 | 3.08440 | | | |
| CV (%) | 11.10 | | | | |
| LSD | 0.6410 | | | | |

# Appendix III.

## Sub experiment-2

### a. Analysis of variance on days to shoot initiation

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 4.40667 | 2.20333 | 5.76 | 0.0401 |
| Error | 6 | 2.29333 | 0.38222 | | |
| Total | 8 | 6.70000 | | | |
| CV (%) | 6.84 | | | | |
| LSD | 1.2352 | | | | |

### b. Analysis of variance on number of shoots at 21 days after initiation

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 0.05716 | 0.02858 | 1.19 | 0.3660 |
| Error | 6 | 0.14360 | 0.02393 | | |
| Total | 8 | 0.20076 | | | |
| CV (%) | 4.95 | | | | |
| LSD | 0.3091 | | | | |

### c. Analysis of variance on number of shoots at 28 days after initiation

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 5.99582 | 2.99791 | 26.44 | 0.0011 |
| Error | 6 | 0.68040 | 0.11340 | | |
| Total | 8 | 6.67622 | | | |
| CV (%) | 7.89 | | | | |
| LSD | 0.6728 | | | | |

### d. Analysis of variance on number of shoots at 35 days after initiation

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 4.58047 | 2.29023 | 49.30 | 0.0002 |
| Error | 6 | 0.27873 | 0.04646 | | |
| Total | 8 | 4.85920 | | | |
| CV (%) | 3.45 | | | | |
| LSD | 0.4306 | | | | |

### e. Analysis of variance on number of shoots at 42 days after initiation

| | Degrees of | Sum of | Mean | F-value | Probability |
|---|---|---|---|---|---|

| | Freedom | squares | Square | | |
|---|---|---|---|---|---|
| Treat | 2 | 7.37776 | 3.68888 | 116.61 | 0.0000 |
| Error | 6 | 0.18980 | 0.03163 | | |
| Total | 8 | 7.56756 | | | |
| CV (%) | 2.44 | | | | |
| LSD | 0.3553 | | | | |

**f. Analysis of variance on length of shoot at 21 days after**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 1.41556 | 0.70778 | 212.33 | 0.0000 |
| Error | 6 | 0.02000 | 0.00333 | | |
| Total | 8 | 1.43556 | | | |
| CV (%) | 2.86 | | | | |
| LSD | 0.1153 | | | | |

**g. Analysis of variance on length of shoot at 28 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 1.74889 | 0.87444 | 27.14 | 0.0010 |
| Error | 12 | 0.38667 | 0.03222 | | |
| Total | 8 | 1.94222 | | | |
| CV (%) | 6.54 | | | | |
| LSD | 0.3586 | | | | |

**h. Analysis of variance on length of shoot at 35 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 4.34889 | 2.17444 | 244.63 | 0.0000 |
| Error | 6 | 0.05333 | 0.00889 | | |
| Total | 8 | 4.40222 | | | |
| CV (%) | 2.52 | | | | |
| LSD | 0.1884 | | | | |

**i. Analysis of variance on length of shoot at 42 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 4.86889 | 2.43444 | 730.33 | 0.0000 |
| Error | 6 | 0.02000 | 0.00333 | | |
| Total | 8 | 4.88889 | | | |
| CV (%) | 1.18 | | | | |
| LSD | 0.1153 | | | | |

**j. Analysis of variance on number of leaves at 21 days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 7.99742 | 3.99871 | 35.76 | 0.0005 |
| Error | 6 | 0.67100 | 0.11183 | | |
| Total | 8 | 8.66842 | | | |
| CV (%) | 6.09 | | | | |
| LSD | 0.6681 | | | | |

**k. Analysis of variance on number of leaves at 28 Days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 9.4189 | 4.70943 | 39.96 | 0.0003 |
| Error | 6 | 0.7071 | 0.11786 | | |
| Total | 8 | 10.1260 | | | |
| CV (%) | 5.26 | | | | |
| LSD | 0.6859 | | | | |

**l. Analysis of variance on number of leaves at 35 Days after initiation**

| | Degrees of Freedom | Sum of squares | Mean Square | F-value | Probability |
|---|---|---|---|---|---|
| Treat | 2 | 4.95476 | 2.47738 | 38.36 | 0.0004 |
| Error | 6 | 0.38753 | 0.06459 | | |
| Total | 8 | 5.34229 | | | |
| CV (%) | 3.38 | | | | |
| LSD | 0.5078 | | | | |