

INTERNSHIP REPORT

On

**“Studies on Effect of BAP on Shoot Proliferation of
Mulberry (*Morus indica L.*) cv. *S₁* By
Using Nodal Explant”
Internship report**



Internship Report Submitted to

Hon. Director, MGM University

Institute of Bioscience and Technology, Aurangabad
in partial fulfillment of the requirement for the degree of

**BACHELOR OF TECHNOLOGY
IN BIOTECHNOLOGY (16Font)**

Submitted by

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Reg. No. 2014/43, Course No.:481

Course Title: Mini Project

Project Guide

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**Professor and Director
Department of Biotechnology
MGMU, IBT, Aurangabad**

-2021-22-

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ACKNOWLEDGEMENT

The success of any project depends largely on the team work and also encouragement and guidelines of many others. I take this opportunity to express our gratitude to the people who have been instrumental in the successful completion of this project.

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Last but not least, I am always thankful to my beloved Parents and Friends along with my family members for their love, blessings and continual support.

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Signature

Khamat Bhausahab Tukaram

Reg. No. 2014/43

ABBREVIATIONS

| | | |
|----------------|---|------------------------------------|
| BAP | : | 6-Benzyl amino purine |
| DNA | : | Deoxyribose Nucleic Acid |
| <i>et. al.</i> | : | Etalia (and associate) |
| FAO | : | Food and Agricultural Organization |
| GA | : | Gibbrelic Acid |
| Gm | : | Gram |
| IAA | : | Indole Acetic Acid |
| IBA | : | Indole Butyric Acid |
| Kin | : | Kinetin |
| LB | : | Luria Bertani |
| mg | : | Miligram |
| mm | : | Milimeter |
| Mm | : | Milimolar |
| mg/L | : | Micromolar |
| NAA | : | Napthalene Acetic Acid |
| Max | : | Maximum |
| Min | : | Minimum |
| ml | : | Mililiter |
| pH | : | log [H] ion concentration |
| SM | : | Selection media |
| <i>viz.</i> | : | Namely |

CONTENT

| Sr. No. | Title / Particulars | Page No. |
|----------------|---|-----------------|
| 01 | Declaration by the Candidate | i |
| 02 | Certificate - I | ii |
| 03 | Certificate - II | iii |
| 04 | Plagiarism Clearance Certificate | iv |
| 05 | First Page of Plagiarism Report | v |
| 06 | Acknowledgement | vi-vii |
| 07 | Abbreviations Used (if any) | viii |
| 08 | Contents | ix |
| 09 | Abstract | x |
| 10 | Chapter - I : Introduction , Objectives, Overview of the Organization | 1-15 |
| 11 | Chapter - II : Weekly Overview of Internship Activities | 16-45 |
| 12 | Chapter - III : Materials and Methods | 46-79 |
| 13 | Chapter - IV : Results | 80-120 |
| 14 | Chapter - V : Analysis | 121-131 |
| 15 | Chapter - VI : Summary and Conclusions | 132-134 |
| 16 | References | 135 -150 |
| 17 | Future Line Work | 151-152 |
| 18 | List of Figures | 155-156 |
| 19 | List of Plates (if any) | 157-158 |
| | Appendix (ces) | 161 -162 |
| | Penalties in case of plagiarism in submission of an Internship Report and dissertations | |

ABSTRACT (FORMAT)

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Methodologies:

We follow a structured methodology for our projects which starts from designing the solution to the implementation phase. Well planned Project reduces the time to deliver the project and any additional ad-hoc costs to our clients, hence we dedicate majority of our time understanding our clients business and

gather requirements. This ground up approach helps us deliver not only the solution to our clients but also add value to your investments.

Key parts of the report:

Under each division we further provide specific industry solutions on focused domains with cutting edge technologies.

Benefits of the Company/Institution through our report:

Under each division we further provide specific industry solution on focused domains with cutting edge technologies. We emphasize on building relationships with our clients by delivering projects on time and within budget.

Keywords: At least five keywords

CHAPTER- I

INTRODUCTION

- i. Short Introduction to the institute
- ii. Introduction to the topic covering its background in view of past literature.
- iii. Literature should be properly cited.
- iv. Detailed description of the project assigned to you.

Internship Objectives

The present internship work entitled “*In vitro* callus induction and plant regeneration of soybean (*Glycine max (L.) Parbhani sona* via seed explant culture” was conducted with the following objectives

- To evaluate the effects of caffeine on soybean seed.
- To initiation of callus by supplementing with BA+ 2,4 D
- To determine the optimal concentrations of BA+NAA for shoot regeneration
- To determine the optimal concentrations of NAA for root regeneration.

Overview of the Organization:

CHAPTER- II

WEEKLY OVERVIEW OF INTERNSHIP ACTIVITIES

| Week | Date | Name of the topic / Module Completed |
|------|------|--------------------------------------|
|------|------|--------------------------------------|

| | | |
|-----------------|------------|-----------------------|
| I st | 08.05.2022 | Isolation of Bacteria |
|-----------------|------------|-----------------------|

| Week | Date | Name of the topic / Module Completed |
|------|------|--------------------------------------|
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| | | |
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| II nd | 08.05.2022 | |
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| Week | Date | Name of the topic / Module Completed |
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| III rd | 08.05.2022 | |
|-------------------|------------|--|

| Week | Date | Name of the topic / Module Completed |
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|------|------|--------------------------------------|

| | | |
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| IV th | 08.05.2022 | |
|------------------|------------|--|

CHAPTER- III

MATERIAL AND METHODOLOGY

- i. Detailed description of the experiments/activities performed by student.
- ii. Detailed description of the tasks assigned to you.

The details of various material and methods were adopted during the course of present internship work are narrated in this chapter under suitable sub-heads.

Experimental situate: The experiment was conducted in Department of Plant Biotechnology MGM College of Agricultural Biotechnology, Gandheli, Aurangabad during 2017-18.

Details of NAA at variable concentrations: The MS basal medium was supplemented with BAP at variable conc. (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L) and constant IAA (2.0 mg/L) nodal culture of mulberry by BAP and IAA.

1.: Source of plant material and explants preparation: The nodal explant of mulberry was obtained from college instructional farm. The explant was collected from college industrial farm, washed several times with tap water and then by using liquid detergent (5% v/v). Against 70% ethanol is used for 30 seconds, after that process 0.1% mercury chloride (HgCl_2) for 3 minutes and rinses the explant several times to remove the trace of HgCl_2 by sterile water.

2. Preparation of shoot initiation MS media: Shoot initiation in MS media was used for the experiments containing CaCl_2 , vitamin and sucrose. The pH 5.6-5.8 was adjusted by 1N NaOH and 1N HCl and volume was made up by autoclaved distilled water. Then added agar and 2 to 3 drops of antifungal and antimicrobial supplement. After that the media was uniformly mixed with magnetic stirrer and autoclave at 121°C for 20 min. The plant growth regulators may get destroyed during autoclaving, such chemicals are therefore, sterilized by filtration through using syringe driven filter membrane 0.22 µm porosity. After autoclaving the MS medium was supplemented with different concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L and IAA 2.0 mg/L) (Murashige and Skoog, 1962).

5. Inoculation: In each slant tube MS medium (20 ml) was supplemented with different concentrations of BAP ranging from 0.5 µM to 3 µM along with the combinations of 2.0 mg/L

IAA for the shoot initiation from nodal explant. Harvested nodal explant was cut in the size of length 1 to 1.5 cm and inoculated in to the test tube.

6. Maintenance of nodal cultures: Nodal cultures was maintain at 2500 Lux light density with photoperiod of 16 hour provided by cool white fluorescent, also for better shoot initiation the culture was kept in dark at 25°C ($\pm 2^\circ\text{C}$) and 70% relative humidity.

Shoot initiation: The numbers of days take to show initial differentiation of shoot from the date of inoculation (DAI) of different explants was recorded and were mean number of days.

Number of shoots: The number of shoots proliferates was measured, after inoculation was recorded.

Number of leaves: The number of leaves proliferate was be measure, after inoculation was recorded.

8. Analysis of data: The data obtained on various observations was analyzed by “Analysis of variance” method (Panse and Sukhatme 1967).

CHAPTER- IV

RESULT

The result obtained in the present investigation on “Studies on Effect of BAP on Shoot Proliferation of Mulberry (*Morus indica L.*) cv. *S₁* By Using Nodal Explant” are presented under the following headings.

Number of days required for shoot initiation: Number of days required for shoot initiation per explants of mulberry as enhanced by different levels of BAP in combination with constant IAA at (30 DAI) is presented in Table 1.

Table1. Response of various concentrations of BAP and IAA on shoot proliferation of mulberry (30 DAI)

| Symbol (T) | Number of days required for shoot initiation 30 DAI (A) | Number of shoot produced per explants (30 DAI) (B) | Number of leaves (30 DAI) (C) |
|----------------|---|--|-------------------------------|
| T ₁ | 8.0 | 3.00 | 3.00 |
| T ₂ | 9.00 | 3.25 | 2.25 |
| T ₃ | 8.75 | 2.00 | 1.00 |
| T ₄ | 7.75 | 2.25 | 1.25 |
| T ₅ | 7.75 | 2.25 | 2.00 |
| T ₆ | 5.50 | 3.25 | 2.75 |
| S.E.± | 0.63 | 0.18 | 0.20 |
| CD | 1.99 | 1.10 | 1.22 |
| Mean | 7.79 | 2.75 | 2.04 |

Data presented in table 1 indicate that the mean number days required for shoot initiation was 7.79. The number days required for shoot initiation was influenced significantly due to different levels of BAP and constant IAA.

Treatment T₆ (BAP 3.0mg/L) was significantly superior over rest of the other treatments and recorded minimum number of days for initiation of shoot i.e.(5.50). The number of required

days for initiation of shoot by treatment, T₅, T₄, T₁, T₃ and T₂ were .7.75,7.75,8.0,8.75 and 9.0 respectively (**Table 1. A**).

Multiple shootlets produced per explants: Numbers of shoot were produced in media supplemented with BAP and IAA (Table 1 B.). Data presented in table.1 B would reveal that mean number of shoots produced per explants of *Morus indica L.* at 30 DAI was 2.75. The shoot number was influenced significantly due to different levels of BAP in combination with constant IAA.

The treatment T₆ (BAP 3.0 mg/L) recorded significantly effective over rest of the treatments and showed maximum result recording to number of shoot i.e (3.25).

Treatments T₂, T₁, T₅, T₄ and T₃ produce 3.25, 3.00, 2.25, 2.25 and 2.00 mean number of shoots (**Table 1.B**).

Number of leaves proliferated: Data presented in table 1C concluded that mean number of leaves per explant of *Morus indica L* at 30 DAI was 2.04. The mean numbers of leaves per explants were influenced significantly by different levels of BAP and IAA at 30 DAI. Treatment T₁, (BAP 0.5mg/L) produced highest number of leaves (3.00) found significantly superior over rest of the treatments. Treatment T₃ produced minimum mean number of leaves per explant (1.00). The number of leaves was produced by treatment T₆, T₂, T₅, T₄ and T₃ were 2.75, 2.25, 2.0. 1.25 and 1.00 respectively (**Table 1. C**).

CHAPTER- V

ANALYSIS

For shoot proliferation, growth regulators especially cytokinins (Lane, 1979; Stolz, 1979; Bhojwani, 1980; Garland and Stolz, 1981) are one of the most important factors affecting the response. A range of cytokinins (Kinetin, BA, 2-ip and zeatin) has been used in micropropagation work (Bhojwani and Razdan, 1992). In white clover (Bhojwani, 1981), hybrid willow (Bhojwani, 1980) and chickpea (Barna and Wakhlu, 1994). BA was the most effective cytokinin for the shoot tip, meristem and bud culture. At higher levels, cytokinins tends to induce adventitious bud formation (McComb, 1978; Zimmerman and Broome, 1980). In the present study also cultures were multiplied using a range of BA concentrations, however 5.0 μM BA was found to be optimum (Table 1). In the present study, it was seen that heat shock (at 50 °C after 7 days of inoculation) gave better shoot proliferation in combination with high sucrose concentration (232 and 290 mM) in comparison to the explants that were not given any heat shock (Table 2). In MS medium supplemented with BA (5 μM), containing different concentration of sucrose, on an average each explant gave rise to 6 -7 shoots (Table 2). Hundred percent cultures showed shoot proliferation on this medium. Beneficial effect of heat shock was also seen on shoot growth on medium containing 174 mM sucrose. It was noticed that heat shock did not had any significant effect on shoot proliferation in medium having low sucrose concentration specifically 58 and 116 mM (Table 2). Beneficial effect of high sucrose concentrations on different events of morphogenesis like shoot multiplication (De Bruyn and Ferreira, 1992), rooting of microshoots (Rahman et al., 1992; Romano et al., 1995) and somatic embryogenesis (Loiseau et al., 1995) has been reported earlier. However, temperature dependent response of different sucrose concentrations, as demonstrated in the present investigation, has been reported in *Gladiolus hybridus* (Kumar et al., 1999, 2002). Researchers are always in search of certain novel compounds that can have wide range of applications, so, in the present study effect of a bioactive compound ('Compound A') was studied on the growth and proliferation of cultures.

CHAPTER VI

SUMMARY & CONCLUSION

Briefly conclude the practical experience, experiments performed and techniques learnt during your internship in the organization.

Note: Internship report are NOT expected to be copied from anywhere, the student must provide information in these sections based on his/her personal observation, learning and experience gained during the internship. Cheating or copying in these sections is NOT acceptable and hence the entire internship report can be completely rejected as per **ZERO TOLERANCE POLICY** of the university. The university may also take a legal action according to plagiarism policy as defined by HEC (Higher Education Commission).

REFERENCES

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Reddy, S.R. (2012). *Principles of agronomy* (4th ed.). New Delhi: Kalyani Publishers.

Gail C. Frank. (2008). *Community nutrition: Applying epidemiology to contemporary practice* (2nd ed.). Boston: Jones and Bartlett Publishers.

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Reddy, T. Yellamanda., & Reddy, G.H.Sankara. (2013). *Principles of agronomy* (4th ed.). New Delhi: Kalyani Publishers.

Damodaram, T. & Hegde, D.M. (2002). *Oilseeds situation: a statistical compendium*. Hyderabad: Directorate of Oilseeds Research.

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Smit, Gerrit.(Ed.). (2005). *Dairy processing: Improving quality*. Boca Raton FL: CRC Press.

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Surname of the editor, initial. & Surname of the editor, initial. (Eds.). (Year of publication). *Title of the book in italic: subtitle of the book in italic-if any* (Edition of the book: If any). Place of publication: Name of the publisher.

Bourne, F.J. & Gorman, N.T. (Eds.). (1984). *Advances in veterinary immunology 1983*. Amsterdam : Elsevier Science Publishers B.V.

More than Two Editors:

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Kundu, S.S., Misra, A.K., & Pathak, P.S. (Eds.). (2004). *Buffalo production under different climate regions* (1st ed.). Lucknow : International Book Distributing Co.

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e Book:**One Author:**

Surname of the author, initial. (Year of publication). *Title of the book in italic: subtitle of the book in italic (if any)*. Retrieved from (URL/Website address). Accessed on (date of accessing / browsing in Name of the Month Date, Year format)

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Mishra, Sanjaya.(2019). Implementing technology enabled learning in Indian universities. *University News*. 57 (34), 8-12.

Print Journal - Two Authors:

Surname of the author, initial., & Surname of the author, initial.(Year of publication). Title of the article. Name of the journal in italic. Vol.No. (Issue No.), page numbers.

Sehgal, Sandeep & Landol, Stanzin. (2019). Intercropping of Andrographics Paniculata under Emblica officinalis in Western Himalayan Subtropics. *The Indian Forester*. 145(12), 1152-1156.

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*&resourceType=1&fromPage=1. Accessed on August 28, 2020.

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Yadav, R., Mewada, K., Raipurohit, S., & Kamboj, R.D. (2020). Valuation and quantification of Non-Timber forest products (NTFPS) available in Baria forest division of Gujrat State, India. *Indian Forester*. 146 (06), 490-495. Retrieved from- <https://jgateplus.com/search/jFarticleDetails>. Accessed on August 28, 2020.

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Print Newspaper:

Surname of the Author (s) with initial. (Year, Month, Day). Title of the article: Subtitle of article. *Title of Newspaper in italic*, page number (s).

Ghosh, Abantika. (2020, March 17). Virus testing in India, elsewhere. *The Indian Express*, 9.

Online:

Surname of the author (Researcher), initial. (Year). *Title of the contribution/ published paper in italic*. In *Name of the Editor (Ed.)*, *Title of the conference (page no of the published article as pp. 110-115)*. Location of the conference. Retrieved from- (URL/Website Address). Accessed on Month Date, Year.

Wijayatunga, Ameesha Ramithanjalee. (2018). *Overstay teacher transfers in public schools in Sri Lanka : Impact on school management and performance*. In *Sheehan, Eugene P. (Ed.)*, *4th International Conference on Education (pp. 39-50)*. Bangkok, Thailand. Retrieved from - https://educationconference.co/wp-content/uploads/2018Conference-Proceedings_Issue- 1.pdf. Accessed on August 20, 2020.

7. Dictionary :

Print:

Surname of the editor. (Ed.). (Year). *Title of the dictionary in italic*. (Edition (if any e.g. 6th ed.)).

Place of Publication: Publisher name.

Wehmeier, Sally. (Ed.). (2003). *Oxford Advanced Learner's Dictionary*. (6th ed.). New York: Oxford University Press.

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Name of the online dictionary. Retrieved from - (URL/Website Address). Accessed on Month Date, Year.

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Online Newspaper:

Surname of the Author(s) with initial. (Year, Month, Day). Title of the article: Subtitle of article. *Title of Newspaper in italic* (Edition if applicable), page number (s) or section letter (s). Retrieved from (URL of article).

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4. An Internship Report :**Print Thesis:**

Surname of the author (Researcher), initial. (Year of submission). *Title of the thesis/dissertation in italic (Master's Thesis / Doctoral Dissertation)*. Name of the University.

Misal, D.R. (2016). *Economics of production and marketing of Maize in Jalna District of Maharashtra State (Master's Thesis)*. Vasantrya Naik Marathwada Krishi Vidyapeeth, Parbhani.

Online Thesis:

Surname of the author (Researcher), initial. (Year of submission). *Title of the thesis/dissertation in italic (Master's Thesis / Doctoral Dissertation)*. Name of the University. Retrieved from - (URL/Website Address). Accessed on Month Date, Year.

Makne, V. G. (1986). *Genetic analysis of oil, protein, yield and other quantitative characters in groundnut (Arachis Hypogaea L.) (Doctoral Dissertation)*. Vasantrya Naik Marathwada Krishi Vidyapeeth, Parbhani. Retrieved from - <https://krishikosh.egranth.ac.in/dis/bitstream?han=1/58>. Accessed on August 28, 2020.

6. Conference/seminar paper:**Print:**

Surname of the author (Researcher), initial. (Year). *Title of the contribution / published paper in italic*. In Name of the Editor (Ed.), *Title of the conference (page no of the published article aspp. 110-115)*. Location of the conference.

Karanjkar, P.N., Namade, T.B., Takankhar, V.G. & Waghmare, Y.N. (2017). *Effect of integrated nutrient management on productivity of Maize (Zea mays L.)*. In Waskar, D.P., Asewar, B.V., Gaikwad, G.K., Pendke, M.S., Khandare, R.N., Jadhav, A.S., Dakhore, K.K. & Perke, D.S. (Eds.), *International Seminar on Global Climate Change : Implications for Agriculture and Water Sectors* (pp. 190). WALMI, Aurangabad, Maharashtra.

In-Text Citations:

In-text citations have two formats: parenthetical and narrative.

In parenthetical citations, the author name and publication date appear in parentheses.

In narrative citations, the author name is incorporated into the text as part of the sentence and the year follows in parentheses.

Parenthetical Citations

Both the author and the date, separated by a comma, appear in parentheses for a parenthetical citation. A parenthetical citation can appear within or at the end of a sentence.

It has been noted that the majority of respondents (65.00%) had high utility perception of AgroTech VNMKV mobile app (Pawar, 2019).

Narrative Citations

The author's surname appears in running text, and the date appears in parentheses immediately after the author's name for a narrative citation. The author's name can be included in the sentence in any place it makes sense.

Pawar (2019) noted that the majority of respondents (65.00%) had high utility perception of AgroTech VNMKV mobile app.

FUTURE LINE OF WORK

LIST OF FIGURES

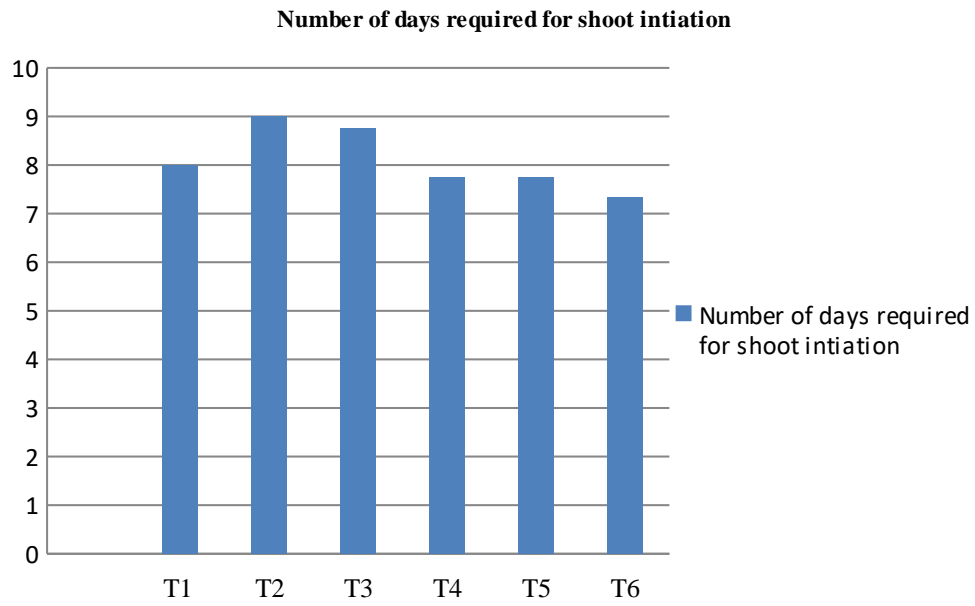


Fig.No.1. Response of BAP in different combination with constant IAA

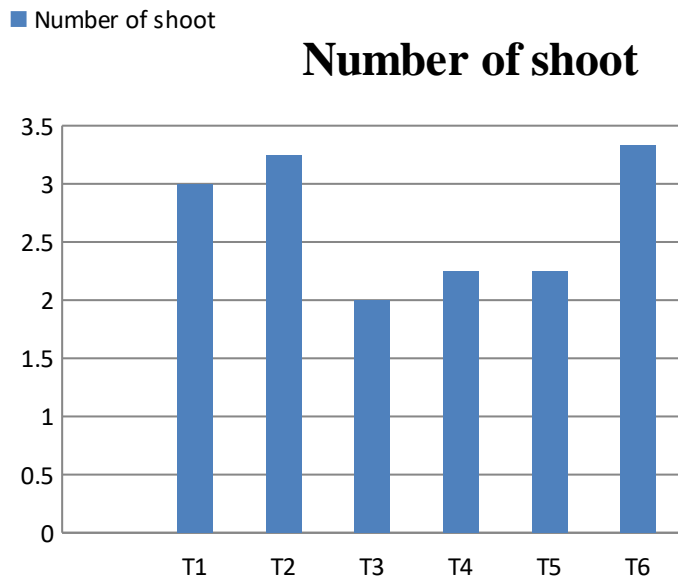


Fig. No. 2 Number of multiple shoots per explants in 30 DAI

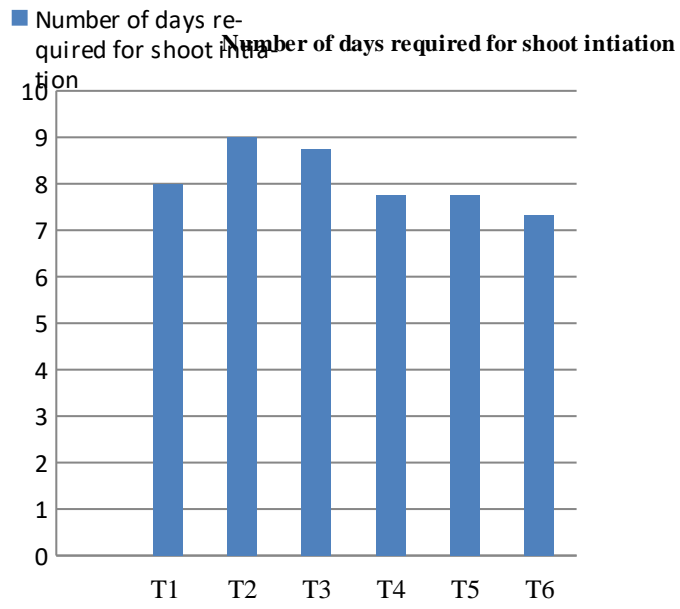


Fig. No. 3 Number of leaves produced.

LIST OF PLATES



1. Shoot initiation from nodal explants
2. Shoot proliferation in BAP with constant IAA
3. Maximum Number of leaves proliferated

APPENDIX I

Composition of Media (Murashige T and Skoog F Medium 1962)

| Sr. No | Elements | Constituents | Milligram/liter(mg/L) |
|--------|----------------|--|---|
| 1. | Macronutrients | NH_4NO_3 KNO_3 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ KH_2PO_4 | 1650.50 1900.00 440.00 180.69 170.00 |
| 2. | Micronutrients | $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ H_3BO_3 Potassium Iodide $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ $\text{CaCl}_2 \cdot .6\text{H}_2\text{O}$ $\text{CuSO}_4 \cdot .7\text{H}_2\text{O}$ | 16.19 8.60 6.20 0.83 0.25 0.025 0.025 |
| 3. | Irons | $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ | 27080 37.30 |
| 4. | Vitamins | Myoinositol Glycine Nicotinic Acid Pyridoxine HCL Thiamine HCL | 100.00 2.00 0.50 0.50 2.00 |

Font : Times New Roman

Title and main heading Font Size : 16 and Bold

Sub Heading: 14 and Bold

Running Script : 12

Spacing : 1.5