DISERTATION

On

"Studies on Effect of BAP on Shoot Proliferation of Mulberry *(Morus indica L.) cv.* S_1 By Using Nodal Explant"

Dissertation 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

Khamat Bhausaheb Tukaram

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

Course Title: Mini Project

Project Guide

Dr. S. N. Harke

Professor and Director

Department of Biotechnology

MGMU, IBT, Aurangabad

-2021-22-

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled, "(**Title of thesis in bold letters**)", submitted by me is based on the actual work carried out by me under the guidance and supervision of **(Full name of the research guide)**. The extent of information derived from the existing literature have been duly cited and referenced. The existing researchwork or its any part is not submitted anywhere else for the award of any degree or diploma**.**

I also hereby declare that no sentence, equation, diagram, table, paragraph or section has been copied verbatim from previous work unless it is cited and duly referenced. There is no plagiarism; the work presented is original and own work of the researcher. No ideas, process, results or words of others have been presented asresearcher's own work.

Place :

Date : DD/MM/YYYY

Signature of the Candidate

(Full name of the candidate in bracket without any salutation e.g. Mr, Ms. etc.) Reg. No:

CERTIFICATE – I

This is to certify that the thesis entitled, **"(Title of thesis in bold letters)"** submitted by **(Full name of the candidate in bold letters with registration number)** inpartial fulfillment of the requirements for the award of the degree of **BACHELOR OF TECHNOLOGY** or **BACHELOR OF SCIENCE (Hons.) or MASTER OF SCIENCE** in the subject of **(Name of the subject in bold letters)** submitted to the MGM University, IBT, Aurangabad is a record of bonafide research work carried out by him / her under my guidance and supervision. The thesis or its any part has not previously formedthe basis for the award of any degree, diploma or other similar title.

Place :

Date : DD/MM/YYYY

Signature of the Candidate

(Full name of the candidate in bracket without any salutation e.g. Mr, Ms. etc.) Reg. No:

CERTIFICATE – II

This is to certify that the thesis entitled, **"(Title of thesis in bold letters)"** submitted by **(Full name of the candidate in bold letters with registration number)** inpartial fulfillment of the requirements for the award of the degree of **BACHELOR OF TECHNOLOGY or BACHELOR OF SCIENCE (Hons.) or MASTER OF SCIENCE** in the subject of **(Name of the subject in bold letters)** to the MGM University, IBT, Aurangabad has been approved by the Student's Advisory Committee after viva voce examination of the student in collaboration with the External Examiner.

Signature

(Name of External Examiner with date)

Signature

(Name of Research Guide)

Research Guide & ChairmanAdvisory Committee

Signature

(Name of the Project Coordinator)
Member

Director

(Note: Name of external examiner, members, research guide start with initial without any salutation e.g. Dr.Mr. etc – P.S. Deshmukh)

PLAGIARISM CLEARANCE CERTIFICATE

This is to certify that thesis entitled, **"Title of thesis in bold letters"** submitted by **(Full name of the candidate with registration number)** has been properly examined by URKUND: Anti plagiarism Software. The percentage of similarities found in the thesis is____%.

No sentence, equation, diagram, table, paragraph or section has been copied verbatim from previous work unless it is duly cited and referenced. The work presented is original and own work of the researcher (i.e. there is no plagiarism). No ideas, process, result or words of other have been presented as researcher's own work.

Signature

(Name of the Student)

Signature

(Name of Research Guide)

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.

To start with, I would like to express my sincere gratitude to my Project Guide Dr. A.B. Kshirsagar, Associate Professor Department of Plant Biotechnology; who offered us guidance and support all along the completion of the project. I am thankful to Dr. S.N.Harke **,** Principal for supporting us during the whole curriculum. I am thankful to Prof. N.M. Maske, Assistant Professor, Department of Crop Sciences for statistical analysis of research comprehensive report.

My special thanks to my classmate friends Akash Doiphode,Prasad Wagh, Akshay Bankar, Dinesh Garudi for their immense wholehearted and sincere help during this study.

I express our sincere thanks to all Academic and Office Staff of this College for their kind helps during training work. I am also grateful to the Authors from past and present whose contributions were of great help to undertaken these investigations.

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.

Place: MGM/CABT.

Date: DD.MM.YEAR

Signature

Khamat Bhausaheb Tukaram

Reg. No. 2014/43

ABBREVIATIONS

BAP	:	6-Benzyl amino purine
DNA	:	Deoxyribose Nucleic Acid
et. al.	:	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
viz.	:	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	Thesis /Dissertation Abstract	x
10	Chapter - I : Introduction and Objectives	1-15
11	Chapter - II : Review of Literature	16-45
12	Chapter - III : Materials and Methods	46-79
13	Chapter - IV : Results	80-120
14	Chapter - V : Discussion	121-131
15	Chapter - VI : Summary and Conclusions	132-134
16	Literature Cited	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 thesis and dissertations	

ABSTRACT (FORMAT)

Title of the thesis :

Full name of the candidate :(without any salutation e.g. Mr./Ms./Mrs. etc)

Full Name of the Research Guide :(without any salutation e.g. Mr./Ms./Mrs. etc)

Department :

College / University :

Degree to be awarded :

Chlorophytum borivilianum (Safed musli) is an important medicinal plant and used world wide in drug industry. Although Chlorophytum propagates through tubers in its natural state, but propagation rate is too slow to meet demand of high quality planting material for commercial cultivation. Micropropagation protocol from selected, elite plants of Safed musli using nodal segment as explant was standardized. Shoot cultures were initiated on MS medium (1962, MS) containing BA (2.5 µM) and NAA (0.5 µM). Shoot proliferation was achieved on medium containing BA (5 µM) within 21 days of culture. Heat shock (50 °C for 1 h after 7 days of culturing) enhanced shoot proliferation at high sucrose concentration (232 and 290 mM). Heat shock also affected shoot length significantly in medium with 174 mM sucrose. 'Compound A' (a bioactive compound) was found to have beneficial effect on shoot proliferation at lower sucrose concentrations. Effect of two gelling agent (agar and phytagel) was compared in the present study. It was found that the phytagel has beneficial effect on the shoot proliferation and growth. However, agar was found to be beneficial for rooting of shoots. 95% rooting of microshoots was obtained on MS medium containing 290 mM sucrose and supplemented with IBA (5 µM). Regenerated plants after hardening were transferred to soil and they showed 24% survival in polyhouse while 90% survival was observed when directly transferred to shade house, thereby reducing the cost of acclimatization of plants. FYM and vermicompost were found to have beneficial effect on acclimatization of in vitro produced plants. The regenerated plants were morphologically similar to control plants and preliminary study indicate that they are also genetically identical.

Keywords: At least five keywords

CHAPTER- I

INTRODUCTION

Mulberry **(***Morus indica L)* is an invaluable tree of immense economic importance in silk industry for its foliage. Which constitutes the chief food for the silkworm bombyx mori L.? The improvement of productivity traits in mulberry plays a vital role in the progress of sericulture industry. However, perennial nature of the plant coupled with prolonged juvenile period slow down the process of mulberry improvement. Further, mulberry is across pollinated crop and hence heterozygosity prevails. Therefore, propagation through seeds dose not conserve stable genetic makeup, which limit the genetic improvement conventional hybridization techniques. During the last three decades, micro propagation techniques have been extensively utilized as a valuable and viable tool for overcoming such constraints in mulberry. Mulberry trees are growing wild and under cultivation in many temperate world region, mulberry is fast growing when young (Kavyashree, 2006).

Mulberry is a huge source of iron, calcium, Vitamin A, C, E and K, Folate, thiamine, Pyridoxine, Niacin and fibre, it is an excellent source of protein. 3 ounces of mulberry has 9 grams of protein. Mulberry is also rich polynutrients like anthocyanin, flavonoids, lutein, zea-xanthin, B carotene and A carotene. The trees can be monoecious or dioecious. The mulberry fruit is a multiple fruit, 2–3 cm (0.79–1.18 in) long. Immature fruits are white, green, or pale yellow. In most species the fruits turn pink and then red while ripening, then dark purple or black, and have a sweet flavour when fully ripe. The fruits of the white-fruited cultivar are white when ripe; the fruit of this cultivar is also sweet, but has a very bland flavour compared with darker varieties (Anonymous 2010) .

Over 90 % of Indian silk is mori silk as mulberry foliage constitutes the chief feed for the silkworm. Incidentally the production cost of mulberry leaves covers more than 60 per cent of the cost of cocoon production in sericulture. Mulberry is conventionally propagated through cuttings. However, many desired cultivars do not root easily or have low rooting ability. Also, propagation viacutting is restricted to only certain months of the year, and the saplings obtained by cuttings when compared with micro propagated plants also show inferior vigour. Mulberry improvement propagation through seeds is undesirable owing to cross-pollination and heterozygosis. Polyploidy in the plants and the dioeciously nature of the genus is a serious barrier to genetic improvement by conventional hybridization technique. Asexual multiplication

is preferred over sexual means as the genetic characters of the parent are maintained and population variation is minimized. Hence, the present study was carried to know the response of BAP and IAA on V1 variety in vitro condition (Anonymous 2010).

Mulberry is also known for their medicinal value, the root bark of mulberry has been used as traditional medicine in Asian countries an exhibits antibacterial activity against food poisoning micro-organism. It also helps to control blood sugar, mulberry leaves is also used to control diabetes and it also protects blood cell membrane. In abroad many countries are indicates their positive approaches for the cultivation of mulberry crop, in china 626000 hector filled under cultivation while in brazil it is 38000 hector. And in the case of India 282244 hector filled is cultivated by mulberry crop, Indian sericulture farms manufacture four types of silk - Mulberry, Tassar, Eri and Muga of which Mulberry silk accounts for 90 per cent of the total silk production in the country.

Studies have been made in mulberry to examine the impact of various growth regulators on in vitro organogenesis and plant regeneration by using different explants viz. leaf, internoddle segment, hypocotyls and cotyledons (Akram, M and Aftab, F 2012) .However, the shoot differentiation from callus is confined only to a few genotypes and re- peatability of protocols developed was not assured due to the recalcitrant nature of the plant (Raghunath *et al.* 2013) .

Tissue culture techniques such as micro-propagation provide fast and dependable method for production large number of uniform plantlets in short time. Present studies wad undertaken to determine the effect of BAP on shoot proliferation of mulberry crop by using nodal explants.

OBJECTIVES

The present investigation entitled "*In vitro* callus induction and plant regeneration of soybean (*Glycine max (L.) Parbhani sona* via seed explant culture" will be 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.

CHAPTER- II

REVIEW OF LITERATURE

The attempts has been made in this chapter to review the work done in past on this aspect of present investigation by eminent scientists in India and abroad.

Murashige and Skoog, (1962) formulated that basal media for the *in vitro* propagation of herbaceous plant species by supplementing it with different combination of growth regulators.

Martin, (1985) demonstrated that, using tissue culture technology, up to 400,000 plant could be cloned, from a single rose plant on annul basis.

Debergh and Read, (1991) demonstrated that micropropagation offers not only quick propagation of plants, but also eliminates diseases and provides scope for development of new cultivars.

R. Radhakrishnan *et al.*,(2007) worked on Callus induction and plant regeneration of Indian soybean (*Glycine max (L.) Merr.* cv. CO3) via half seed explant culture.They demonstrated that Callus initiation was observed in all media evaluated and the highest cell proliferation was obtained from explants cultivated in the presence of 13.3 µ M BAP and 13.5 µ M 2,4-D.

Ebony Y Joyner *et al.*,(2010) worked on callus Induction and Organogenesis in Soybean [*Glycine max (L.) Merr.*] cv.Pyramid from Mature Cotyledons and Embryos.They reported that 5 µM BAP was the most effective for that purpose Fully developed plants.

Phetole Mangena *et al.*,(2015) worked on In-vitro Multiple Shoot Induction in Soybean.They revealed that the highest number of multiple shoots, from both explant types, was obtained on MS media supplemented with 1.57 and 2.00 mg L^{1} BA.

CHAPTER- II

MATERIAL AND METHODOLOGY

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

Experimental situate: The experiment was conduct 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. The details of statistical design were applied for above said objectives.

Statistical design **:** Completely Randomized Design (CRD).
No. of treatments **:** 06
No. of replications **:** 04
Treatment details **:** Concentrations of BAP

Treatments (T)	Concentration of BAP (mg/L)
T_1	0.5
T_2	1.0
T_3	1.5
T_4	2.0
T_5	2.5
T_6	3.0

Note: The MS medium (20ml) and IAA (2mg/L) is constant from $T_{1\ to}$ T_6.

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 time with tap water and then by using liquid detergent (5% v/v). Against 70% ethanol is use for 30 second, after that process 0.1% mercury chloride ($HgCl_2$) for 3 minutes and rinses the explant several time 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 1N HCl and volume was make 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 121ºC 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 mg/L porosity. After autoclaving the MS medium was supplement with different concentrations of BAP (0.5, 1.0, 1.5, 2.0,2.5 and 3.0mg/L and IAA 2.0 mg/L) (Murashige and Skoog, 1962).

5. Inoculation: In each slant tube MS medium (20 ml) was supplement 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 (±2°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- III

RESULT

The result obtained in the present investigation on "Studies on Effect of BAP on Shoot Proliferation of Mulberry *(Morus indica L.) cv.* S_1 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 T_6 (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, $T_{5,}$, $T_{4,}$, $T_{1,}$ T_3 and T_2 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 T_6 (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 $T_{2,}$, $T_{1,}$, $T_{5,}$ T_4 and T_3 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 $T_{1,}$ (BAP 0.5mg/L) produced highest number of leaves (3.00) found significantly superior over rest of the treatments. Treatment T_3 produced minimum mean number of leaves per explant (1.00). The number of leaves was produced by treatment $T_{6,}$, $T_{2,}$, $T_{5,}$ T_4 and T_3 were 2.75, 2.25, 2.0. 1.25 and 1.00 respectively **(Table 1. C)**.

CHAPTER- V

DISSCUSION

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 morhogenesis 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

Chlorophytum borivilianum is a medicinal plant of considerable importance. It is widely used in drug industry and its demand is increasing day by day. The tuberous roots of Safed musli are the only propagule which can either be sold in the market for economic gains or saved for commercial cultivation year after year. This has created a severe shortage of planting material for large scale cultivation. To fill the gap of demand and supply, and to provide genetically uniform planting material from a known source, micropropagation is the best alternative. Therefore, efforts are required to develop efficient micropropagation protocol for safed musli. The objectives of the present study was to standardize conditions for establishment of axenic culture from elite germplasm, shoot proliferation, rooting of micro shoots, hardening and transfer of plants to soil. For the identification of any possible somaclones, in addition to their comparison with in terms of morphology, experiments were planned to carry out genetic analysis using DNA based markers. For this purpose we isolated DNA from both mother plant as well as plants regenerated through tissue culture. The conclusions Drawn from this study are,

1. Surface sterilization with HgCl2 (0.1% for 5-minutes) with 70% alcohol dip was best for the surface sterilization of the explants.
2. For the initiation of the culture, MS medium with BA 2.5 µM with NAA 0.5 µM was used.
3. Best shoot proliferation after heat shock was achieved on MS medium 10% sucrose and supplemented with BA (5.0 µM).
4. For shoot proliferation, medium with phytagel as gelling agent was found to be better than medium gelled with agar.
5. ‘Compound A’ (1 mg/l) enhanced shoot proliferation in the present study showing beneficial effect on shoot proliferation.
6. In the present study, IBA (5.0 µM) was found to promote rooting in basal medium containing different concentrations of sucrose (particularly 290 mM) and heat shock further promoted the rooting in microshoots. Higher sucrose concentrations were also found to be beneficial for rooting.
7. 95% shoots showed rooting response on PGR -free medium.
8. Agar as gelling agent was beneficial for rooting of microshoots as compared to phytagel.

9. In vitro raised plantlets showed 24% survival if transferred to polyhouse conditions and about 90% survival when directly transferred to shade house avoiding poly house stage.

10. FYM and vermicompost showed beneficial effect on hardening of In vitro raised plantlets.

11. Based on preliminary study using RAPD and iSSR markers, regenerated plants were found to be similar to the mother plant.

REFERENCES

Important Instructions:

- Use APA (Sixth Edition) – Citation Style
- Do not write any salutation e.g. Dr., Mr., Ms., Prof., Padmabhushan etc. before the surnameof the author / editor.
- If there is any confusion or doubt in the surname of the author, write the author name as it is printed on the book.
- Always take updated year of publication for the book.
  e.g First Edition 2010, Second Edition 2012, Third Edition 2015, Reprinted on 2017. In suchcases take the updated year of publication i.e. 2017 and consider the latest edition i.e. Third.
- All references and sources cited in all chapters from Introduction to Summary and Conclusion should be included in the Literature Cited of the thesis

Writing of references as per different sources

1. Book:

One Author:

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

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

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

Two Authors:

Surname of the author, initial, & Surname of the author, initial. (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.

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

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

More than Two Authors:

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

One Editor:

Surname of the editor, initial. (Ed.). (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.

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

Two Editors:

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:

Surname of the author, initial, editor., 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.

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

Swanson, Burton E., Bentz, Robert P. & Sofranko, Andrew J. (Eds.). (2005). *Improving agricultural extension: A reference manual* (1^{st}ed.) . Delhi : Daya Publishing House.

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)

Chapter in an edited book:

Surname of the author, initial. (Year of publication). *Title of the chapter.* In Name of the editor/s (Ed./ Eds.), *Title of the edited book in italic* (Edition if any, page number/s pp.). Place of publication: Publisher.

2. JournalArticle:

Print Journal - OneAuthor:

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

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.

Print Journal – More than Two Authors:

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

Sood, P., Sharma, A., Chahota, R. & Bansal, S. (2020). Evaluation of certain minerals and seminal plasma proteins in Jersey bulls having major sperm morphological defects. *Indian Journal of Animal Research.* 54 (01), 6-10.

Shinde, G.N., Zinjarde, R.M. & Wankhede, Bhavana. (2018). Feeding and management practices adopted by Kathani cattle in Armori tahsil of Gadchiroli district. *Journal of Soils & Crops*.28 (01), 172-176.

e- Journal:

Surname of the author, initial. (Year of publication). Title of the article. *Name of the journal in italic.* Vol. No. (Issue No.), page numbers. Retrieved from - (URL/Website address). Accessed on(date of accessing / browsing in Name of the Month Date, Year format)

Tiwari, D.N. (2020). Sustainable forest management and avoiding pandemic crisis. *Indian Forester.* 146(06). 475-478. Retrieved from - https://jgateplus.com/search/jFArticle Detai

ls_new/? current Context =al l Journal& f=journal_id[% 27140238 % 27] *&resourceType=1&fromPage=1. Accessed on August 28, 2020.

e- Journal: More than one author:

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.

3.News Paper:

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.). Placeof Publication: Publisher name.

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

Online:

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

Online Cambridge Dictionary. Retrieved from - https://dictionary.cambridge.org/. Accessed on August 20, 2020.

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).

Mehata, Pratap Bhanu. (2020, March 18). Pandemic and politics: Coronavirus crisis calls for solidarity but it also begets deeper conflicts. *The Indian Express.* Opinion Colum. Retrieved from – https://indianexpress.com/article/colum. Accessed on August 28, 2020.

4. Thesis:

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)*. Vasantrao 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 quantitive characters in groundnut (Arachis Hypogaea L.) (Doctoral Dissertation).* Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Retrieved from - https://krishikosh. egranth.ac.in / dispbitstream?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.

Karanjikar, 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

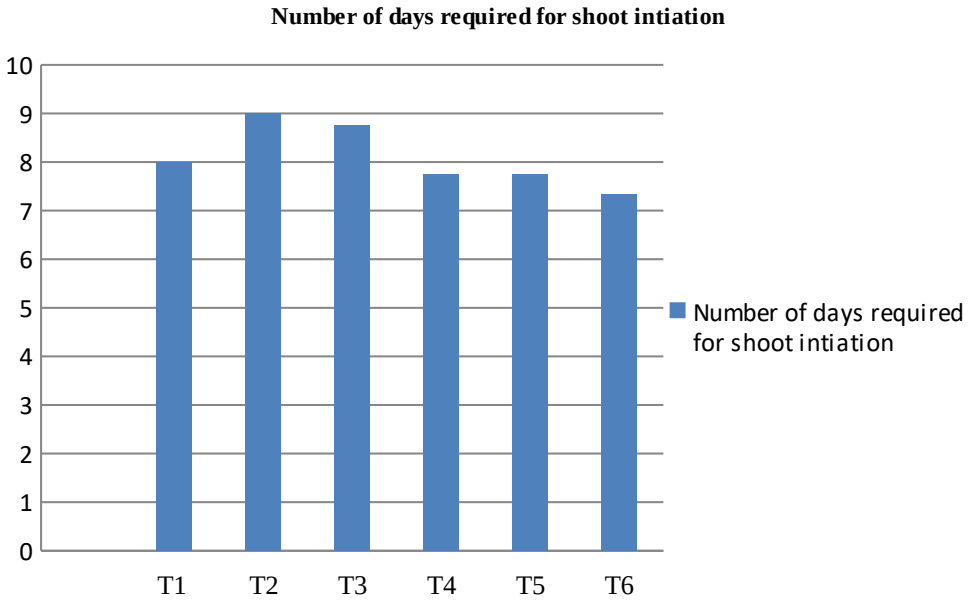

Fig. No. 3 Number of leaves produced.

LIST OF PLATES

1. Shoot initation from nodal explants
2. 2. Shoot proliferation in BAP with constant IAA
3. 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 $CaCl_2 2H_2o$ $MgSO_4 7H_2O$ KH_2PO_4	1650.50 1900.00 440.00 180.69 170.00
2.	Micronutrients	$MnSO_4 4H_2O$ $ZnSO_4 7H_2O$ H_3BO_4 Potassium Iodide $Na_2MoO_4 2H_2O$ $CaCl_2.6H_2O$ $CuSO_4.7H_2O$	16.19 8.60 6.20 0.83 0.25 0.025 0.025
3.	Irons	$FeSO_4 7H_2O$ $Na_2EDTA.2H_2O$	27080 37.30

4.	Vitamins	Myoinositol Glycine Nicotinic Acid Pyridoxine HCL Thiamine HCL	100.00 2.00 0.50 0.50 2.00
5.	Sugar (Energy source)	Sucrose	30000
6.	Solidifying Agent	Agar	800

Font : Times New Roman

Title and main heading Font Size : 16 and Bold

Sub Heading: 14 and Bold

Running Script : 12

Spacing : 1.5